African Journal of Agricultural Research

Volume 13 Number 5 1 February, 2018
ISSN 1991-637X

Academic Journals

ABOUT AJAR

The African Journal of Agricultural Research (AJAR) is published weekly (one volume per year) by Academic Journals.

African Journal of Agricultural Research (AJAR) is an open access journal that publishes high-quality solicited and unsolicited articles, in English, in all areas of agriculture including arid soil research and rehabilitation, agricultural genomics, stored products research, tree fruit production, pesticide science, postharvest biology and technology, seed science research, irrigation, agricultural engineering, water resources management, marine sciences, agronomy, animal science, physiology and morphology, aquaculture, crop science, dairy science, entomology, fish and fisheries, forestry, freshwater science, horticulture, poultry science, soil science, systematic biology, veterinary, virology, viticulture, weed biology, agricultural economics and agribusiness. All articles published in AJAR are peer- reviewed.

Contact Us

Editorial Office: ajar@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJAR

Submit manuscript online http://ms.academicjournals.me/

Editorial Board

African Journal of Agricultural Research

Table of Contents: Volume 13 Number 5, 1 February, 2018

ARTICLES

academicJournals

Vol. 13(5), pp. 220-227, 1 February, 2018
DOI: 10.5897/AJAR2017.12818
Article Number: F5A965C55861
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Review

Sustainable and technological strategies for basic cereal crops in the face of climate change: A literature review

Silvia Soledad Moreno Gutiérrez*, Alfredo Toriz Palacios, Jorge A. Ruiz-Vanoye and Sócrates López Pérez

[1]Department of Strategic Planning and Technology Direction, Autonomous Popular University of the State of Puebla, Puebla, Mexico.
[2]Department of Planning and Economic Development, Autonomous University of the State of Hidalgo, Pachuca Hidalgo, Mexico.

Received 22 October, 2017; Accepted 4 January, 2018

At the international level, corn, wheat and rice are basic grains considered to be the most important food source for humans, as they are a fundamental part of the daily diet and represent more than 55% of the caloric intake. They make up the greater production and consumption in the world. Due to Climate Change, these highly vulnerable crops to extreme temperatures have suffered a reduction in quality and quantity of yields. In addition, there is an increase in the risk of production especially for small farmers who provide more than 75% of the world production. According to the projections for the year 2050, basic cereals will continue to be essential for food security and global survival. In turn, the temperature will continue to increase and will cause a decrease of up to 10% in yield for each increased degree celsius, in the absence of sustainable adaptation. The present work consists of a review of the literature of adaptation strategies in the face of Climate Change, which contributes to reestablishing the damage caused by the green revolution on basic cereal crops, the environment, and biodiversity, the technological strategies review was also included considering that it is a tool that offers valuable support to the farmer in the decision making inherent in the planning and improvement of their cereal crops.

Key words: Sustainable adaptation strategies, basic cereals, climate change, technological strategies.

INTRODUCTION

Basic cereals such as corn, wheat and rice are foods that are grown all over the world, are part of the daily diet of humans and constitute foods that provide more than 55% of the calories consumed worldwide. According to Reynolds et al. (2016), in per capita terms this represents the most important food in the world. About 75% of the total cereal production is generated by small farmers (Torres, 2017). Demand for basic grains will continue to

*Corresponding author. E-mail: silviasoledad.moreno@upaep.edu.mx. Tel: 7711275849.

Table 1. Yield by crop and management system caused by Climate Change.

Region	CSIRO Model	NCAR Model
Corn under irrigation		
Developing countries	-2.0	-2.8
Developed countries	-1.2	-8.7
Corn (rain)		
Developing countries	0.2	-2.9
Developed countries	0.6	-5.7
Rice under irrigation		
Developing countries	-14.4	-18.5
Developed countries	-3.5	-5.5
Rice (rain)		
Developing countries	-1.3	-1.4
Developed countries	17.3	10.3
Wheat under irrigation		
Developing countries	-28.3	-34.3
Developed countries	-5.7	-4.9
Wheat (rain)		
Developing countries	-1.4	-1.1
Developed countries	3.1	2.4

Change in % between the yield with climate of 2000 and yield with climate 2050. Weighted average.
Source: Own elaboration with data of Nelson et al. (2009).

rise (although slightly) from the years 2016 to 2025 mainly in developing countries, based on population growth with the exception of sub-Saharan Africa where it will increase by 4.9% and its consumption by 8.3% (OECD/FAO, 2017).

In line with the goal to ensure food security in the face of the threat of Climate Change, from a few years ago and nowadays, large producers in different countries were able to increase the production and yield of their cereal crops through the new agriculture identified as the green revolution. In Mexico, for example, 33% of cereal consumption is imported from the United States, a high dependence that has raised import costs (Wise and Garvey, 2012).

By 2050, the demand for basic grains will increase on the basis of world population growth and will require 800 million tonnes more than now (Reeves et al., 2016). However, the Climate Change whose origin is anthropogenic and emerges with the modern era and its processes of industrialization, according to Graß et al. (2015), will continue to cause global temperature increases, from 1.4 to 4.0°C by the year 2050. There will be extreme climatic events of greater frequency and intensity, new pests and diseases, water shortage, losses of biodiversity, among others, causing crop reduction, yield and quality FAO (2016). It is estimated that production shortfalls from a number of countries will reach 25% of the world's total (Nelson, 2009). The Table 1 shows the crop yield according to two management systems (CSIRO and NCAR), shows the difference between the one reached during the year 2000 and the projection to the year 2050.

Risks that are added to the consequences of the green revolution, which even when it provided benefits, used unsustainable adaptation strategies that require greater consumption of energy and economic resources, capable of causing damage to human health, deterioration of the environment by the excessive use of fertilizers, chemical pesticides, high cost of seeds and technology (dependence), new pests, increased water consumption and non-adaptation of traditional crops (Reeves et al. 2016). Thus, even in developed countries, Climate Change continues worsening the uncertainty and instability of cereal grain production.

If we continue without sustainable adaptation strategies that contribute to restoring optimal cultivation conditions, the catastrophic scenarios that can be seen in the year 2050, in the immediate future could become a reality. They include loss of human health, production and yield deficits, irreversible damage to environment and food security, loss of biodiversity, global adverse socioeconomic losses (Ye et al., 2013; Tesfaye et al., 2015; Jones and Phillips, 2016).

LITERATURE REVIEW

The cereal sector and climate change

Of the world's basic diet foods, one of the most important groups are basic cereals and are grown worldwide. Family farmers or small farmers provide up to 75% of total food production (Eitzinger et al., 2013; Reeves et al., 2016).

Due to the strong dependence between Climate Change and crop development, cereal species are among the most affected by extreme temperatures (Jones and Phillips, 2016).

In Mexico, a third of the national consumption of basic grains such as corn and wheat is imported mainly from the United States and this strong dependence of Mexico (34% from 2006 to 2008) has led to an increase in import costs (Cal and Ciesas, 2012). Even though in the first half of the century the projections may seem to be favorable in economic and food terms for some countries, this is not the case for the world (OECD/FAO, 2017).

Satisfactory results of the green revolution have nevertheless caused serious damage of various kinds in environmental and soil aspects, such as land degradation, depletion of soil micronutrients, increased

soil toxicity, high incidence and resistance of pests and diseases and increased greenhouse gas emissions, which in the medium term will contribute to the disappearance of fields and cereal species, due to the inherent consequences of the green revolution and exhaustive use of the technology (Reeves et al., 201.6). Research and the construction of sustainable adaptation strategies continue to be a priority task at the international level, however, most of the work developed shows valuable local orientations. However studies with a global focus are still required (Arnell, 2016).

Impact of Climate Change on crop development

Due to the need of the cereal sector to provide and protect their crops in a sustainable way, the participation of researchers has been fundamental to the scientific demonstration of phenological stages of greater resistance and sensitivity to Climate Change and extreme factors, identifying anthesis with greater sensitivity, observing a decrease in yield, growth and physiological indicators.

The omission of crops that require timely attention, will lead to problems of yield and quality (Rawson and Macpherson, 2001). Frederiks et al. (2015) evaluated damage caused by radiant frost, identifying it after the emergency phase; Crimp (2016) studied frost using the Stevenson screen at 2°C at ground level and temperature threshold; similar work is done by Perry et al. (2017), who used evaluation sensors before achieving it with the naked eye. As mentioned earlier, the results consisted of identifying the most sensitive and the most resistant stages. However, the study is unable to provide data related to the time of early evaluation. In summary, the results of these studies were able to identify those stages of greater sensitivity and those of greater resistance to meteorological factors.

Ji et al. (2017) analyzed the impact of low temperatures on wheat yield, identified the stages of ear formation and grain filling as being of greater sensitivity in cultivars of controlled environment. With the same conclusions but for high temperatures, Pimentel et al. (2015) observed the 79% reduction in yield. Boutraa et al. (2015) evaluated the growth of Saudi wheat under heat stress in a controlled environment, observing a reduction in physiological and growth indicators. Sanad et al. (2016) evaluated spring wheat in the drought, concluding greater phenological resistance during emergence and sensitivity in anthesis, under controlled conditions.

Sharifi et al. (2016) evaluated the effect of the temperature depending on the stage and found out that the time from the rice planting to the initiation of the panicle is more sensitive. Ihsan et al. (2016) analyzed the phenological development of wheat crops, their growth, yield and water use efficiency in arid soil in Western Arabia, observing the relationship between growth and yield at the beginning of the reproduction phase and also observed greater sensitivity to drought and to the dates of planting during anthesis.

Chen et al. (2017) analyzed growth time and leaf area index of the winter wheat crop in their reproductive phase under extreme temperatures from 2001 to 2008 in China and detected shortening of reproductive growing, acceleration of senescence and damages to leaf area.

Impact of Climate Change on yield and quality of basic cereals

The OECD/FAO (2016) predict that the increase in cereal production will correspond to a 90% increase in yield, which, like the quality of the seed depends on the climate of production or environment, in addition to the genotype (Sosa et al., 2015). However, the relationship between Climate Change and yield levels and quality of basic cereal crops is the reverse, Figure 1 expresses this in the case of rice in agronomic areas of America, as temperature increases the yield goes down (Higuera and Monroy, 2014).

In the world, 21% of the yield variability of basic cereal crops is explained by the increase in temperatures that exceed the optimal margins (Iizumi and Ramankutty, 2016). Nowadays basic grains are among the species most affected by extreme temperatures according to Jones and Phillips (2016). Recently Crimp et al. (2016) in Australia, observed that the risk of wheat production has increased by 30% due to Climate Change. Drastic changes in temperature have resulted in decreased yields and quality of corn, wheat and rice crops, as well as economic losses in various parts of the world (Giroux et al., 2016).

At the international level, however, projections indicate that crop yields in cereals will benefit in some cases during the first half of the century (Krimly et al., 2016). Since the year 2014, an increase was observed that broke record, however, most of the crop was obtained in few but large production areas, where farmers pay the consequences of the intensive monoculture, with the degradation of soils, reduction of groundwater, marked reduction of yield increase, plus major damage to the environment with effect on their crops (Reeves et al., 2016).

In other regions of North America and the Caribbean, declining yields are expected according to Pérez and Omar (2015), in North Africa declining wheat, according to Chourghal et al. (2016), corn in the United States (from 1.6 to 2.7% per decade) according to Basche et al. (2016), as well as in some regions of Europe where there will be an increase in the frequency of extreme events and population of the continent (Trnka et al., 2015).

In other countries, the decline in yield is expected from 2050, in the case of West Africa according to Ahmed et al. (2015), some regions of Canada at the end of this century. According to Rose et al. (2016), supported by

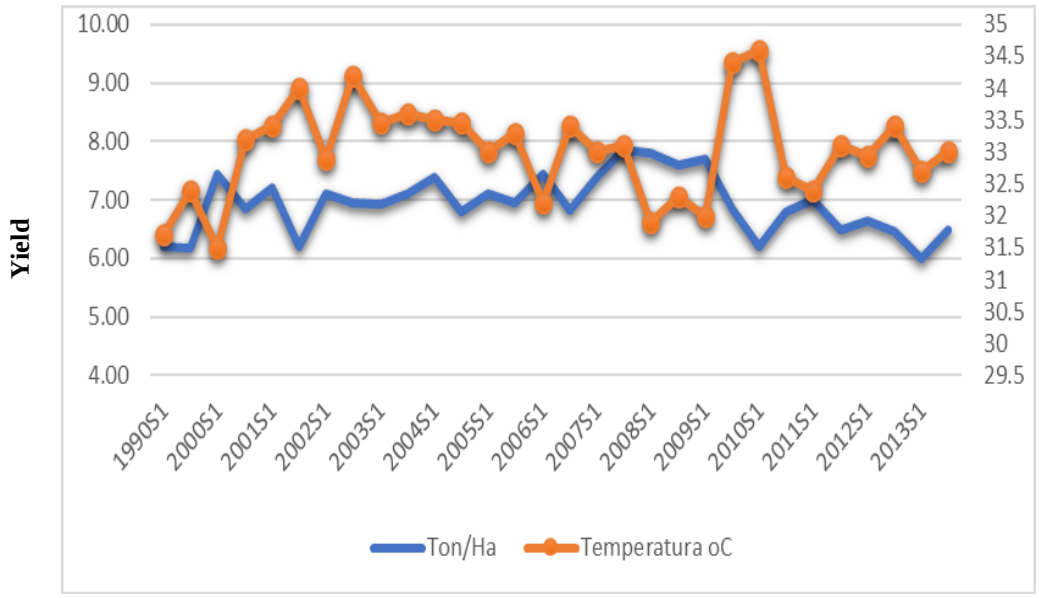

Figure 1. Temperature vs Yield.
Source: own elaboration with data of Higuera and Monroy (2014).

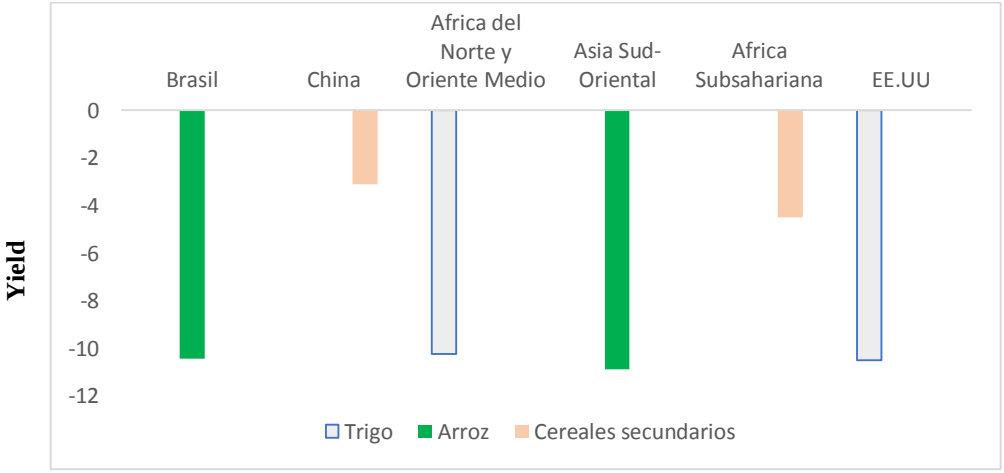

Figure 2. The projected decline in cereal yields due to Climate Change in 2050, without adaptation (%).
Source: Own elaboration with data of Reeves et al. (2016).

Zhao et al. (2017), there will be a decrease in world cereal yield greater than 10% and adverse socioeconomic consequences on the cereal sector according to Pérez and Omar (2015) of greater intensity for small farmers (Eitzinger et al., 2013).

Worldwide, wheat yield has decreased by 5.5% and corn by 3.8%, since 1980 due to Climate Change. By the year 2050, unadapted cereal yields will be reduced even further in developed and developing countries (Figure 2).

Particularly in the case of corn, when it is basically dry, its yield will decrease due to droughts and floods in sub-Saharan Africa and Asia, as well as incidence, severity and distribution of diseases. Similar situation to rice in the tropics since the current high yield varieties are intolerant to abiotic tensions, probably intensified by Climate Change, threatened by an increase in thunderstorms.

Wheat yield at high-temperature frequency will have serious effects, as crops in South, West Asia and North Africa will suffer from heat stress, water shortage, pest increase and soil pathogens, even in the Indo-Gangetic plains by 2050. Climate Change could reduce nutritional content of cereal (Reeves et al., 2016).

Sustainable adaptation strategies

Sustainable adaptation research agrees to support the small farmer and traditional crop management as the strategy with the greatest possibilities at a global level, whose objective is the adaptation and at the same time the mitigation of the Climate Change in a sustainable way, taking care of the environment and increasing the crops resilience, practice based on the experience and customs of the region, preserved from centuries ago (García and Del Fabro, 2015).

Roncancio et al. (2016), support polyculture systems, Reeves et al. (2016) propose the guide based on the Food and Agriculture Organization (FAO) model "Save for growth" in basic crops such as corn, rice and wheat, through ecosystem-based agricultural systems, with participation and support for family farmers, increasing cereal yield and resilience to Climate Change, a proposal that has been implemented in several countries with favorable adaptation and mitigation results.

Similarly, Sapkota et al. (2015), among other authors, support conservation farming that is attentive to the soil, profitable and sustainable, protecting the environment, supporting sustainability. Conde (2014) proposed the eco-friendly farming that reduces ecological impact and intelligent farming that seeks sustainable increase of production and income, greater resilience, adaptation and mitigation.

Unsustainable adaptation strategies

On the other hand, biotechnology is an alternative with several proposals for the improvement of seed that increases yield, but not its quality. However, the disadvantages exist and the risks to crop, environment, biodiversity and even to human health are generally serious. However, biotechnology has been widely implemented in the cereal sector and today they experience the consequences (Reeves et al., 2016).

Trigo (2015) worked in the development of new corn and wheat varieties with greater resistance. He et al. (2014) assert that the use of germplasm in the production of cereals and the use of molecular technologies will be useful for cereal production in the future. Robles et al. (2015) analyzed the hybrid corn and proposed the use of improved seed. Constantinescu (2017) developed a neuro-diffuse system based on environmental factors that proposes the appropriate corn hybrid per year.

In general, strategies for adaptation are observed that promote increased production and economic income, but avoid those that cause damage to the life and to the planet.

Technological strategies

In support of the cereal sector the technological strategies implemented at the local level have been multiple, and have made use of traditional technologies (mathematics and statistics) and intelligent technologies (based on Artificial intelligence techniques) basically. The approaches are oriented to obtain information of support to the farmer in the decisive decision making that contributes to reducing the vulnerability of its crops, all supported in information technologies. The most used forecasting approach has made possible the reduction of uncertainty about future events, a priority for the proper management of crops and the development of contingency plans.

Traditional technological strategies

Planning models have been used to analyze temporal variables of corn in order to identify adequate planting dates and increase crop yields in Mexico (Covarrubias et al., 2014).

Prediction models have been used to obtain timely information that favors the development of appropriate action plans. Wallach et al. (2017) predicted the impact of climate warming on rice development time, estimating the mean squared error (MSE), use generalized least squares and statistical analysis that separates model uncertainty with MSE. On the other hand, Bogard et al. (2014) constructed a model to predict the locally adapted wheat ideotype according to its phenology, based on ecophysiological models and statistical analyzes.

Prediction based on plant phenology provides accurate information for its protection. Torres et al. (2012) developed a mathematical prediction model for corn and armyworm to reduce pest damage supported by the measuring instruments, Sakamoto et al. (2013) made predictions of the same crop through statistical analysis. Lv et al. (2015) used mathematical analysis and a wheat phenology model to estimate parameters of specific cultivars and predict their development.

The models of crop simulation, of wide antiquity and use, have provided possible scenarios and very useful data, even when they show weaknesses, such as lack of data, basic parameters not considered or considered static when in fact they are dynamic. The CERES system, developed in 1896, updated today, is the most used to evaluate corn and wheat crops, considering two scenarios of Climate Change. Gallo (2015) performed daily calculations on phenological aspects, growth index, distribution of biomass, the HERMES model of Kersebaum in 1989, simulated monocultures and double corn cultivation along with predictions regarding the year 2100 as well as a decrease in yield in summer periods from 2050 (Graß et al., 2015).

The DSSAT deterministic model (Decision Support System for Agrotechnology Transfer) of the International Consortium for Agricultural Systems Applications (ICASA) more than 25 years ago, updated today, simulates corn growth, nitrogen dynamics in soil, water

and temperature at the global field scale. The WOFOST model of quantitative analysis of annual crop production based on physio-ecological processes considers phenology, transpiration, respiration, CO_2 absorption, water simulation and daily growth.

Basche et al. (2016) analyzes the Simulator model of Agricultural Production Systems (APSIM), simulate the behavior of the production of corn in winter for a period of 45 years, associating temperature variability to decreased performance per decade. The deterministic mathematical model AquaCrop simulates the development of corn cultivation for various regions of the world, considers total and deficit irrigation, determines optimal planting dates, and simulates biomass and yield according to water availability (Bernal et al., 2013).

The soil-plant-atmosphere systems for corn were developed with the objective of maximizing yield and minimizing the water used during evapotranspiration (Serio, 2015).

Prediction models are used with different approaches as predicting crop yield is the most exploited. Farjam et al. (2014) estimated the yield of corn seeds and grains on 144 farms using Artificial Neural Network models (ANN) taking into consideration fertilizers, biocides, seeds, human work, gas oil and machinery. Matsumura et al. (2015) developed a model based on precipitation and fertilizers; also, Lv et al. (2015) constructed a Neuronal Gray Network for rice and corn, as well as Gandhi et al. (2016), for rice.

Tripathi et al. (2015) created a model for wheat considering soil types and pond ash; Bose et al. (2016) constructed the Neuronal Score Network for the basic crops and Ravari et al. (2016) predicted yield variability index as well as wheat tolerance to soil salinity.

In the occurrence of weeds, prediction models are also developed for rice crops according to Barrero et al. (2016) for wheat crops by Mansourian et al. (2017).

In quality aspects, Al-Mahasneh et al. (2014) developed a collective prediction model of moisture absorption isotherms for 12 cereals and 5 legumes. Also, Lal and Varma (2014) constructed a model to identify functional aspects of cereal proteins according to structural composition.

Goyal (2013) mentions the great support that ANN's represent for the cereal sector, as well as intelligent models that have superiority of precision of results on the traditional models (Beigi et al., 2016; Mansourian et al., 2017).

In the classification, the intelligent models have supported the identification of quality wheat grains by means of ANN developed by Khoshroo et al. (2014), as well as Zhang et al. (2014) for damaged grains of wheat according to parameters of shape, color and texture.

Other models used are those oriented to monitoring and estimation. Mao et al. (2014) created a model that measures protein content in wheat, using particle swarm optimization algorithm, by grain analysis and internal composition. Yang et al. (2016) created an ANN for Nitrogen content in rice leaf, as related to production, whereas Nuñez et al. (2016) performed optimization of the culture medium that maximizes the production of amylase in wheat bran through an ANN and genetic algorithm.

Donné et al. (2016) performed segmentation of the corn plant using a convolutional ANN. Shi et al. (2016) analyzed losses of quality and quantity of wheat seeds due to insects and created internal infestation detection model by means of pattern recognition techniques. Other technologies and systems of agroclimatic monitoring, systems of monitoring of anomalies through image processing are also employed (Mora et al., 2016).

CONCLUSIONS

Climate Change is still a present and future threat to the world's basic cereal crops because their healthy development depends on having the best meteorological conditions. The forecasts indicate that the Climate Change will continue its course and if there is no adaptation and mitigation, it will leave consequences of greater intensity over time.

Large producers and developed countries have been able to compensate the effects of Climate Change on their crops and increase their production, achieving yield trends upwards for the next 10 years at least; however, their management of the crop called the green revolution has given rise to risks greater for biodiversity, food security, human health and of course for crops.

Because of this, farmers now face two challenges: the adverse effects of the Climate Change and the consequences of the green revolution, which far from reducing the problem has increased it. The planet therefore requires adaptation strategies that not only increase production but also avoid risks and damages to the environment and reestablish optimal conditions of cultivation. Otherwise catastrophic forecasts will become a reality in the immediate future.

The literature states that only some of the proposed strategies respond to the needs of the planet, for example, those that coincide in support to the environment, the small farmer and traditional practices, which makes them sustainable and capable of contributing to mitigation.

Technological strategies (more precisely intelligent models) offer the valuable farmer data and a broader picture of the behavior of his crops before the Climate Change.

Therefore, sustainable adaptation strategies should be used in a complementary way with the technological strategies to increase the success in the results considering that the farmer acts in present time according to his knowledge and experience. In turn, the technological strategy show data and future possibilities

of broad support even for farmers with little or no experience, joint alternatives suitable to achieve adaptation to Climate Change and contribute to the mitigation of Greenhouse Gases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmed K, Wang G, Yu M, Koo J, You, L (2015). Potential impact of Climate Change on cereal crop yield in West Africa. Climatic Change 133(2):321-334.

Al-Mahasneh M, Alkoaik F, Khalil A, Al-Mahasneh A, El-Waziry A, Fulleros R, Rababah T (2014). A generic method for determining moisture sorption isotherms of cereal grains and legumes using artificial neural networks. J. Food Process. Eng. 37(3):308-316.

Arnell N (2016). The global-scale impacts of Climate Change: the QUEST-GSI project. Clim. Change 134(3):343-352.

Barrero O, Rojas D, González C, Perdomo S (2016). Weed detection in rice fields using aerial images and neural networks. In Signal Processing, Images and Artificial Vision (STSIVA), Symposium. IEEE 21:1-4.

Basche A, Archontoulis S, Kaspar T, Jaynes D, Parkin T, Miguez F (2016). Simulating long-term impacts of cover crops and Climate Change on crop production and environmental outcomes in the Midwestern United States. Agric. Ecosyst. Environ. 218:95-106.

Beigi M, Harchegani M, Eshkaftaki M (2016). Prediction of paddy drying kinetics: a comparative study between mathematical and artificial neural network modeling. Chem. Ind. Chem. Eng. Q. pp. 39-39.

Bernal J, Díaz E, Méndez J, Cortés C, Boshell F, Mejías P, Stedutto P. (2013). Uso del modelo AquaCrop para estimar rendimientos para el cultivo de maíz en los departamentos de Córdoba, Meta, Tolima y Valle del Cauca. FAO Colombia, Bogotá P 161.

Bogard M, Ravel C, Paux E, Bordes J, Balfourier F, Chapman S, Allard V (2014). Predictions of heading date in bread wheat (Triticum aestivum L.) using QTL-based parameters of an ecophysiological model. J. Exp. Bot. P. eru328.

Bose P, Kasabov N, Bruzzone L, Hartono R (2016). Spiking Neural Networks for Crop Yield Estimation Based on Spatiotemporal Analysis of Image Time Series. IEEE Trans. Geosci. Remote Sens. 54(11):6563-6573.

Boutraa T, Akhkha A, Al-Shoaibi A (2015). Evaluation of growth and gas exchange rates of two local saudi wheat cultivars grown under heat stress conditions. Pak. J. Bot. 47(1):27-34.

Chen Y, Zhang Z, Tao F, Palosuo T, Rötter R (2017). Impacts of heat stress on leaf area index and growth duration of winter wheat in the North China Plain. Field Crops Res. In Press. https://www.sciencedirect.com/science/article/pii/S0378429017302150

Chourghal N, Lhomme J, Huard F, Aidaoui A (2016). Climate Change in Algeria and its impact on durum wheat. Reg. Environ. Change 16(6):1623-1634.

Conde C (2014). Cambio climático y agrobiodiversidad. Rev. Colomb. Investig. Agroind. 1(1):72-79.

Constantinescu A (2017). Neuro-fuzzy concepts applied for planning of the cereal crops: applications to the maize hybrids growing in a Romanian region. J. Biol. Dyn. 11(1):1-7.

Covarrubias M, Bustamante W, Delgado C, Ibarra E (2014). Análisis de cuatro variables del período de lluvias asociadas al cultivo maíz de temporal. Rev. Mex. Cienc. Agríc. 5(1):101-114.

Crimp S, Zheng B, Khimashia N, Gobbett, D, Chapman S, Howden M, Nicholls N (2016). Recent changes in southern Australian frost occurrence: implications for wheat production risk. Crop Pasture Sci. 67(8):801-811

Donné S, Luong Q, Goossens B, Dhondt S, Wuyts N, Inzé D, Philips W (2016). Machine learning for maize plant segmentation. In Belgian-Dutch Conference on Machine Learning (BENELEARN). https://biblio.ugent.be/publication/8132925

Eitzinger J, Thaler S, Schmid E, Strauss F, Ferrise R, Moriondo M, Olesen J (2013). Sensitivities of crop models to extreme weather conditions during flowering period demonstrated for maize and winter wheat in Austria. J. Agric. Sci. 151(06):813-835.

Farjam A, Omid M, Akram A, Niari Z (2014). A neural network based modeling and sensitivity analysis of energy inputs for predicting seed and grain corn yields. J. Agric. Sci. Technol. 16(4):767-778.

Food and Agriculture Organization (FAO) (2016). El estado mundial de la agricultura y alimentación. Cambio climático, agricultura y seguridad alimentaria. http://www.fao.org/3/a-i6030s.pdf

Frederiks T, Christopher J, Sutherland M, Borrell A (2015). Post-head-emergence frost in wheat and barley: defining the problem, assessing the damage, and identifying resistance. J. Exper. Bot. 66(12):3487-3498.

Gallo A (2015). Assessment of the Climate Change impact and adaptation strategies on Italian cereal production using high resolution climate data. Climatic Change 132:321-336.

Gandhi N, Petkar O, Armstrong L (2016). Rice crop yield prediction using artificial neural networks. In Technological Innovations in ICT for Agriculture and Rural Development (TIAR), 2016 IEEE (pp. 105-110). IEEE.

García A, Del Fabro L (2015). Experience on food purchase from family farming by Brazil´ s School Lunch Program: design determining factors. Investigación Agraria 17(1):3-17.

Giroux M, Bourgeois G, Dion Y, Rioux S, Pageau D, Zoghlami S, Vanasse A (2016). Evaluation of forecasting models for Fusarium head blight of wheat under growing conditions of Quebec, Canada. Plant Dis. 100(6):1192-1201.

Goyal S (2013). Predicting properties of cereals using artificial neural networks: A review. Sci. J. Crop Sci. 2(7):95-115.

Graß R, Thies B, Kersebaum K, Wachendorf M (2015). Simulating dry matter yield of two cropping systems with the simulation model HERMES to evaluate impact of future Climate Change. Eur. J. Agron. 70:1-10.

He Z, Xia X, Peng S, Lumpkin T (2014). Meeting demands for increased cereal production in China. J. Cereal Sci. 59(3):235-244.

Higuera A, Monroy D (2014). Variabilidad climática de la temperatura Impacto económico en el EBITDA de los cultivos de arroz riego mecanizado en la zona centro de Colombia para el periodo 1999-2013: medidas de adaptación. http://www.fao.org/docrep/006/x8234s/x8234s00.htm#Contents

Ihsan M, El-Nakhlawy F, Ismail S, Fahad S (2016). Wheat phenological development and growth studies as affected by drought and late season high temperature stress under arid environment. Front. Plant Sci. P 7.

Iizumi T, Ramankutty N (2016). Changes in yield variability of major crops for 1981–2010 explained by Climate Change. Environ. Res. Lett. 11(3):034003.

Ji H, Xiao L, Xia Y, Song H, Liu B, Tang L, Liu L (2017). Effects of jointing and booting low temperature stresses on grain yield and yield components in wheat. Agric. For. Meteorol. 243:33-42.

Jones A, Phillips A (2016). Historic Food Production Shocks: Quantifying the Extremes. Sustainability 8(5):427.

Khoshroo A, Arefi A, Masoumiasl A, Jowkar G (2014). Classification of wheat cultivars using image processing and artificial neural networks. Agric. Commun. 2:17-22.

Krimly T, Apfelbeck J, Huigen M, Dabbert S, Reichenau T, Lenz-Wiedemann V, Schneider K (2016). Effects of Future Climate Changes on Yields, Land Use and Agricultural Incomes. In Regional Assessment of Global Change Impacts. Springer International Publishing pp. 609-614.

Lal S, Varma S (2014). Identification of abiotic stress related cereal proteins based on their structural composition using back propagation networks. J. Agroecol. Nat. Resour. Manage. 1(4):270-274.

Lv H, Lei T, Huang X, Zhang Y (2015). Application of an Improved Grey Neural Network in Grain Yield Prediction.

Mansourian S, Darbandi E, Mohassel M, Rastgoo M, Kanouni H (2017). Comparison of artificial neural networks and logistic regression as potential methods for predicting weed populations on dryland chickpea and winter wheat fields of Kurdistan province, Iran. Crop

Prot. 93:43-51.
Mao X, Sun L, Hui G, Xu L (2014). Modeling research on wheat protein content measurement using near-infrared reflectance spectroscopy and optimized radial basis function neural network. J. Food Drug Anal. 22(2):230-235.
Matsumura K, Gaitan C, Sugimoto K., Cannon A, Hsieh W (2015). Maize yield forecasting by linear regression and artificial neural networks in Jilin, China. J. Agric. Sci. 153(03):399-410.
Mora J, Hurtado D, Sandoval O (2016). Preliminary monitoring of physiopathies incidence using digital image processing. Biotecnol. Sector Agropecu. Agroind. 14(1):45-52.
Nelson G, Rosegrant M, Koo J, Robertson R, Sulser T, Zhu T, Magalhaes M (2009). Cambio Climático: El impacto en la agricultura y los costos de adaptación. IFPRI. http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=INIA.xis&method=post&formato=2&cantidad=1&expresion=mfn=020722
Nuñez E, Barchi A, Ito S, Escaramboni B, Herculano R, Mayer C, Neto P (2016). Artificial intelligence approach for high level production of amylase using Rhizopus microsporus var oligosporus and different agro-industrial wastes. J. Chem. Technol. Biotechnol. 92(3):684-692.
Organisation for Economic Co-operation and Development/Food and Agriculture Organization (OECD/FAO) (2017). OECD-FAO Agricultural Outlook 2017-2026. Publishing, Paris. http://dx.doi.org/10.1787/agr_outlook-2017- en
Pérez V, Omar F (2015). Efecto del cambio climático en el maíz y frijol de Honduras evidenciado a través de la matriz de contabilidad social. https://bdigital.zamorano.edu/bitstream/11036/4489/1/AGN-2015-029.pdf
Perry E, Nuttall J, Wallace A, Fitzgerald G (2017). In-field methods for rapid detection of frost damage in Australian dryland wheat during the reproductive and grain-filling phase. Crop Pasture Sci. 68(6):516-526.
Pimentel A, Rocha J, Souza M, Ribeiro G, Silva C, Oliveira I (2015). Characterization of heat tolerance in wheat cultivars and effects on production components. Rev. Ceres 62(2):191-198.
Ravari S, Dehghani H, Naghavi H (2016). Assessment of salinity indices to identify Iranian wheat varieties using an artificial neural network. Ann. Appl. Biol. 168(2):185-194.
Rawson H, Macpherson G (2001). Trigo regado: Manejo del cultivo. Food Agric. Org. http://www.fao.org/docrep/006/x8234s/x8234s00.htm
Reeves T, Thomas G, Ramsay G (2016). Ahorrar para crecer en la práctica: maíz, arroz, trigo. Guía para la producción sostenible de cereales. http://www.fao.org/3/a-i4009s.pdf
Robles M, Hernández J, Ortega J, Martínez J, Castillo I, Sosa E, Torres A (2015). Obtención de híbridos de maíz para grano en la Comarca Lagunera y Aguascalientes, México. Ecosist. Recurs. Agropecu. 2(6):245-254.
Roncancio S, Muñoz J, Sánchez F (2016). Policultivos y sistemas silvopastoriles. Estrategias para la adaptación al cambio climático. ISBN: 978-958-763-156-2 file:///C:/Users/maest/Downloads/12_Policultivos_y_sistemas_silvopastoriles.pdf
Rose G, Osborne T, Greatrex H, Wheeler T (2016). Impact of progressive global warming on the global-scale yield of maize and soybean. Clim. Change 134(3):417-428.
Sakamoto T, Gitelson A, Arkebauer T (2013). MODIS-based corn grain yield estimation model incorporating crop phenology information. Remote Sens. Environ. 131:215-231.
Sanad M, Campbell K, Gill K (2016). Developmental program impacts phenological plasticity of spring wheat under drought. Bot. Stud. 57(1):35.
Sapkota T, Jat M, Aryal J, Jat R, Khatri-Chhetri A (2015). Climate Change adaptation, greenhouse gas mitigation and economic profitability of conservation agriculture: Some examples from cereal systems of Indo-Gangetic Plains. J. Integr. Agric. 14(8):1524-1533.
Serio L (2015). Desarrollo y validación de un modelo del sistema suelo-planta-atmósfera para la estimación de la evapotranspiración real del cultivo de maíz (Doctoral dissertation, Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires).
Sharifi H, Hijmans R, Hill J, Linquist B (2016). Using Stage-Dependent Temperature Parameters to Improve Phenological Model Prediction Accuracy in Rice Models. Crop Sci. 57(1):444-453.
Shi W, Jiao K, Liang Y, Wang F (2016). Efficient detection of internal infestation in wheat based on biophotonics. J. Photochem. Photobiol. B: Biol. 155:137-143.
Sosa R, Carballo A, Mir H, Livera A (2015). Calidad de la semilla de trigo de temporal en función del ambiente de producción. Rev. Mex. Cienc. Agríc. 6(6):1239-1251.
Tesfaye K, Gbegbelegbe S, Cairns J, Shiferaw B, Prasanna B, Sonder K, Robertson R (2015). Maize systems under Climate Change in sub-Saharan Africa: Potential impacts on production and food security. Int. J. Clim. Change Strat. Manage. 7(3):247-271.
Torres I (2017). La crisis alimentaria Mundial: causas y perspectivas para su entendimiento|| World Food Crisis: Causes and Prospects for their Understanding. Razón Palabra 20(3-94):602-621.
Torres J, Landeros F, Enciso T, Sañudo M (2012). Phenological prediction models for white corn (*Zea mays* L.) and fall armyworm (Spodoptera frugiperda JE Smith). Agrociencia (Montecillo) 46(4):399-410.
Trigo Y (2015). El centro de investigación y mejoramiento del maíz y el trigo (CIMMYT). Sociol. Méx. P 16.
Tripathi R, Kalyani V, Ram L, Jha S (2015). Prediction of Wheat Yield from Pond Ash Amended Field by Artificial Neural Networks. J. Hazard. Toxic Radioact. Waste 19(4):04015001.
Trnka M, Hlavinka P, Semenov M (2015). Adaptation options for wheat in Europe will be limited by increased adverse weather events under Climate Change. J. Royal Soc. Interf. 12:112.
Wallach D, Nissanka S, Karunaratne A, Weerakoon W, Thorburn P, Boote K, Jones J (2017). Accounting for both parameter and model structure uncertainty in crop model predictions of phenology: A case study on rice. Eur. J. Agron. Elsevier 88:53-62.
Wise T, Garvey E (2012). Mexican Rural Development Research Reports. http://www.ase.tufts.edu/gdae/Pubs/wp/12-03TurrentMexMaizeSpan.pdf
Yang J, Gong W, Shi S, Du L, Sun J, Song S (201). Estimation of nitrogen content based on fluorescence spectrum and principal component analysis in paddy rice. Plant Soil Environ. 62:178-183.
Zhao C, Liu B, Piao S, Wang X, Lobell D, Huang Y, Durand J, Elliott J, Ewert F, Janssens IA, Li T, Lin E, Liu Q, Martre P, Müller C, Peng S, Peñuelas J, Ruane AC, Wallach D, Wang T, Wu D, Liu Z, Zhu Y, Zhu Z, Asseng S (2017). Temperature increase reduces global yields of major crops in four independent estimates. Proc. Natl. Acad. Sci. 114(35):9326-9331.

academicJournals

Vol. 13(5), pp. 228-238, 1 February, 2018
DOI: 10.5897/AJAR2017.12856
Article Number: 1A6396E55865
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Litter production in a natural stand of Brazil nut trees (*Bertholletia excelsa* Bonpl.)

Quêzia Leandro de Moura Guerreiro[1*], Raimundo Cosme de Oliveira Júnior[2], Maria de Lourdes Pinheiro Ruivo[3], Katia Emidio da Silva[4], Troy Patrick Beldini[5], Marcelino Carneiro Guedes[6], Amanda Fabricia Leão Mota[7], Brenda Lohana Teixeira de Moraes[7], Paulo Roberto Brasil Santos[7] and Izabela Moura Duin[8]

[1]Graduate Program in Environmental Sciences, Federal University of Pará, Brazil.
[2]East Amazon Agroforestry Research Center, Brazilian Agricultural Research Corporation, Brazil.
[3]Department of Earth Sciences and Ecology, Emílio Goeldi Museum, Brazil.
[4]Agroforestry Research Center of the Western Amazon, Brazilian Agricultural Research Corporation, Brazil.
[5]Institute of Biodiversity and Forests, Federal University of Western Pará, Brazil.
[6]Amapá Agroforestry Research Center, Brazilian Agricultural Research Corporation, Brazil.
[7]Institute of Science and Technology of Natural Water Resources, Federal University of Western Pará, Brazil.
[8]Bacteriologia Labor, Agronomic Institute of Parana, Brazil.

Received 3 November, 2017; Accepted 10 January, 2018

This research estimated litter production and analyzed its relation to environmental variables such as maximum temperature, insolation, and rainfall. The study was conducted on a 300 × 300 m experiment as part of the project titled mapping of native Brazil nut stands and socio-environmental and economic characterization of Brazil nut production systems (MapCast), in the Tapajós National Forest (FLONA Tapajós). Every 30 days for one full year (August 2015 to July 2016), litterfall was collected and stored in a laboratory. After drying, the material was separated into leaves, wood, flowers and fruits, and miscellaneous and weighed. Statistical tests conducted were Shapiro-Wilk (5%), Principal coordinate analysis, t-test, Pearson's linear correlation, cross-correlation, and canonical redundancy analysis. Rainfall and temperature data were inferior and superior, respectively, to normal climate conditions in the region, and data for solar insolation had an abnormal pattern compared to normal climate conditions. Leaf production varied between 169.9 and 965.6 kg ha^{-1} month^{-1}, and that of wood between 26.7 and 501.3 kg ha^{-1} month^{-1}, while that for flowers and fruit varied from 0.6 to 19.6 kg ha^{-1} month^{-1}. The greatest leaf production was measured during the months with the lowest amount of rainfall and highest temperatures, and variation in leaf production and total litterfall was partially explained by temperature and insolation.

Key words: Litter, *Bertholletia excelsa*, Amazon, El Niño, FLONA Tapajós.

INTRODUCTION

Forest litter is considered the most dynamic portion of the litter-soil system (Scoriza et al., 2012), being composed of leaves, stems, flowers and fruits, and detritus (Golley, 1978). Giácomo et al. (2012) relate that litter has the function of reducing water loss through evaporation and temperature fluctuation at the soil surface, and protecting soils from erosion, excessive solar radiation, compaction, and nutrient leaching. In tropical regions it is considered

the principal source of nutrients entering the soil due to its decomposition and subsequent reabsorption of nutrients by the vegetation, a process that supports ecosystem sustainability (Calvi et al., 2009).

There are currently a lot of studies on the effect of soil compaction on limiting root growth of plants. Plants are the source of life in the living world. They perform many ecological functions in their environment, and they shape the life of living things in the environment where they live. The life of living things in the world is directly or indirectly dependent on plants (Sevik and Cetin, 2015; Cetin, 2016). The ability of plants to fulfil their functions primarily depends on the availability of appropriate climatic and edaphic conditions (Cetin, 2015). Therefore, soil is one of the absolutely necessary conditions for plant existence, which is essential for the life of living things.

Some studies shows that it examined the change of the soil structure in the forests according to the tree species. An attempt to determine some soil characteristics based on tree species and depth of soil was made within the scope of the study. Soil is important for forest and landscape. Enzymes in the soil structure ensure that they are alive in forest areas (Sevik and Cetin, 2015; Cetin, 2016; Cetin, 2015).

Processes related to litter production are of great importance in forests that grow on soils of naturally low fertility, such as those in a large part of the Amazon basin (Quesada et al., 2011). Almeida et al. (2015) detailed the need for more research to elucidate the factors that influence litter production in the Amazon biome due to the fact that existing studies are limited in geographical scope and therefore insufficient with respect to the scale and heterogeneity of the Amazon system.

In productive forest ecosystems, such as in natural stands of Brazil nut trees (*Bertholletia excelsa* Bonpl.), a species native to the Amazon and of great importance to economic sustainability of the region (Salomão, 2014), the study of litter production can help to understand the nutrient dynamics in these stands (Lima et al., 2015; Proctor, 1983) and direct management techniques. Godinho et al. (2014) also emphasize the importance of quantifying litter production in pristine ecosystems that are threatened by human activities, such as the case of stands of native Brazil nut trees along the BR 163 (Santarém-Cuiabá) highway (Scoles et al., 2016).

Besides human disturbance, litter production can be influenced by meteorological variables, soil fertility, plant genetic factors, and forest successional stage and species composition (Almeida et al., 2015).

Meteorological factors are frequently included in research on litterfall, principally air temperature and precipitation (Bianchin et al., 2016; Chave et al., 2010; Ferreira et al., 2015; Santos Neto et al., 2015; Zhang et al., 2014) and also evapotranspiration (Wagner et al., 2016). Borchert et al. (2015) related that insolation is also a relevant climatic factor for litter production. The degree of influence of these environmental factors can vary depending on the region studied (Godinho et al., 2015; Wagner et al., 2016).

In order to understand the functioning of tropical forests in relation to climatic variables it is necessary to understand how these will respond to climate change (Bi et al., 2015). Meir et al. (2009) and Malhi et al. (2009) emphasize that knowledge of Amazonian forest ecosystems during the dry season should be a central focus of research due to the risks of forest integrity posed by regional climate change predictions. Godinho et al. (2015) explain that there should be more attention paid to forests in countries that are in the process of development, such as Brazil, because they suffer from intense human activities, principally due to the use of fire, clear cutting, and over-exploitation of forest resources. In the Tapajós region, Pyle et al. (2008) found elevated values for woody materials in forest litter and attributed this result to a change in forest environmental equilibrium that could be happening in the study region. Furthermore, the variability in Amazonian precipitation can be partially explained, principally during the dry season, by the ENSO (El Niño Southern Oscillation) (Yoon and Zeng, 2010).

The aim of this study was estimate litter production and analyzed its relation to environmental variables such as maximum temperature, insolation, and rainfall in a *B. excelsa* stand used by the local population for Brazil nut harvesting.

MATERIALS AND METHODS

Study area

This study was developed in an area that is part of the larger study area of the project titled Mapping of native Brazil nut stands and socio-environmental and economic characterization of Brazil nut production systems (MapCast), at km 84 in the Tapajós National Forest (FLONA).

The study site was 300 m × 300 m and was installed in an area that had a natural stand of *B. excelsa* (Figure 1). In 2015, there were 92 Brazil nut stems with DBH (Diameter at Breast Height) above 10 cm (Figure 1), and the density of these stems on the study site was 10 stems/ha. According to Mori and Prance (1990), this density is considered high for humid tropical forests. Precipitation data (mm), maximum temperature (°C), and insolation (hours) corresponding to the litter sampling period were obtained from a conventional weather station in Belterra-Pará.

The FLONA Tapajós has several classes of soil; however, Yellow Oxisols are predominant (Carvalho, 1992) throughout the FLONA.

*Corresponding author. E-mail: queziamoura@hotmail.com.

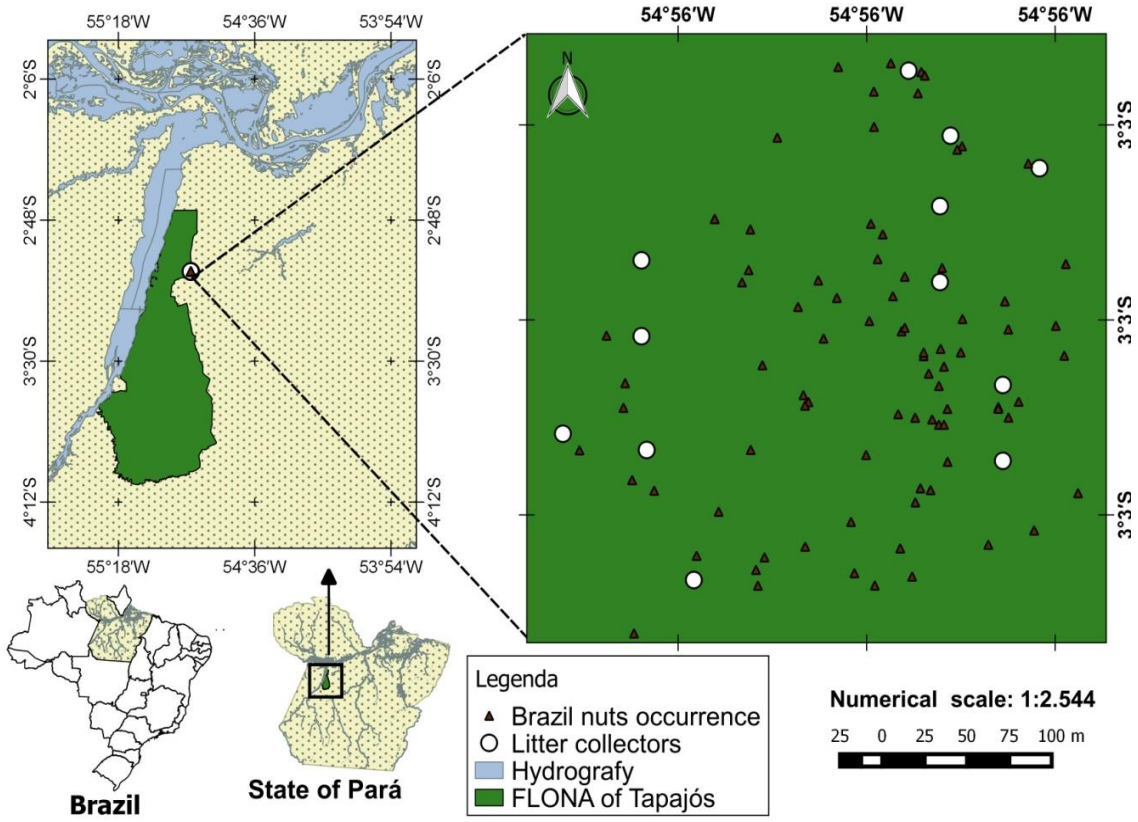

Figure 1. Location of the Tapajós National Forest, the MapCast project study site, the *Bertholletia excelsa* stems, and the litter collection points.

Guerreiro et al. (2017) present the principal physico-chemical characteristics of the MapCast project study site using a geostatistical analysis. The majority of the vegetation of the FLONA is characterized dense ombrophilous forest with emergent trees reaching 50 m in height (Pinho et al., 2004). The canopy is dense, closed, and compact, and is about 25 to 30 m in height (Veloso et al., 1991).

According to Radambrasil (1976) and Espírito-Santo et al. (2005), the FLONA Tapajós was subdivided into 16 classes divided in two great phytophysiognomy groups: Dense Tropical Forest, and Open Tropical Forest. The first has a subcategory lowland dense tropical forest, which occurs in lowland areas with clay soil and has as dominant species: *Diplotropis* species, *Minquartia guianensis*, *B. excelsa*, and *Goupia glabra*. The second subcategory is submontane dense tropical forest and is formed by trees of smaller stature such as *Mouriri brevipes*, *Mezilaurus itauba*, *Qualea* species, and *Manilkara huberi*.

The second of the great phytophysiognomy groups occurs on the intensely dissected plateus with erosion prevalent on the slopes, narrow valleys, and soils with a medium texture where forests with lianas and various palm species are found. The works of Loureiro et al. (1979), Maués (2002), Locatelli et al. (2005), Salman et al. (2008), and Scoles (2010) describe the principal characteristics of the species *B. excelsa* found on the MapCast study site.

The altitude of the FLONA is approximately 175 m above sea level and the relief is strongly undulating (Ibama, 2004). The climate is rainy with average annual rainfall of 2,300 mm (Pinho et al., 2004). The average annual temperature is 25°C, with an average minimum of 18.4°C and maximum of 32.6°C, and average air relative humidity is 86% (Carvalho, 2001).

Litter collection and processing

Litterfall collection was conducted using 12 circular nylon collectors (1 m^2) with a 2 mm mesh. Collectors were installed 50 cm above the soil surface and randomly distributed in the study area (Figure 1). Every 30 days during one full year from September 1st, 2015 to August 1st, 2016 litterfall was collected and stored in properly identified Kraft paper bags. In the laboratory, the material was dried in an oven at 40°C for 24 h.

The litter was separated into four classes: (1) leaves, including leaflets and petiole; (2) wood, including bark, small pieces of branches and twigs of all sizes, including those larger than 2 cm; (3) flowers and fruits (reproductive structures); and (4) miscellaneous unidentifiable vegetation material. The material was dried at 80°C for 48 h until reaching constant weight. The material was weighed on an analytical balance with three digits and was conducted for each collector, by material class and month. Using the dry weight values, the monthly production for each class and for total production was calculated in $kg.ha^{-1}$. The procedures used for drying, separation, and weighing followed those used by the soils laboratory at Embrapa-Amazônia Oriental in Santarém, Pará.

Meteorological data

Data for precipitation (mm), maximum temperature (°C), and insolation (hours) corresponding to the litter sampling period were obtained from a conventional weather station in Belterra-Pará, located at 38 km from the study site. Data for precipitation and insolation were summed individually for each month and daily

maximum temperature values were presented as monthly averages.

Statistical analysis

Data for litter and meteorological variables were tested for normality using the Shapiro-Wilk test at a 5% level of significance (Zar, 1999). In order to describe the similarity and the ordination of the litter production data, principal coordinate analysis (PCO) was employed, using the Euclidian distance. The outliers found by this test were discarded before further analysis.

In order to determine if there was a significant difference at a probability level $\alpha \leq 0.05$ between production of leaves, woody material, flowers and fruits, and total litterfall (sum of the four classes) during the periods determined by the PCO analysis, a t-test was applied to data that were transformed using the Neperian logarithm (ln) (Valentin, 2012).

Verification of relationships between meteorological variables and litter production was conducted using the Pearson's Linear Correlation test, and to test for time lags between litter production and meteorological variables, a cross-correlation test was used (Davis, 1986). Each of these tests used non-transformed data.

The canonical redundancy analysis (CRA) test was used to quantify the influence (%) of the meteorological variables on the production of litter in each class using the "Forward selection" method with the Monte Carlo permutation test using the ranging standardization to indicate which variables were significant. The Shapiro-Wilk, PCO, t-test, and the correlations were conducted using the Past statistical program, version 3.14 (Veloso et al., 1991), and the CRA was done using the program Canoco, version 4.5 (Ter Braak and Smilauer, 2002). The choice of these statistical tests is supported by the analyses done in Valentin (2012), Vasconcellos et al. (2013) and Wagner et al. (2016).

RESULTS

Meteorological data

Rainfall data (Figure 2A), temperature (Figure 2B) and insolation (Figure 2C) are presented together with litter production data for each class. The rainfall data for the study period from August 2015 to July 2016 were below the normal rate for the region, except for September wherein one rainfall event was responsible for the rainfall registered for the entire month, thus conferring to the month of September a rainfall total above the historical average for this month (Figure 2A).

Average maximum temperatures were above normal during the entire study period with December 2015 and January 2016 at 3.1 and 3.6°C above the average for the last 45 years, respectively, (Figure 2B).

Insolation data for August 2015 to July 2016 also did not follow the normal pattern for the region, alternating between a greater number of hours (August and September, 2015; December 2015 to May 2016; and July 2016) and a lower number of hours of insolation (October to November, 2015 and June 2016) (Figure 2C).

Litter production

Leaf production as litterfall varied between 169.9 (in May 2016) and 965.6 kg ha^{-1} $month^{-1}$ (in September 2015), woody material from 26.7 (in December 2015) to 501.3 kg ha^{-1} $month^{-1}$ (in September/2015), and for flowers and fruits between 0.6 (in January 2016) and 19.6 kg ha^{-1} $month^{-1}$ (in April 2016) (Figure 2A). During the months with the lowest rainfall (Figure 2A) and highest temperatures (Figure 2B) leaf litter fall production was the highest.

Wood production had the largest peaks in the rainy season, except for September. The litter class flowers and fruits had its largest production in the months of September (18.3 kg ha^{-1} $month^{-1}$), October 2015 (15.8 kg ha^{-1} $month^{-1}$) and April 2016 (19.6 kg ha^{-1} $month^{-1}$). Leaf production was always greater than production in the other classes for all 12 months (Table 1).

Statistical analysis

The PCO analysis yielded two groups, with the first group composed of the months of February, March, April, May, June, and July, 2016, and the second group formed by the months of August, October, November, and December, 2015, and January, 2016 (Figure 3). These groups were designated as the rainy and dry periods, respectively, and the month of September was considered and outlier by the PCO analysis.

After litter data transformation normality was corrected and then a t-test was conducted to test for significant variation between the rainy and dry periods for total leaf production ($t=5.49$; $p<0.01$) (Figure 4A) and for total litter production ($t=4.28$; $p<0.01$) (Figure 4D).

The litter classes wood and flowers and fruits showed no significant variance ($p>0.05$) (Figure 4B and C) and no significant correlation with any meteorological variable (Table 2).

Leaf production had a time lag of one month for the variable rainfall (Lag: 1, $r = -0.65$) and insolation (Lag: 1, $r = 0.80$), and total litterfall showed a time lag of one month (Lag: 1, $r = 0.78$) for insolation (Table 2). There was no time lag for production of wood and flowers and fruits, and the vegetation showed no response, at a monthly scale, to temperature variation.

The three meteorological variables analyzed explain 38.24% of the temporal variation in litterfall production (Figure 5). The first axis explains 32.4% of production of leaves and total production of litter, related to temperature and insolation, forming a gradient from the dry to the rainy period. Rainfall comprises the second axis which had a lower value for percentage of explanation of the variation in production data. Wood production had no relation with any meteorological variable, result that is in agreement with that from the Pearson linear correlation test and the t-test. The Monte Carlo permutation test using the "Forward selection" method indicated that temperature explained 26.5% of the variation in production data, with this being the only meteorological variable considered significant ($p \leq 0.05$).

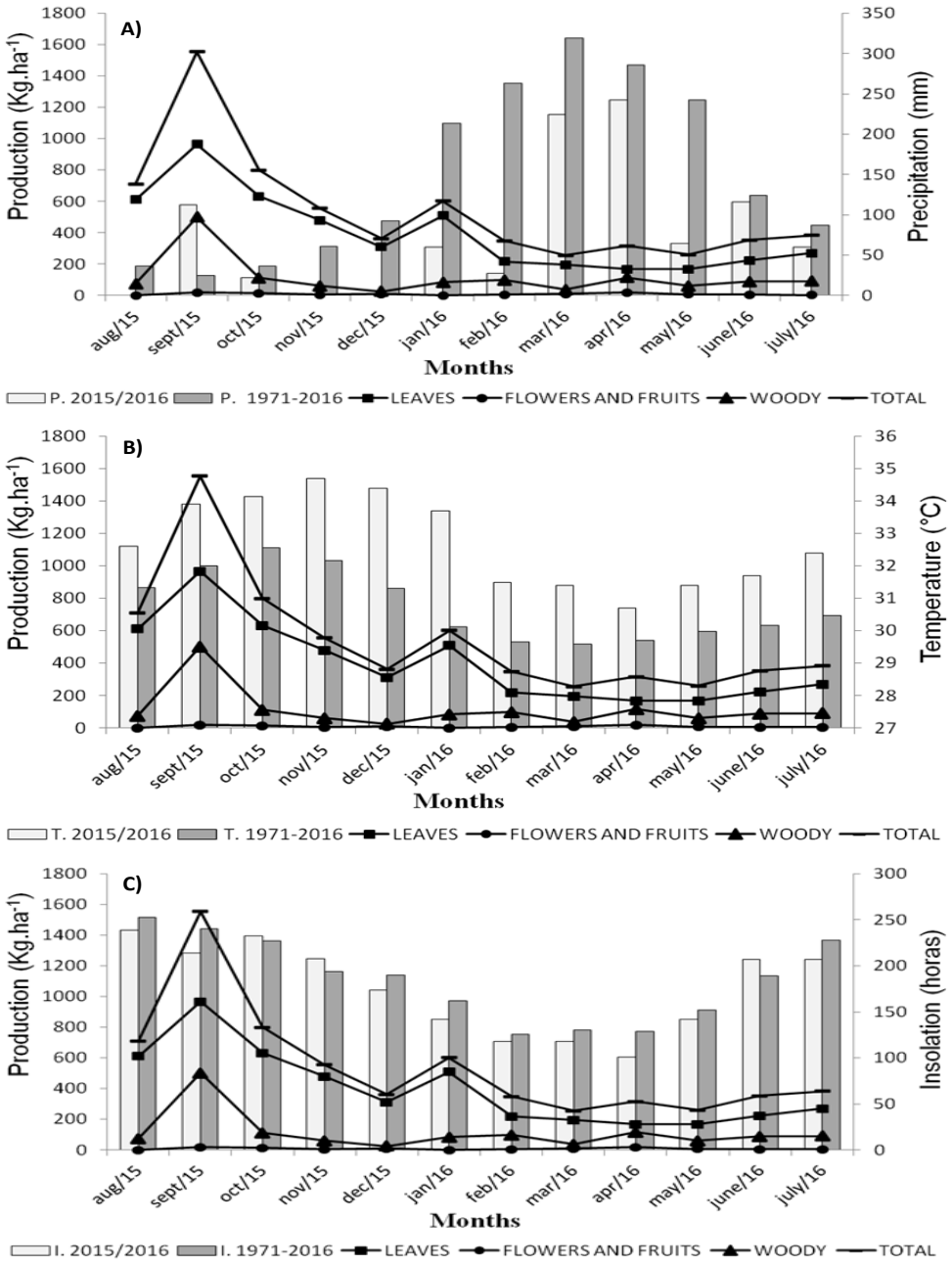

Figure 2. Monthly values for litter production for leaves, woody material, and flowers and fruits, and data for rainfall (A), temperature (B) and insolation (C), in a natural Brazil nut tree stand in the FLONA Tapajós, Pará. Continuous lines represent production data for the four classes of litterfall; bars represent meteorological variables P.: precipitation, T.: temperature, I.: insolation. The average historic values for climatic variables are identified as "1971-2016".

Table 1. Contribution of each litterfall class in relation to total monthly production deform August 2015 to July 2016.

Parameter	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	July
			2015						2016			
Leaf (%)	85.8	62.1	79.1	86.1	85.7	84.6	62.5	75.8	53.5	65.2	63.5	70.3
Wood (%)	10.5	32.2	14.1	11.2	7.4	13.9	28.6	14.8	36.5	23.2	25.6	23.9
Flowers and fruits (%)	0.2	1.2	2.0	1.0	3.2	0.1	1.7	4.0	6.2	3.1	1.3	1.1
Miscellaneous (%)	3.5	4.5	4.8	1.7	3.8	1.5	7.3	5.3	3.9	8.4	9.6	4.7

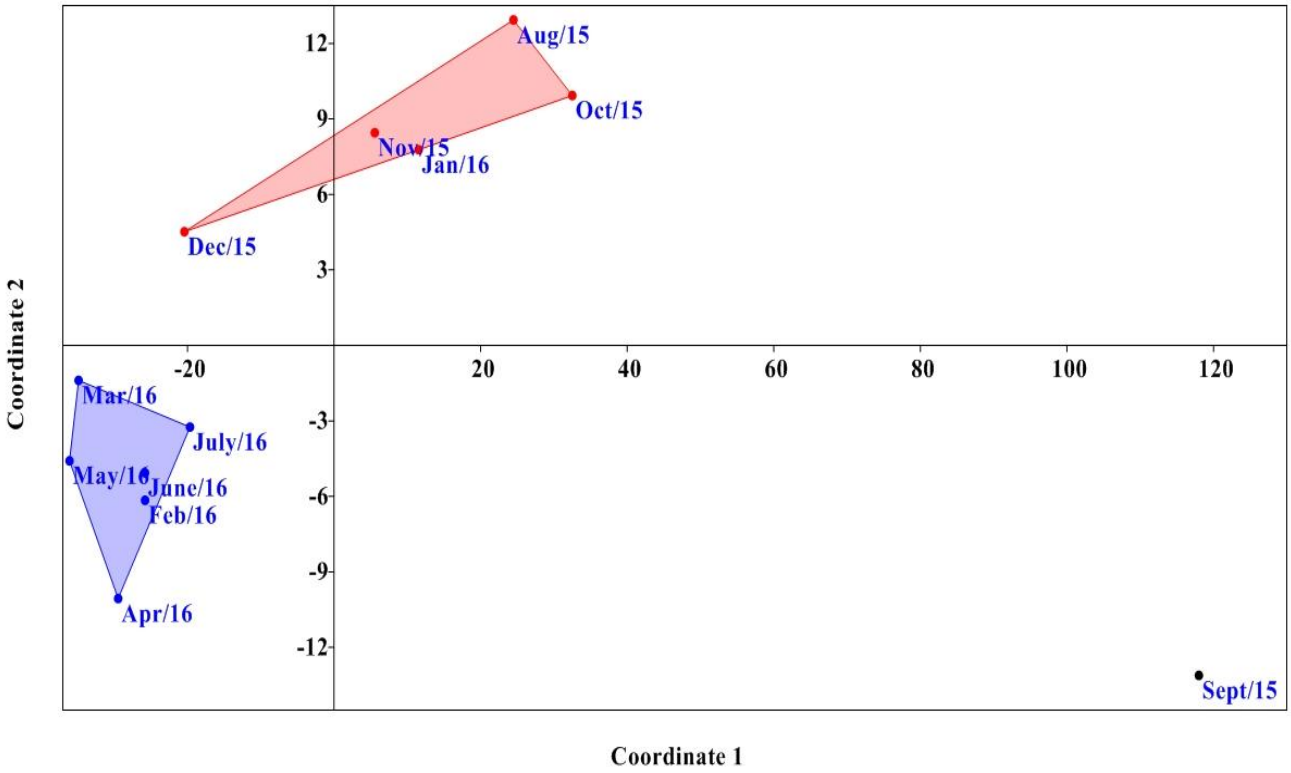

Figure 3. Similarity groups yielded by the PCO analysis using litter production data from a native Brazil nut tree stand in the FLONA Tapajós, Pará. The group in red is formed by the months of July 2016 (July/16), August (Aug/15), October (Oct/15), November (Nov/15) and December (Dec/15) 2015 and represents the dry period. The group in blue is formed by the months of January (Jan/16), February (Feb/16), March (Mar/16), April (Apr/16), May (May/16) and June (June/16) 2016 and represents the rainy period. September (Sept/15) had no similarity with the two groups.

DISCUSSION

Temporal variation of the meteorological data and litter production

The sampling period for litter production (August 2015 to July 2016) coincided with a very intense El Niño event according to the Oceanic Niño Index (ONI) (Golden, 2016). The comparison between the historical climate averages for rainfall and temperature for the past 40 years and the data from this study shows a strong anomaly caused by this event in the FLONA Tapajós.

The average monthly production of the leaf and wood classes was higher than that reported by Silva and Oliveira Júnior (2010) in 2007 and by Silva (2014) in 2002 and 2003, in dense ombrophilous forest on terra firme at km 67 in the FLONA Tapajós wherein flowers and fruits showed inferior values to those in the current study, principally for months with less rainfall. The results from the current study are greater than those reported by Ferreira et al. (2015) in humid tropical forest in the FLONA Caxiuanã in the eastern Amazon.

The high values for leaf and wood production could be due to influence of the temperature and rainfall anomalies related to the El Niño event that occurred during 2015 and 2016. For example, in 2007 the temperature of the eastern portion of the Pacific ocean, which is a variable that influences the precipitation rate in the Amazon, was stable, and therefore there was no El Niño event in this year, but in years 2002 and 2003 there was a moderate and weak El Niño, respectively (INMET, 2010). Costa et al. (2014), studying litter production in 2009 and 2010 in the FLONA Caxiuanã, reported a significant increase in litter production coinciding with an El Niño event.

The low value for flower and fruit production in El Niño years was also reported by Silva (2014). The high temperatures (Figure 2B) and the subsequent reduction or absence of water in the system in the current study could have altered reproduction processes of the vegetation in the study area. Chagas et al. (2012) related, about the National Forest of Caxiuanã, also in Pará State, that a reduction in rainfall significantly affected all the parameters of vegetation development, and Guerreiro (2017) conducted a socioeconomic study with extrativists of *B. excelsa* who collected Brazil nuts in the area around km 84 of the FLONA Tapajós, and these local people related that production of Brazil nuts and other fruits in 2016 was extremely low compared to preceding years. They associated this reduction to the intense dry period and frequent fires that occurred in the forest in the

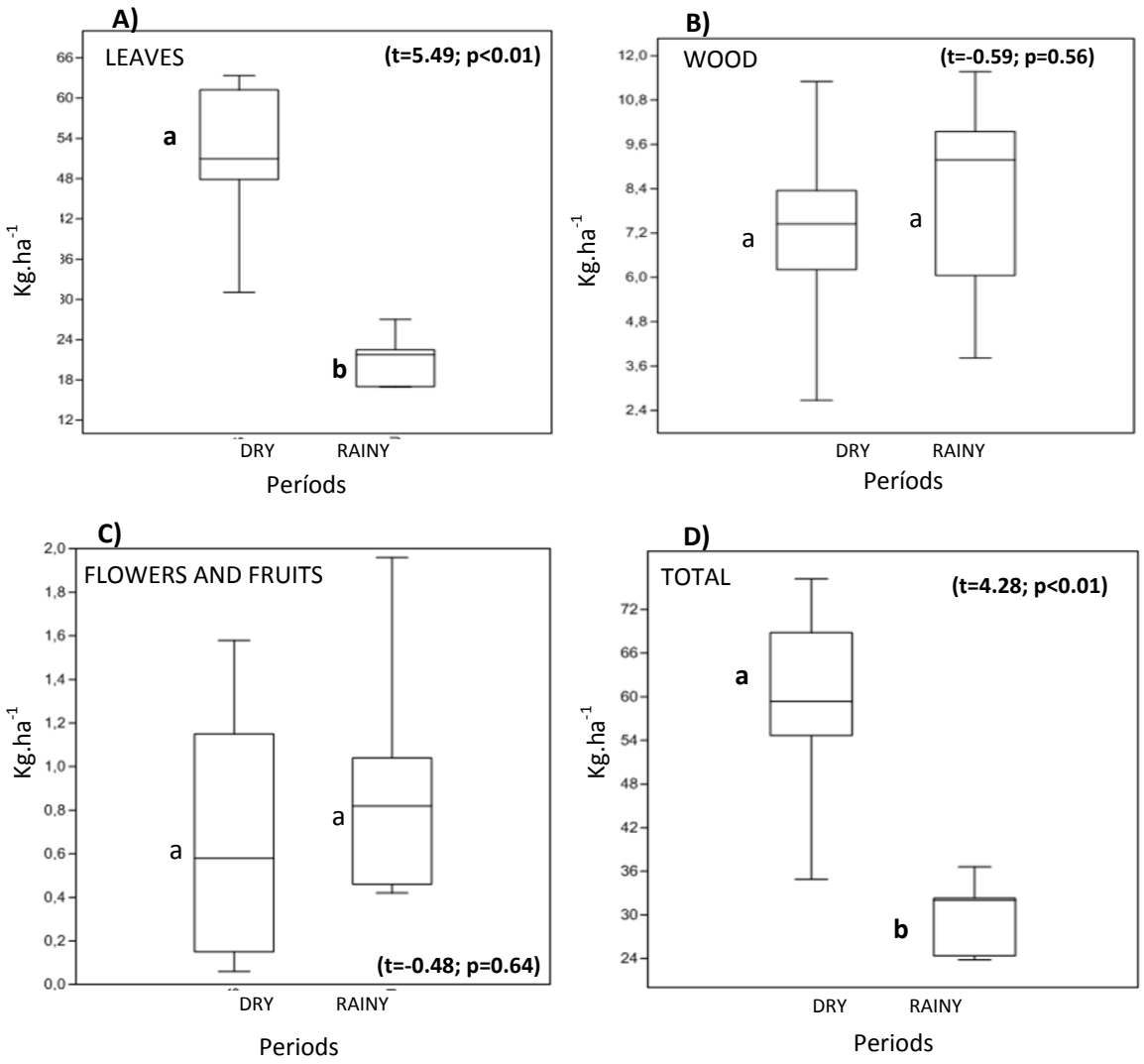

Figure 4. Variation in the production of leaves, wood, flowers and fruits, and total litter (sum of all four classes) in relation to the rainy and dry periods in a native Brazil nut tree stand in the FLONA Tapajós, Pará. Periods with the same letter are not significantly different by the *t-test* at a 5% significance level. Error bars represent standard deviation.

Table 2. Results from the Pearson Linear Correlation and cross correlation tests conducted using litter production data and meteorological variables in the study area in the FLONA Tapajós, Pará.

Parameter	Pearson's correlation			Cross correlation	
	Precipitation	Temperature	Insolation	Precipitation	Insolation
				Lag: 1	**Lag: 1**
Leaves	*r* = -0.60 p = 0.05	*r* = 0.73 p = 0.01	*r* = 0.69 p = 0.02	*r* = -0.65	*r* = 0.80 p<0.01
					Lag: 1
Total production	ns -	*r* = 0.67 p = 0.03	*r* = 0.67 p = 0.02	p = 0.04 sd	*r* = 0.78 p<0.01

r: Correlation coefficient; p: probability level of significance; ns: insignificant (p > 0.05); sd: no time lag (p > 0.05); Lag: time lag (time lag for the response of litter production in relation to environmental variables, in months).

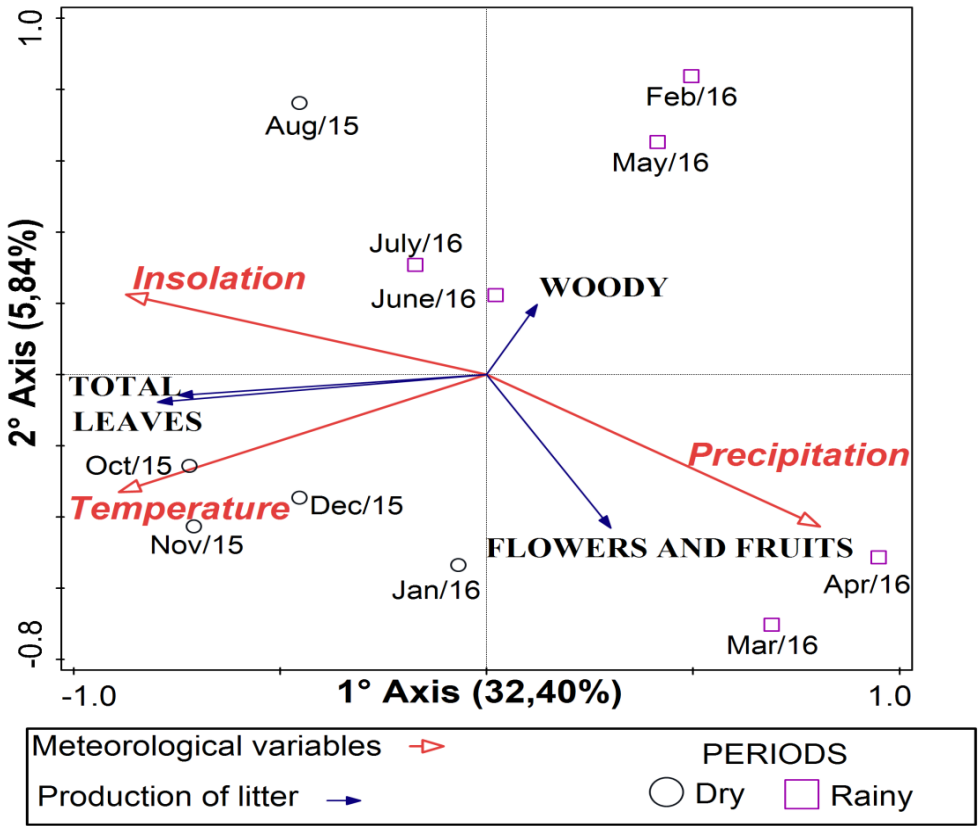

Figure 5. Diagram of the RDA ordination with meteorological variables and litter production data from the study area in the FLONA Tapajós, Pará. The response variables (Precipitation, Temperature and Insolation) are represented by the red arrows, the dependent variables (estimated production for the classes leaves, wood, flowers and fruit, and total production) are represented by the blue arrows. Circled months represent samples from the dry period, and squares represent samples from the rainy period.

second half of 2015.

The peak of litter production occurred in September 2015 and coincided with a single rainfall event that exceeded the historic average rainfall amount for that month. Frequent torrential rainfall and strong winds that occur in the Amazon region cause a larger production of litter (Godinho et al., 2015). Wood production in this month was also high and Moraes (2002) related that the first strong rains after a prolonged dry period stimulates the fall of dry branches that are still attached to the tree and this might have occurred in the current study since the preceding month of August registered no rainfall.

The larger contribution of leaves to total litter production in all collection months has been reported in other studies in tropical forests (Almeida et al., 2015; Ferreira, 2014; Ferreira et al., 2015; Silva et al., 2009; Silva and Oliveira Júnior, 2010; Silva, 2014).

Litter production and meteorological variables

The significant variation between the rainy and dry periods for leaf and total litter production agrees with the results from the correlations done between these classes and precipitation, temperature, and insolation. Silva (2014) also showed a correlation between litter production and temperature, and Santos Júnior (2008) related that environmental forcing factors present a well-defined pattern during the entire year with larger values for temperature and insolation and a smaller volume of water during the dry period compared to the rainy period, and this is also reflected in the dynamics of the vegetation.

The association of water stress and high temperatures with the greater number of hours of sunlight without interference of clouds during the dry period could have caused a large pulse of litter production due to physiological stimulation, dispersion of older materials, or natural breakage of parts of the trees (Silva, 2013). The falling of leaves during the period of reduced rainfall is considered a defense mechanism in order to reduce water loss due to evapotranspiration (Parolin et al., 2010; Ourique et al., 2016).

Flower and fruit production in areas of high plant

diversity, such as the FLONA Tapajós (Andrade et al., 2015; Gonçalves and Santos, 2008), often present a well-defined seasonality of litter production because different species possess different phenological aspects (O'brien et al., 2008). The peaks in production registered for woody materials in both the rainy and dry periods increased the standard deviation and impeded a significant result for the t-test in spite of the fact that wood production was much larger during the dry period. Malhi et al. (2009), analyzing data from three experimental areas located on terra firme forests under deep Oxisols, highly leached and under high plains, all located in the eastern region of the Amazon, also identified an abnormal production of woody litter and suggested that possible alteration of environmental variables influenced this biological variable.

The time lag indicates the time that the vegetation took to respond to changes in environmental factors. Studies by Restrepo-Coupe et al. (2013) and Borchert et al. (2015) explain that in tropical forests light interacts with adaptive mechanisms to indirectly determine photosynthetic capacity through leaf production and seasonality of litterfall. The phenology of many tropical trees is highly correlated with seasonal variation in insolation (Rivera et al., 2002).

In contrast to the studies by Silva (2014), Ourique et al. (2016) and Mochiutti et al. (2006), conducted in the Amazon region, the current study did not identify a relationship between rainfall and litter production through the redundancy analysis. The results from the Pearson's correlation also support this result since the correlation was weak only for leaf production. Hayashi (2006) also did not register a correlation between litter production and rainfall in a primary forest in the Amazon. The greater influence of temperature on total litter production and leaf production is explained by Kapos et al. (1997) as being an adaptation of tree species to low levels of variation of the abiotic factors wherein leaf loss is increased when trees experience abnormal or brusque changes in these factors. In the present study, the temperature data were substantially higher than the historical climatic average for the region.

Conclusion

Litter production varied during the year-long sampling period and the highest litter production by class was leaves in the dry period and wood in the rainy period.

The variation of leaf and total litter production is partially explained by temperature variation and insolation. The meteorological variables examined in this study do not explain the variation in production of reproductive material or wood that occurred between August 2015 and July 2016. The El Niño event 2015/2016 was responsible for the anomalies in rainfall and temperature data with respect to the historical climatic averages for these variables.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This work was supported by the Brazilian Agricultural Research Corporation.

REFERENCES

Almeida EJ, Luizão F, Rodrigues DJ (2015). Produção de serrapilheira em florestas intactas e exploradas seletivamente no sul da Amazônia em função da área basal da vegetação e da densidade de plantas. Acta Amaz. 45(2):157-166.

Andrade DF, Gama JRV, Melo LO, Ruschel AR (2015). Inventário florestal de grandes áreas na Floresta Nacional do Tapajós, Pará, Amazônia, Brasil. Biota Amaz. 5(1):109-115.

Bi J, Knyazikhin Y, Choi S, Park T, Barichivich J, Ciais P, Fu R, Ganguly S, Hall F, Hilker T, Huete A, Jones M, Kimball J, Lyapustin AI, Mõttus M, Nemani RR, Piao S, Poulter B, Saleska SR, Saatchi SS, Xu L, Zhou L, Myneni RB (2015). Sunlight mediated seasonality in canopy structure and photosynthetic activity of Amazonian rainforests. Environ. Res. Lett. 10:064014.

Bianchin JE, Marques R, Britez RM, Capretz RL (2016). Deposição de fitomassa em formações secundárias na Floresta Atlântica do Paraná. Floresta Ambient. 23(4):524-533.

Borchert R, Calle Z, Strahler AH, Baertschi A, Magill RE, Broadhead JS, Kamau J, Njoroge J, Muthuri C (2015). Insolation and photoperiodic control of tree development near the equator. New Phytol. 205:7-13.

Calvi GP, Pereira MG, Espíndula AJR (2009). Produção de serapilheira e aporte de nutrientes em áreas de floresta atlântica em Santa Maria de Jetibá, ES. Ciênc. Florest. 19(2):13-138.

Carvalho JOP (1992). Structure and dynamics of a logged over Brazilian Amazonian rain forest. PhD thesis, University of Oxford, Oxford. 215p.

Carvalho JOP (2001). Estrutura de matas altas sem babaçu na Floresta Nacional do Tapajós. In: Silva JNM, Carvalho JPde, Yared JG (Ed.) A silvicultura na Amazônia Oriental: contribuições do projeto Embrapa/DFID. Belém: Embrapa Amazônia Oriental/DFID. pp. 277-290.

Cetin M (2015). Evaluation of the sustainable tourism potential of a protected area for landscape planning: a case study of the ancient city of Pompeipolis in Kastamonu. Int. J. Sustain. Dev. World Ecol. 22(6):490-495.

Cetin M (2016). Sustainability of urban coastal area management: A case study on Cide. J. of Sustainable Forestry. 35(7):527-541.

Chagas GFB, Silva VPR, Costa ACL, Dantas VA (2012). Impactos da redução da pluviometria na biomassa aérea da Floresta Amazônica. Rev. Bras. Eng. Agríc. Ambient. 16(1):72-79.

Chave J, Navarrete D, Almeida S, Álvarez E, Aragão LEOC, Bonal D, Châtelet P, Silva-Espejo JE, Goret JY, Hildebrand PV, Jiménez E, Patiño S, Peñuela MC, Phillips OL, Stevenson P, Malhi Y (2010). Regional and seasonal patterns of litterfall in tropical South America. Biogeosciences 7(1):43-55.

Costa MC, Costa ACL, Coelho LTS, Silva TML, Azevedo AF (2014). Correlação entre precipitação pluviométrica E umidade do solo na produção de serapilheira em Caxiuanã (PA). Rev. Ibero-Americana de Ciênc. Ambient. 5(1):170-179.

Davis JC (1986). Statistics and Data Analysis in Geology. Nova York: John Wiley & Sons. 646p.

Espírito-Santo FDB, Shimabukuro YE, Aragão LEOC, Machado ELM (2005). Análise da composição florística e fitossociológica da Floresta Nacional do Tapajós com o apoio geográfico de imagens de satélites. Acta Amaz. 35(2):155-173.

Ferreira LS, Cattânio JH, Jardim MAG (2015). Efeito da topografia e da precipitação na florística e na produção de liteira em Caxiuanã, Pará. Rev. Árv. 39(6):995-1005.

Ferreira ML (2014). Incremento diamétrico arbóreo em diferentes grupos funcionais e produção de serapilheira em duas florestas brasileiras. PhD thesis, University of São Paulo, São Paulo. 201p.
Giácomo RG, Pereira MG, Machado DL (2012). Aporte e decomposição de serapilheira em áreas de Cerradão e Mata Mesofítica na Estação Ecológica de Pirapitinga – MG. Ciênc. Florest 22(4):669-680.
Godinho TO, Caldeira MVW, Brun EJ (2015). Ciclagem de nutrientes via serapilheira em ecossistemas florestais naturais no Brasil. In: Faria ÁBdeC, Brun EJ, Ferrari F (Org). Ciências Florestais e Biológicas. Curitiba: UTFPR. pp. 13-52.
Godinho TO, Caldeira MVW, Rocha JHT, Caliman JP, Trazzi PA (2014). Quantificação de biomassa e nutrientes na serapilheira acumulada em trecho de Floresta Estacional Semidecidual Submontana, ES. Cerne. 20(1):11-20.
Golden GWS (2016). El Niño and La Niña Years and Intensities Based on Oceanic Niño Index (ONI). Accessed in: http://ggweather.com/enso/oni.htm.
Golley FB (1978). Ciclagem de minerais em um ecossistema de floresta tropical úmida. Editora da Universidade de São Paulo: São Paulo. 105p.
Gonçalves FG, Santos JR (2008). Composição florística e estrutura de uma unidade de manejo florestal sustentável na Floresta Nacional do Tapajós, Pará. Acta Amaz. 38(2):229-244.
Guerreiro QLM, Oliveira Júnior RC, Santos GR, Ruivo MLP, Beldini TP, Carvalho EJM, Silva KE, Guedes MC, Santos PRB (2017). Spatial variability of soil physical and chemical aspects in a Brazil nut tree stand in the Brazilian Amazon. Afr. J. Agric. Res. 12(4):237-250.
Hammer O, Haper DAT, Ryan PD (2001). Past: paleontological statistics software package for education and data analisys. Palaeontol. Eletr. 4:1-9.
Hayashi SN (2006). Dinâmica da serapilheira em uma cronossequencia de florestas no município de Capitão Poço-Pa. MSc thesis, University Rural of da Amazônia, Belém 75p.
Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) (2004). Floresta Nacional do Tapajós – Plano de Manejo. Belterra: IBAMA, 373p.
Intituto Nacional de Meteorologia (INMET) (2010). Ocorrência de La nina e El Niño. Accessed in: http://enos.cptec.inpe.br/tab_elnino.shtml.
Kapos V, Wandelli E, Camargo JL, Ganade G (1997). Edge-related changes in environmental and plant responses due to forest fragmentation in central Amazonia. In: Laurance WF, Bierregard Jr RO (Eds.). Tropical forest remnants: ecology, management, and conservation of fragmented communities. Chicago: University of Chicago Press pp. 33-44.
Lima RP, Fernandes MM, Fernandes MRM, Matricardi EAT (2015). Aporte e decomposição da serapilheira na Caatinga no Sul do Piauí. Floresta Ambient. 22(1):42-49.
Locatelli M, Vieira AH, Martins EP, Souza VF, Macedo RS (2005). Crescimento em diâmetro de castanha-do-brasil (*Bertholletia excelsa* H.B.K.) cultivada em solo de baixa fertilidade. PortoVelho: Embrapa 4 p.
Loureiro AA, Silva MF, Alencar JC (1979). Essências florestais madeireiras da Amazônia. Manaus: INPA. 245 p.
Malhi Y, Saatchi S, Girardin C, Aragão LEOC (2009). Produção, estoques e fluxo de carbono nas florestas amazônicas. Geophys. Monogr. 186:355-371.
Maués MM (2002). Reprodutive phenology and pollination of the Brazil nut tree (*Bertholletia excelsa* Humb. & Bompl. Lecythidaceae) in eastern Amazonia, In: Kevan P, Imperatriz-Fonseca VL (Ed.). *Pollination bees:* the conservation link between agriculture and nature. Brasília: Ministério do Meio Ambiente pp. 245-254.
Meir P, Brando PM, Nepstad D, Vasconcelos S, Costa ACL, Davidson E, Almeida S, Fisher RA, Sotta ED, Zarin D, Cardinot G (2009). Os efeitos da seca nas florestas chuvosas Amazônicas. Geophys. Monogr. 186:429-449.
Mochiutti S, Queiroz JAL, Melém Junior NJ (2006). Produção de serapilheira e retorno de nutrientes de um povoamento de taxi-branco e de uma Floresta Secundária no Amapá. Bol. Pesqui. Florest. 52:3-20.
Moraes RM (2002). Ciclagem de nutrientes na floresta do PEFI: produção e decomposição da serapilheira. In: Bicudo D, Forti M, Bicudo C (Ed.). Parque Estadual das Fontes do Ipiranga, unidade de conservação que resiste à urbanização de São Paulo. São Paulo: Secretaria de Estado do Meio Ambiente pp. 133-142.
Mori SA, Prance GT (1990). Taxonomy, ecology, and economic botany of the Brazil nut (*Bertholletia excelsa* Humb. & Bonpl.: Lecythidaceae). Adv. Econ. Bot. 8:130-150.
O'brien JJ, Oberbauer SF, Clark DB, Clark DA (2008). Phenology and stem diameter increment seasonality in a Costa Rican wet tropical forest. Biotrópica 40(2):151-159.
Ourique LK, Silva RO, Souza CAS, Higushi N (2016). Relação da produção de serapilheira com incremento em diâmetro de uma floresta madura na Amazônia Central. Scientia Forestalis. 44(112):875-886.
Parolin P, Lucas C, Piedade MTF, Wittmann F (2010). Drought responses of flood-tolerant trees in Amazonian floodplains. Ann. Bot. 105(1):129-139.
Pinho GSC, Fiedler NC, Lisbôa CDJ, Rezende AV, Martins IS (2004). Efeito de diferentes métodos de corte de cipós na produção de madeira em tora na Floresta Nacional do Tapajós. Ciênc. Florest. 14(1):179-192.
Proctor J (1983). Tropical Forest Litterfall, In: Sutton SL, Whitmore TC, Chadwick AC (Ed.) Tropical rain forest: Ecology and management. Oxford: Blackwell Scient. Public. pp. 267-273.
Pyle EH, Santoni GW, Nascimento HEM, Hutyra LR, Vieira S, Curran DJ,Haren JV, Saleska SR, Chow VY, Carmago PB, Laurance WF, Wofsy SC (2008). Dynamics of carbon, biomass, and structure in two Amazonian forests. J. Geophys. Res. 113:1-20.
Quesada CA, Lloyd1 J, Anderson LO, Fyllas NM, Schwarz M, Czimczik CI (2011). Soils of Amazonia with particular reference to the RAINFOR sites. Biogeosciences 8(6):1415-1440.
Restrepo-Coupe N, Rochac HR, Hutyra LR, Araujo AC, Borma LS, Christoffersen B, Cabral OMR, Camargo PB, Cardoso FL, Costa ACL, Fitzjarrald DR, Goulden ML, Kruijt B, Maia JMF, Malhi YS, Manzi AO, Miller SD, Nobre AD, Randow CV, Sá LDA, Sakai RK, Tota J, Wofsy SC, Zanchi FB, Saleskaa SR (2013). What drives the seasonality of photosynthesis across the Amazon basin? A cross-site analysis of eddy flux tower measurements from the Brasil flux network. Agric. For. Meteorol. 82(1):28-44.
Rivera G, Elliott S, Caldas LS, Nicolossi G, Coradin VT, Borchert R (2002). Increasing day-length induces spring-flushing of tropical dry forest trees in the absence of rain. Trees 16: 445-456.
Salman AKD, López GFZ, Bentes-Gama MM, Andrade CMS (2008). Espécies arbóreas nativas da Amazônia Ocidental Brasileira com potencial para arborização de pastagens. Porto Velho: Embrapa. (Boletim técnico, 127). 20p.
Salomão RP (2014). A castanheira: história natural e importância socioeconômica. Bol. Mus. Para. Emílio Goeldi, sér. Ciênc. Nat. 9(2):259-266.
Santos Junior UM (2008). Fisiologia e indicadores de estresse em árvores crescendo em ambientes alagados pela hidroelétrica de Balbina na Amazônia Central. PhD thesis, National Institute for Space Research, Manaus. 144p.
Santos Neto AP, Barreto PAB, Gama-Rodrigues EF, Novaes AB, Paula A (2015). Produção de serapilheira em floresta estacional semidecidual e em plantios de *Pterogyne nitens* Tul. E *Eucalyptus urophylla* S. T. Blake no sudoeste da Bahia. Ciênc. Florest. 25(3):633-643.
Scoles R (2010). Ecologia e extrativismo da castanheira (*Bertholletia excelsa*, lecythidaceae) em duas regiões da Amazônia brasileira. PhD thesis, National Institute for Space Research, Manaus. 209p.
Scoles R, Canto MS, Almeida RG, Vieira DP (2016). Sobrevivência e frutificação de *Bertholletia excelsa* Bonpl. em áreas desmatadas em Oriximiná, Pará. Florest. Ambient. 23(4):555-564.
Scoriza RN, Pereira MG, Pereira GHA, Machado DL, Silva EMR (2012). Métodos para coleta e análise de serrapilheira aplicados à ciclagem de nutrientes. Florest. Ambient. 2(2):1-18.
Sevik H, Cetin M (2015). Effects of water stress on seed germination for select landscape plants. Pol. J. Environ Stud. 24(2):689-693.
Silva AD (2014). Produção e concentração de nutrientes via deposição de liteira na Floresta Nacional do Tapajós, Belterra – PA. MSc thesis, University of West of Pará, Santarém 90p.
Silva AD, Oliveira Junior RC (2010). Produção de liteira na Floresta

Nacional do Tapajós no ano de 2007. Espaço Científico 11(1/2):1-12.
Silva RM, Costa JMN, Ruivo MLP, Costa ACL, Almeida SS (2009). Influência de variáveis meteorológicas na produção de liteira na Estação Científica Ferreira Penna, Caxiuanã, Pará. Acta Amaz. 39(3):573-582.
Silva WR (2013). Produção de liteira final em uma área de contato capinarama-Floresta Ombrófila na Amazônia setentrional. MSc thesis, University of Roraima, Boa Vista 43p.
Ter Braak CJF, Smilauer P (2002). Canoco reference manual and canodraw for Windows user's guide, software for canonical comunity ordination (version 4.5). Netherlands: Biometris, Wageningen and Ceske Budejovice 500p.
Valentin JL (2012). Ecologia Numérica: Uma introdução à análise multivariada de dados ecológicos. Rio de Janeiro: Interciência 168 p.
Vasconcellos RLF, Bini D, Paula AM, Andrade JB, Cardoso EJBN (2013). Nitrogênio, carbono e compactação do solo como fatores limitantes do processo de recuperação de matas ciliares. Rev. Bras. Ciênc. Solo 37:1164-1173.
Veloso HP, Rangel Filho AL, Lima JCA (1991). Classificação da vegetação brasileira, adaptada a um sistema universal. Rio de Janeiro: IBGE.
Wagner FH, Hérault B, Bonal D, Stahl C, Anderson LO, Baker TR, Becker GS, Beeckman H, Souza DB, Botosso PC, David MJ, Bowman S, Bräuning A, Brede B, Brown FI, Camarero JJ, Camargo PB, Cardoso FCG, Carvalho FA, Castro W, Chagas RK, Chave J, Chidumayo EN, Clark DA, Costa FRC, Couralet C, Mauricio PHS, Dalitz H, Castro VR, Milani JEF, Oliveira EC, Arruda LS, Devineau JL, Drew DM, Dünisch O, Durigan G, Elifuraha E, Fedele M, Fedele LF, Filho AF, Finger CAG, Franco AC, Freitas Júnior JL, Galvão F, Gebrekirstos A, Gliniars R, Graça PMLA, Griffiths AD, Grogan J, Guan K, Homeier J, Kanieski MR, Kho LK, Koenig J, Kohler SV, Krepkowski J, Lemos-Filho JP, Lieberman D, Lieberman ME, Lisi CS, Santos TL, Ayala JLL, Maeda EE, Malhi Y, Maria VRB, Marques MCM, Marques R, Chamba HM, Mbwambo L, Melgaço KLL, Mendivelso HA, Murphy BP, O'Brien JJ, Oberbauer SF, Okada N, Pélissier R, Prior LD, Roig FA, Ross M, Rossatto DR, Rossi V, Rowland L, Rutishauser E, Santana H, Schulze M, Selhorst D, Silva WR, Silveira M, Spannl S, Swaine MD, Toledo JJ, Toledo MM, Toledo M, Toma T, Filho MT, Hernández JIV, Verbesselt J, Vieira SA, Vincent G, Castilho CV, Volland F, Worbes8 M, Zanon MB, Aragão LEOC (2016). Climate seasonality limits leaf carbon assimilation and wood productivity in tropical forests. Biogeosciences 13:2537-2562.
Yoon JH, Zeng N (2010). An Atlantic influence on Amazon rainfall. Clim. Dyn. 34:249-264.
Zar JH (1999). Biostatistical analysis. New Jersey: Prentice Hall, Upper Saddle River 662p.
Zhang H, Yuan W, Dong W, Liu S (2014). Seasonal patterns of litterfall in forest ecosystem worldwide. Ecol. Complex. 20:240-247.

academicJournals

Vol. 13(5), pp. 239-247, 1 February, 2018
DOI: 10.5897/AJAR2017.12878
Article Number: AE2D38755869
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Higher K^+/Na^+ and lower reactive oxygen species and lipid peroxidation are related to higher yield in maize under saline condition

Rohman M. M.[1*], Islam M. R.[1, 2], Mahmuda B. M.[3], Begum S.[1], Fakir O. A.[4] and Amiruzzaman M.[1]

[1]Molecular Breeding Laboratory, Plant Breeding Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.
[2]Department of Biotechnology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh.
[3]Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh.
[4]Agricultural Research Station, Benerpota, Satkhira, Bangladesh.

Received 12 November, 2017; Accepted 15 December, 2017

Thirty six single cross hybrids with six commercial checks were evaluated for yield performance along with earliness, plant height and ear height. The mean performance for days to tasseling and days to silking were significantly different among the hybrids. Considering the yield, Kaberi 50 and Pioneer 3396 were found as the best and the second best check, respectively. None of the hybrids produced higher yield compared to the best check Kaberi 50. On the other hand, $L_1 \times T_1$, $L_3 \times T_1$, $L_5 \times T_3$, $L_6 \times T_3$, $L_{10} \times T_1$ and $L_1 \times T_3$ produced higher yield compared to 2nd best check Pioneer 3396 whereas $L_5 \times T_3$ and $L_{11} \times T_3$ were earlier than the checks. The seven lines involving these crosses were selected for next generation evaluation. Better performing crosses showed higher K^+/Na^+ and lower contents of $O_2^{\bullet-}$, H_2O_2 and melondialdehyde (MDA) than the susceptible crosses suggesting their better stress tolerance ability and thus better performance in yield.

Key words: Maize yield, saline sensitivity, K^+/Na^+, superoxide, H_2O_2, melondialdehyde.

INTRODUCTION

Salinity, a severe threat for crop growth and production, has been increasing due to global climate. It is predicted that due to salinity, each year about 12 billion US$ will be lost globally by reducing agricultural production (Flowers

*Corresponding author. E-mail: motiar_1@yahoo.com.

et al., 2010). The possibility of salinity increasing is more in arid and semi-arid regions due to water scarcity together with high temperature. Salinity stress causes morphological, physiological, biochemical and molecular changes in cell which causes functional loss of cell organelles and finally cell death (Gill and Tujeta, 2010). Accumulation of Na^+ under salinity stress competes with K^+ binding with proteins causing inhibition in protein synthesis and metabolic enzymes (Schachtman and Liu, 1999; Pardo and Quintero, 2002). Besides, high concentration of NaCl outside the roots reduces the water potential which hampers water up-take resulting in osmotic stress.

High salt level in leaves causes stomatal closure, impairs electron transport and damages the photosynthetic apparatus which ultimately reduces photosynthesis and productivity (Deinlein et al., 2014). The ionic imbalance, osmotic stress causes over production of reactive oxygen species (ROS) in plants (Chawla et al., 2013). A large number of studies established that tolerant plants contain lower magnitude of ROS and melondialdehyde (MDA) under salinity and other abiotic stress as compared to sensitive ones. Therefore, ROS like superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and secondary metabolites of ROS like MDA can be an important biomarkers for selection of tolerant plants under abiotic stress including salinity. On the other hand, genotypes showing a high K^+/Na^+ are tolerance to salinity (Chen et al., 2017).

Maize (*Zea mays* L.) is the 3rd most important cereal crop after rice and wheat and is grown under a wide spectrum of soil and climatic conditions. It is an important C_4 plant from the poaceae family and considered to have wider adaptability to growing environment (Chinnusamy et al., 2005). In Bangladesh, maize is becoming an important crop due to its high market demands with comparatively lower production cost and high yield. It continues to expand rapidly at an average rate of 20% yearly. A growing popularity for maize in the country is due to huge demand, particularly for poultry feed industry. The acreage and production of maize have an increasing tendency with the introduction of exotic hybrids due to high yield potentials.

In Bangladesh, about 1 million hectare of land is under saline coastal belt and there is an opportunity to expand maize cultivation in this area. However, the crisis of saline tolerant maize variety is a major limitation to introduce this crop in this area. Therefore, attention has been given in developing saline tolerant maize. With this objective, 36 single cross hybrids along with 6 commercial checks were evaluated in saline area of Agricultural Research Station, Benarpota, Satkhira to find suitable line for producing hybrid. For selecting suitable line for saline environment, parameters like plant height, days to tasseling, days to silking, ear height and yield were emphasized. At the same time, some biochemical parameters like K^+/Na^+, ROS and MDA were examined as selection criteria.

MATERIALS AND METHODS

Plant materials and crop management

The materials for this experiment consisted of 36 single cross hybrids producing from 12 lines: Pac60/S_4-3, 9MG/S_4-6, 7074/S_3-2, 7074/S_3-5, 7074/S_3-6, 7074/S_3-13, 7074/S_3-18, 7074/S_3-21, 7074/S_3-26, 7074/S_3-30, QY11/S_3-24 and CML-427 considered as L_1, L_3, L_5, L_6, L_7, L_8, L_9, L_{10} and L_{11}, L_{12} L_{13} and L_{14}, respectively, and 3 testers: 9MS/S_6-14, BIL-110, BIL-113 as T_1, T_2 and T_3, respectively. L_2 and L_4 did not germinate. The produced hybrids were evaluated atBenerpota, Satkhira, a saline costal region (Latitude 21°48´-22°58´N, Longitude 88°55´-89°55´E and Altitude 16 feet from sea level), in winter 2016 to 17 following RCB design with 3 replications.

Six commercial hybrids (BHM-9, 981, Sunshine, Pioneer3396, Kaberi 50 and Pacific 99Super) were used as check variety. Each entry planted in one row of 4 m long plot. The spacing between rows was 60 cm and plant to plant distance was 25 cm. One healthy seedling per hill was kept after proper thinning.

Fertilizers were applied @ 250, 55, 110, 40, 5 and 1.5 kg/ha of N, P, K, S, Zn, B, respectively. Standard agronomic practices were followed and plant protection measures were taken as required. Ten randomly selected plants were used for recording observations on plant height, ear height, days to tasseling, days to silking and grain yield were recorded on whole plot basis. ROS and MDA were measured from flag leaves at grain filling stage.

Measurement of soil salinity

Salinity level of the maize growing field was measured by a conductivity meter (HI-993310).

Measurement of ROS and MDA

Measurement of the $O_2^{\bullet-}$ generation rate

Superoxide radical was determined in flag leaves according to the method of Elstner and Heupel (1976) with modifications. Leaves (0.3 g) were homogenized in 3 ml of 65 mmol phosphate buffer (pH 7.8) on an ice bath and then centrifuged at 4°C and 5,000 × g for 10 min. The supernatants (0.75 ml) were mixed with 0.675 ml of 65 mM phosphate buffer (pH 7.8) and 0.07 ml of 10 mM hydroxylamine chlorhydrate and placed at 25°C. After 20 min, 0.375 ml of 17 mM sulfanilamide and 0.375 ml of 7 mM α-naphthylamine were added, and the mixture was placed at 25°C for another 20 min before it was mixed with 2.25 ml of ether. The absorbance was measured at 530 nm and the $O_2^{\bullet-}$concentration was calculated from a standard curve of $NaNO_2$.

Measurement of H_2O_2

H_2O_2 was assayed according to the method described by Yu et al. (2003). Flag leaf tissue (0.5 g) was homogenized in 3 ml of 50 mM K-P buffer (pH 6.5) at 4°C. The homogenate was centrifuged at 11,500×g for 15 min. The supernatant (3 ml) was mixed with 1 ml of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v), and the mixture was then centrifuged at 11,500×g for 15 min at room temperature. The optical absorption of the supernatant was measured spectrophotometrically at 410 nm to determine the H_2O_2 content (ϵ= 0.28 μM^{-1} cm^{-1}) and expressed as micromoles per gram FW.

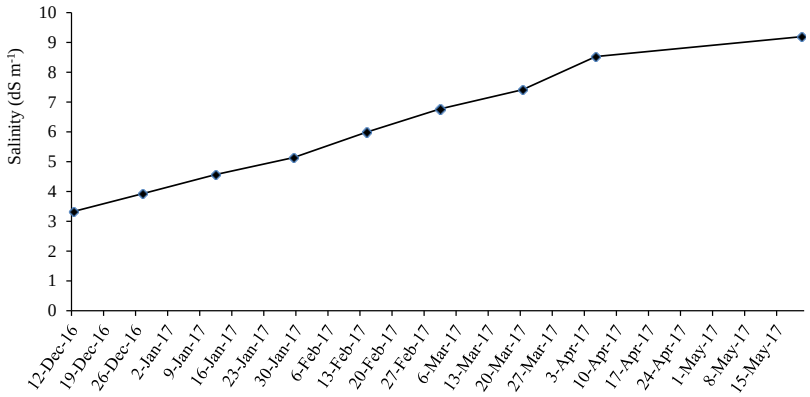

Figure 1. Salinity level of the maize field during study period.

Measurement of MDA

The level of lipid peroxidation was measured by estimating melondialdehyde (MDA), a decomposition product of the peroxidized polyunsaturated fatty acid component of the membrane lipid, using thiobarbituric acid (TBA) as the reactive material (Heath and Packer, 1968). Briefly, flag leaf tissue (0.5 g) was homogenized in 3 ml 5% (w/v) trichloroacetic acid (TCA), and the homogenate was centrifuged at 11,500×g for 10 min. The supernatant (1 ml) was mixed with 4 ml of TBA reagent (0.5% of TBA in 20% TCA). The reaction mixture was heated at 95°C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged at 11,500 ×g for 15 min.

The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. The concentration of MDA was calculated by using the extinction coefficient of 155 mM^{-1} cm^{-1} expressed as nanomole of MDA per gram FW.

Measurement of K^+/Na^+

The sap was extracted from leaves and was put on compact Na^+ ion meter (Horiba-731, Japan) and compact K^+ ion meter (Horiba-722, Japan) to estimate the Na^+ and K^+ ions in leaves. The K^+/Na^+ ratio was measured from the estimated values.

Statistical analysis

Mean performance of different characters and comparison with 2 best checks were analyzed using Statistix 10 software by least significant difference (LSD) following RCB design.

RESULTS AND DISCUSSION

The salinity levels in coastal area of Bangladesh increased after November which increases upto May. It is related with soil moisture. After November, the land dries due to lack of rain. During this study period, the salinity started from 3.3 dSm^{-1} and at harvesting, the level was almost 10 dSm^{-1} (Figure 1). Therefore, it is clear that the crop growth and development was hampered due to salinity at salinity level >4 dSm^{-1} which become injurious for crop growth.

During selection of maize, usually the important traits like days to tasseling (DT), days to silking (DS), plant height (PH), ear height (EH) and yield are given importance. In comparison of these desired traits, 6 commercial hybrids (BHM-9, 981, Sunshine, Pioneer 3396, Kaberi 50 and Pacific 99 Super) were included as check (Ck) in this study. Among the Cks, Kaberi 50 was found as the best check followed by Pioneer 3396 as the second best Ck. Notably, during selection of maize genotypes, yield is considered as most important. It is assumed that genotypes with comparatively lower plant height and ear height have lower possibility to lodge.

Hence, these 2 traits were monitored carefully. On the other hand, early harvesting facilitates to escape higher salinity and traits like early tasseling and silking which are important for maize cultivation in saline area. The mean performance of the crosses was significantly different for DT and DS (Table 1). In comparison of performance for DT with best check, Kaberi 50, 2 crosses $L_{11}\times T_2$ and $L_{13}\times T_3$ were significantly earlier, for DS, 3 crosses $L_{11}\times T_2$, $L_{13}\times T_3$ and $L_{14}\times T_1$ were found earlier. However, significant difference was not found among the genotypes for plant height (PH), ear height (EH) and yield/ha (Yld). Interestingly, most of the crosses had shorter plant height than both of the best checks, although the difference was not statistically significant.

In case of plant height, most of the genotypes were shorter than the best and 2nd best check (Table 1). On the other hand, considering the ear height genotypes, $L_1\times T_1$, $L_3\times T_1$, $L_3\times T_2$, $L_6\times T_3$ were shorter than the best check. $L_1\times T_3$ and $L_{11}\times T_1$ were considerable over the 2nd best check. Since none of the genotypes were over yielder than the best check Kaberi 50, the genotypes showing higher yield over the 2nd best check was considered for selection. Among the crosses, comparing the yield of the best check, yield of $L_1\times T_1$, $L_3\times T_1$, $L_3\times T_2$, $L_5\times T_3$, $L_{10}\times T_1$ and $L_{11}\times T_3$ was higher or similar. However, all the desired traits rarely correlate with higher yield.

Though, considering yield (over the 2nd best check), earliness for $L_1\times T_1$, $L_5\times T_3$ and $L_{10}\times T_1$ was sacrificed.

Table 1. Performance of single cross hybrids for days to tasseling, days to silking and plant height under saline condition.

Genotype	Days to tasseling			Days to silking			Plant height (cm)		
	Mean	Comparison with best Ck	Comparison with 2nd best Ck	Mean	Comparison with best Ck	Comparison with 2nd best Ck	Mean	Comparison with best Ck	Comparison with 2nd best Ck
$L_1 \times T_1$	78.00	2.00	1.33	85.00	1.33	4.67	159.5	-24.37	-28.35
$L_1 \times T_2$	73.33	-2.67	-3.33	80.67	-3.00	0.33	155.3	-28.62	-32.60
$L_1 \times T_3$	80.00	4.00	3.33	87.67	4.00	7.33	161.0	-22.93	-26.91
$L_3 \times T_1$	78.67	2.67	2.00	86.33	2.67	6.00	159.9	-23.95	-27.93
$L_3 \times T_2$	68.67	-7.33	-8.00	76.33	-7.33	-4.00	164.7	-19.23	-23.21
$L_3 \times T_3$	70.33	-5.67	-6.33	78.33	-5.33	-2.00	170.7	-13.25	-17.23
$L_5 \times T_1$	75.67	-0.33	-1.00	80.67	-3.00	0.33	173.3	-10.63	-14.61
$L_5 \times T_2$	69.33	-6.67	-7.33	75.33	-8.33	-5.00	172.9	-10.93	-14.91
$L_5 \times T_3$	73.00	-3.00	-3.67	75.33	-8.33	-5.00	181.5	-2.41	-6.39
$L_6 \times T_1$	77.00	1.00	0.33	84.33	0.67	4.00	170.7	-13.23	-17.21
$L_6 \times T_2$	80.00	4.00	3.33	82.67	-1.00	2.33	166.4	-17.54	-21.52
$L_6 \times T_3$	76.33	0.33	-0.33	83.00	-0.67	2.67	173.4	-10.51	-14.49
$L_7 \times T_1$	74.33	-1.67	-2.33	81.00	-2.67	0.67	171.9	-11.95	-15.93
$L_7 \times T_2$	71.00	-5.00	-5.67	77.33	-6.33	-3.00	166.4	-17.48	-21.46
$L_7 \times T_3$	77.00	1.00	0.33	84.00	0.33	3.67	177.3	-6.59	-10.57
$L_8 \times T_1$	75.00	-1.00	-1.67	82.00	-1.67	1.67	159.7	-24.24	-28.22
$L_8 \times T_2$	68.33	-7.67	-8.33	79.33	-4.33	-1.00	162.2	-21.73	-25.71
$L_8 \times T_3$	73.33	-2.67	-3.33	80.33	-3.33	0.00	159.1	-24.81	-28.79
$L_9 \times T_1$	75.00	-1.00	-1.67	79.00	-4.67	-1.33	172.5	-11.42	-15.40
$L_9 \times T_2$	69.00	-7.00	-7.67	75.33	-8.33	-5.00	176.7	-7.17	-11.15
$L_9 \times T_3$	68.33	-7.67	-8.33	75.00	-8.67	-5.33	180.2	-3.68	-7.66
$L_{10} \times T_1$	79.67	3.67	3.00	80.00	-3.67	-0.33	182.4	-1.55	-5.53
$L_{10} \times T_2$	68.67	-7.33	-8.00	77.00	-6.67	-3.33	168.6	-15.27	-19.25
$L_{10} \times T_3$	67.00	-9.00	-9.667*	74.00	-9.67	-6.33	177.2	-6.75	-10.73
$L_{11} \times T_1$	76.67	0.67	0.00	83.67	0.00	3.33	164.9	-18.93	-22.91
$L_{11} \times T_2$	65.33	-10.667*	-11.333*	72.00	-11.67*	-8.33	169.2	-14.75	-18.73
$L_{11} \times T_3$	67.67	-8.33	-9.00	74.33	-9.33	-6.00	166.3	-17.59	-21.57
$L_{12} \times T_1$	75.67	-0.33	-1.00	81.67	-2.00	1.33	181.2	-2.67	-6.65
$L_{12} \times T_2$	67.00	-9.00	-9.667*	74.33	-9.33	-6.00	173.6	-10.29	-14.27
$L_{12} \times T_3$	70.00	-6.00	-6.67	74.00	-9.67	-6.33	167.9	-15.92	-19.90
$L_{13} \times T_1$	68.33	-7.67	-8.33	75.00	-8.67	-5.33	174.0	-9.90	-13.88
$L_{13} \times T_2$	67.00	-9.00	-9.667*	74.33	-9.33	-6.00	162.9	-21.02	-25.00

Table 1. Contd.

$L_{13}\times T_3$	66.33	-9.667*	-10.333*	73.00	-10.67*	-7.33	170.63	-13.28	-17.26
$L_{14}\times T_1$	68.00	-8.00	-8.67	72.00	-11.67*	-8.33	175.43	-8.47	-12.45
$L_{14}\times T_2$	79.00	3.00	2.33	85.33	1.67	5.00	171.55	-12.35	-16.33
$L_{14}\times T_3$	75.67	-0.33	-1.00	79.67	-4.00	-0.67	168.15	-15.75	-19.73
BHM-9	79.00	3.00	2.33	81.67	-2.00	1.33	178.05	-5.85	-9.83
981	76.33	0.33	-0.33	82.67	-1.00	2.33	164.69	-19.21	-23.19
Sunshine	77.00	1.00	0.33	83.33	-0.33	3.00	181.39	-2.52	-6.50
Pioneer3396	76.67	0.67	-	80.33	-3.33	-	187.89	3.98	-
Kaberi 50	76.00	-	-0.67	83.67	-	3.33	183.91	-	-3.98
Pacific 99Super	75.33	-0.67	-1.33	83.00	-0.67	2.67	174.69	-9.21	-13.19
SE	-	2.90	-	-	3.11	3.11	-	13.51	-
F-test	*	-	-	*	-	-	NS	-	-
CD	-	9.19	-	-	9.85	9.85	-	42.81	-

*P=0.05

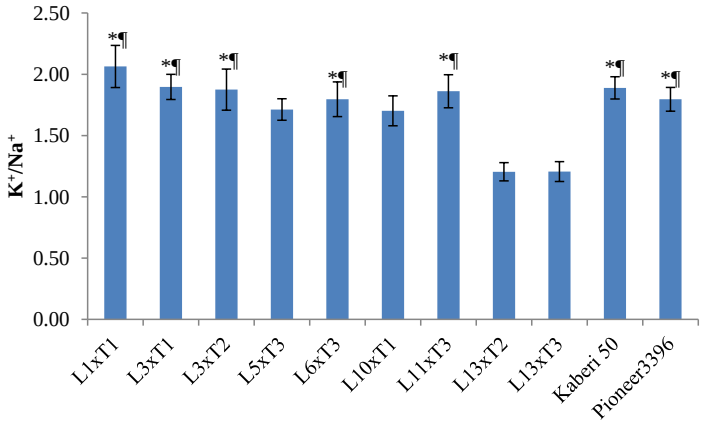

Figure 2. K^+/Na^+ in flag leaves of the selected maize genotypes. $L_{13}\times T_2$ and $L_{13}\times T_3$ were lower yielder genotype and Kaberi 50 and Poineer 3396 were the 2 best standard check*, ¶ mean significantly different over check Kaberi 50 and Pioneer3396, respectively.

During gain filling stage K+/Na+, ROS and MDA were measured. For convenience, the results were presented for higher yielder, 7 genotypes, 2 most lower yielder, 2 genotypes and 2 best checks (Figure 2). The ratio of K^+ to Na^+(K^+/Na^+) in the measured genotypes differed significantly.

Table 2. Performance of single cross hybrids for days to tasseling, days to silking and plant height under saline condition.

Genotype	Ear height			Yield (ton/ha)		
	Mean	Comparison with best Ck	Comparison with 2nd best Ck	Mean	Comparison with best Ck	Comparison with 2nd best Ck
$L_1 \times T_1$	58.24	-3.87	-9.81	5.30	-1.10	0.07
$L_1 \times T_2$	55.36	-6.75	-12.69	4.65	-1.75	-0.57
$L_1 \times T_3$	65.13	3.03	-2.92	4.80	-1.60	-0.42
$L_3 \times T_1$	61.51	-0.59	-6.54	5.52	-0.88	0.30
$L_3 \times T_2$	59.81	-2.29	-8.24	5.23	-1.17	0.00
$L_3 \times T_3$	65.11	3.01	-2.94	4.91	-1.48	-0.31
$L_5 \times T_1$	77.09	14.98	9.03	4.32	-2.08	-0.90
$L_5 \times T_2$	75.18	13.07	7.13	4.85	-1.55	-0.38
$L_5 \times T_3$	74.89	12.79	6.84	5.24	-1.16	0.02
$L_6 \times T_1$	64.14	2.03	-3.91	4.20	-2.20	-1.02
$L_6 \times T_2$	62.21	0.10	-5.85	5.16	-1.24	-0.06
$L_6 \times T_3$	61.75	-0.36	-6.31	5.53	-0.86	0.31
$L_7 \times T_1$	71.16	9.05	3.11	4.47	-1.93	-0.75
$L_7 \times T_2$	64.49	2.39	-3.56	4.77	-1.63	-0.45
$L_7 \times T_3$	74.89	12.78	6.83	4.40	-2.00	-0.82
$L_8 \times T_1$	61.43	-0.68	-6.63	4.63	-1.77	-0.59
$L_8 \times T_2$	58.00	-4.11	-10.05	3.91	-2.48	-1.31
$L_8 \times T_3$	57.18	-4.93	-10.87	4.56	-1.84	-0.67
$L_9 \times T_1$	70.47	8.36	2.41	3.53	-2.87*	-1.69
$L_9 \times T_2$	64.55	2.45	-3.50	4.20	-2.20	-1.02
$L_9 \times T_3$	70.35	8.25	2.30	4.74	-1.66	-0.49
$L_{10} \times T_1$	66.56	4.45	-1.49	5.97	-0.43	0.75
$L_{10} \times T_2$	61.76	-0.35	-6.29	4.14	-2.26	-1.08
$L_{10} \times T_3$	63.68	1.57	-4.37	4.98	-1.42	-0.25
$L_{11} \times T_1$	63.43	1.32	-4.63	5.17	-1.23	-0.06
$L_{11} \times T_2$	57.96	-4.15	-10.09	4.55	-1.85	-0.67
$L_{11} \times T_3$	64.62	2.51	-3.43	5.33	-1.06	0.11
$L_{12} \times T_1$	74.15	12.05	6.10	4.85	-1.54	-0.37
$L_{12} \times T_2$	63.75	1.65	-4.30	5.17	-1.22	-0.05
$L_{12} \times T_3$	65.98	3.87	-2.07	4.54	-1.86	-0.68
$L_{13} \times T_1$	66.22	4.11	-1.83	4.36	-2.04	-0.86
$L_{13} \times T_2$	59.23	-2.87	-8.82	3.15	-3.25*	-2.07
$L_{13} \times T_3$	65.31	3.21	-2.74	3.20	-3.12*	-2.02

Table 2.

$L_{14}\times T_1$	69.63	7.52	1.57	4.48	-1.92	-0.74
$L_{14}\times T_2$	75.94	13.83	7.89	4.51	-1.89	-0.71
$L_{14}\times T_3$	67.52	5.41	-0.53	4.87	-1.53	-0.35
BHM-9	57.74	-4.37	-10.31	5.13	-1.26	-0.09
981	60.21	-1.90	-7.85	4.06	-2.34	-1.16
Sunshine	65.90	3.79	-2.15	4.60	-1.80	-0.62
Pioneer3396	68.05	5.95	-	5.22	-1.18	-
Kaberi 50	62.11	-	-5.95	6.40	-	1.18
Pacific 99Super	60.31	-1.79	-7.74	4.66	-1.74	-0.56
SE	-	6.55	-	-	0.88	0.88
F-test	NS	-	-	NS	-	-
CD	-	20.76	-	-	2.80	2.80

*P=0.05.

The leaves of most of the tolerant genotypes contained significantly higher K^+/Na^+ ratio than the 2 sensitive genotypes ($LT_{13}\times T_2$ and $LT_{13}\times T_3$). However, the ratio in $L_5\times T_3$ and $L_{10}\times T_1$ was statistically similar to that in sensitive genotypes. Importantly, genotypes like $L_1\times T_1$ contented comparatively higher K^+/Na^+ than the best Ck Kaberi 50, although they are statistically similar.

Saline stress eventually leads to reduction in crop yield which varies substantially in different crops. The relative yield loss which varied greatly depends on salinity levels and the degree of tolerance (Maas, 1983). In this study, a linear increase in salinity level was found upto maturity. However, it could be assumed that lower possibility to hamper germination as the salinity level was very low (<4 dSm^{-1}). Na^+ is the principal toxic ion for maize interfering with K uptake and transport, leading to alter of stomata modulations which cause water loss and necrosis (Fortmeier and Schubert, 1995; Sümer et al., 2004). Saline stress causes competition between K^+ and Na^+ which reduces severely K^+ content in both leaves and roots of maize (Kaya et al., 2010; de Azevedo Neto and Tabosa, 2000). However, reduction of K uptake varies with genotypes where resistant maize hybrids have higher K^+/Na^+ ratio than the sensitive ones (Farooq et al., 2015; Akram et al., 2007; Akram et al., 2010). In this experiment, the cross with higher yield (Table 2 and Figure 2) showed comparatively higher K^+/Na^+ than the most sensitive genotypes, depicting their higher tolerance to salinity.

Like the ratio of potassium to sodium ion, $O_2^{\bullet-}$, H_2O_2 and MDA differed significantly among the selected genotypes (Figure 3). Importantly, the levels of $O_2^{\bullet-}$, H_2O_2 and MDA in susceptible genotypes were significantly higher than those in most of the higher yielding genotypes. It was also remarkable that the levels of $O_2^{\bullet-}$, H_2O_2 and MDA seemed to be slightly lower in Ck genotypes compared to higher yielding genotypes. Plants are most sensitive to over production of ROS and MDA, a lipid peroxidative product (Gill and Tujeta, 2010). The levels of ROS, MDA and their scavenging antioxidants are used as, selection criteria of genotypes under salinity (Azooz et al., 2009). In this study, the levels of $O_2^{\bullet-}$, H_2O_2 and MDA were compared in 7 higher yield genotypes and 2 lower yielded genotypes ($L_{13}\times T_2$ and $L_{13}\times T_3$) along with 2 best Ck (Kaberi 50 and Pioneer 3396) (Figure 3).

Osmotic effect due to salinity changes in general metabolic system, leads to over production of ROS and causes oxidative stress in maize (Menezes-Benavente et al., 2004). ROS are highly toxic and damages cell organelles like proteins, DNA, lipids and carbohydrates. The apparatus of photosynthesis system in chloroplast is the major target of ROS which compelled the cell organelles to functional loss and eventually cell death (Gill and Tuteja, 2010). Among the ROS, singlet oxygen $^1O_2$, H_2O_2 and $O_2^{\bullet-}$ are very much important and cause severe damage to cell. In this study, H_2O_2 and $O_2^{\bullet-}$ were compared in flag leaves of some tolerant and susceptible genotypes (as shown in Figure 3), where the contents were higher in the sensitive genotypes. Higher concentrations of H_2O_2 and $O_2^{\bullet-}$

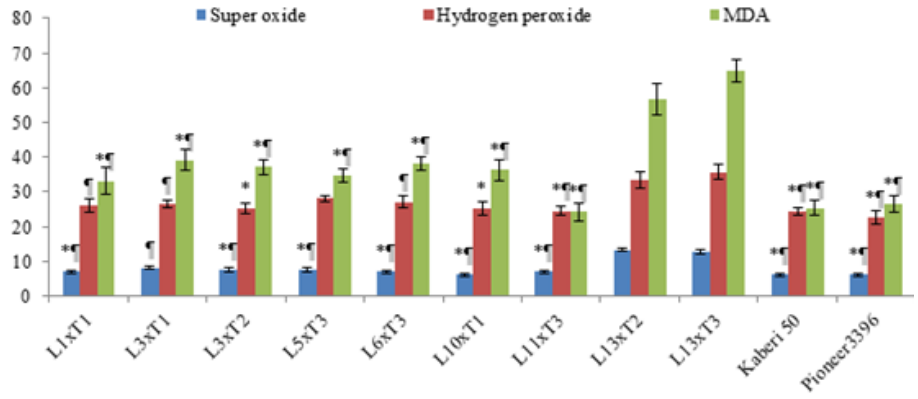

Figure 3. Levels of $O_2^{\bullet-}$, H_2O_2 and MDA in the selected genotypes. $L_{13}\times T_2$ and $L_{13}\times T_3$ are lower yielder genotype and Kaberi 50 and Pioneer 3396 are the 2 best standard check*, ¶ mean significantly different over check Kaberi 50 and Pioneer 3396, respectively.

under salinity can modify proteins and DNA components and alter metabolic process (Gill and Tujeta, 2010) which limits growth and yield.

The higher ROS cause veins of maize to collapse due to leakage into neighboring cells (Menezes-Benavente et al., 2004). They attack cell wall by interacting with polyunsaturated fatty acid and cause peroxidation product as MDA. Therefore, sensitive genotypes are most likely to loss cell organelles due to higher possibility of cell membrane leakage, as the MDA content was higher in sensitive genotypes (Figure 3). The MDA content in sensitive maize genotypes was also reported in seedling stage in our previous studies (Rohman et al., 2016) which might be due to differential antioxidant system (Hichem et al., 2009; Rohman et al., 2016).

Conclusion

Considering earliness, plant height, ear height and yield above 2nd best check, $L_1\times T_1$, $L_1\times T_2$, $L_3\times T_1$, $L_3\times T_2$, $L_5\times T_3$, $L_6\times T_3$, $L_{10}\times T_1$ and $L_{11}\times T_3$ were considered as promising for saline area. Therefore, the lines involving these crosses were selected and advanced for next generational experiment. Importantly, higher yielding crosses showed lower contents of $O_2^{\bullet-}$, H_2O_2 and MDA than the susceptible crosses suggesting their better stress tolerance ability.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Akram M, Ashraf MY, Ahmad R, Rafiq M, Ahmad I, Iqbal J (2010). Allometry and yield components of maize (*Zea mays* L.) hybrids to various potassium levels under saline conditions. Arch. Biol. Sci. Belgrade. 62:1053-1061.

Akram M, Malik MA, Ashraf MY, Saleem MF, M. Hussain (2007). Competitive seedling growth and K^+/Na^+ ratio in different maize (*Zea mays* L.) hybrids under salinity stress. Pak. J. Bot. 39:2553-2563.

Azooz MM, Ismail AM, Elhamd MFA (2009). Growth, lipid peroxidation and antioxidant enzyme activities as a selection criterion for the salt tolerance of three maize cultivars grown under salinity stress. Int. J. Agric. Biol. 11:21-26.

Chen CL, van der Schoot H, Dehghan S, Alvim Kamei CL, Schwarz KU, Meyer H, Visser RGF, van der Linden CG (2017). Genetic diversity of salt tolerance in Miscanthus. Front. Plant Sci. 8:187.

Chinnusamy V, Jagendorf A, Zhu JK (2005). Understanding and Improving Salt Tolerance in Plants. Crop Sci. 45:437-448.

De Azevedo Neto AD, Tabosa JN (2000). Salt stress in maize seedlings: II. Distribution of cationic macronutrients and its relation with sodium. Rev. Bras. Eng. Agric. Amb. 4:165-171.

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. Trends Plant Sci. 19(6):371-9.

Elstner EF, Heupel A (1976). Inhibition of nitrite formation from hydroxyl ammonium chloride: a simple assay for superoxide dismutase. Anal. Biochem. 70(2):616-20.

Farooq M, Hussain M, Wakeel A, Kadambot H, Siddique M (2015). Salt stress in maize: effects, resistance mechanisms, and management. A review. Agron. Sustain. Dev. 35(2):461-481.

Fortmeier R, Schubert S (1995). Salt tolerance of maize (*Zea mays* L.): the role of sodium exclusion. Plant Cell Environ. 18:1041-1047.

Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48:909-930.

Hichem H, Mounir D, Naceur EA (2009). Differential responses of two maize (*Zea mays* L.) varieties to salt stress: changes on polyphenols composition of foliage and oxidative damages. Ind. Crops Prod. 30:144-151.

Kaya C, Tuna AL, Okant AM (2010). Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. Turk. J. Agric. For. 34:529-538.

Menezes-Benavente L, Kernodle SP, Margis-Pinheiro M, Scandalios JG (2004). Salt-induced antioxidant metabolism defenses in maize (*Zea mays* L.) seedlings. Redox Rep. 9:29-36.

Pardo JM, Quintero FJ (2002). Plants and sodium ions, keeping company with the enemy. Genome Biol. 3:1017.1–1017.4.

Rohman MM, Talukder MZA, Hossain MG, Uddin MS, Amiruzzaman M, Biswas A, Ahsan AF MS, Chowdhury MAZ (2016). Saline sensitivity leads to oxidative stress and increases the antioxidants in presence of proline and betaine in maize (*Zea mays* L.) inbred. Plant Omics J. 9(1):35-47.

Schachtman D, Liu W (1999). Molecular pieces to the puzzle of the

interaction between potassium and sodium uptake in plants. Trends Plant Sci. 4(7):281-287.
Sümer A, Zörb C, Yan F, Schubert S (2004). Evidence of sodium toxicity for the vegetative growth of maize (*Zea mays* L.) during the first phase of salt stress. J. Appl. Bot. 78:135-139.
Yu CW, Murphy TM, Lin CH (2003). Hydrogen peroxide-induces chilling tolerance in mungbeans mediated through ABA-independent glutathione accumulation. Func. Plant Biol. 30:955-963.

academicJournals

Vol. 13(5), pp. 248-257, 1 February, 2018
DOI: 10.5897/AJAR2015.10350
Article Number: 65B7BCC55871
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Genetic diversity study of some banana genotypes collected from various parts of India through RAPD analysis

Prasenjit Kundu[1], Fatik Kumar Bauri[1] and Sutanu Maji[2*]

[1]Department of Fruits and Orchard Management, Faculty of Horticulture, Bidhan Chandra KhrishiViswavidyalaya, Nadia, West Bengal - 741252, India.
[2]Department of Applied Plant Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh -226025, India.

Received 26 August, 2015; Accepted 29 October, 2015

Globally, banana is the fourth most important fruit crop and is grown in more than 130 countries across the world. Traditional procedure to characterize banana plants by morphological descriptors has many limitations, and different communities refer to the same local cultivars by different names due to lack of clear clone identity resulting in unnecessary duplication with regard to cultivation, conservation and research. To alleviate this problem application of modern finger printing technology through DNA studies have been recommended for accurate selection of banana clones. Here, 16 genotypes of banana collected from different districts of West Bengal and other parts of India were characterized by RAPD analysis to assist the selection of characters for banana breeding. It was observed that among the 25 randomly selected markers, OPE 1, 4, 7, 9, 20 and 25 showed 100% polymorphism under annealing temperatures of 27 and 29°C. Their genetic diversity study revealed that sixteen germplasms were grouped into eight clusters namely Malbhog, Martaman, Kalibhog and Sobri in Cluster-I; Alapan, Poovan and Champa in Cluster-II; Amritpani, Bamandeshi in Cluster-III; Dudhsagar, Rasthali in Cluster-IV; Krishna Vazai, Manohar in Cluster-V; Chang Monua in Cluster VI; Kanai Bansi in Cluster VII and Nendran in Cluster VIII.

Key words: Banana, molecular characterization, genetic diversity.

INTRODUCTION

Banana (*Musa* sp.) is the maximum distributed fruit crop and is the fourth most important commodity in the world. It is grown in more than 130 countries across the world, continuously exhibiting a spectacular growth pattern worldwide, producing 120 million tonnes (Anonymous, 2014) of which 56% is shared by Asia. India is the largest producer of banana in the world with the production of 29.72 million tonnes during 2013-14 (Anonymous, 2015). There are diverse germplasms of banana traditionally cultivated in different regions of India having remarkable

*Corresponding author. E-mail: majisutanu@gmail.com.

genetic differences, in spite of their overall genomic grouping variations still existing in the same group. Despite the importance of bananas in trade and commerce, there is little information on the genetics for its agronomically important traits (Loh et al., 2000). Traditional procedure to characterize banana plants by morphological descriptors has many limitations. Many improved varieties released have a complex genealogy involving several wild species and landraces. However, barriers such as intractable fertilization, moderate to high levels of female sterility and triploidy have made the identification of desired banana cultivars a key issue for these crop improvement programmes (Bhat et al., 1995). In view of developing efficient breeding schemes, additional data needs to be generated on the complex genome structure of hybrids and cultivars. To this end, the characterization of indigenous germplasms will offer a precise means of formulating taxonomic, phylogenetic and heterotic groupings within the family Musaceae (Crouch et al., 1998).

Cheesman (1948) first suggested that cultivated bananas originated from intra and interspecific hybridization between the two wild diploid species *Musa acuminata* Colla and *Musa balbisiana* Colla, each contributing the A and B genomes, respectively. The identification of *Musa* cultivars has traditionally been based upon various combinations of morphological, phenological and floral criteria. Simmonds and Shepherd (1955) devised a scoring technique based on 15 diagnostic morphological characters to differentiate *M. acuminate* clones from *M. balbisiana* cultivars and their hybrids into 6 genome groups. The taxonomy of cultivated bananas has long been a contentious issue and because it relies heavily on morphology, the literature shows many contradictions. For instance, based on molecular data, Pillay et al. (2000) showed that the clones 'Monthan Saba' and 'Bluggoe', previously classified as BBB based on morphological characteristics but, actually belong to the ABB group. Similarly, tetraploid 'KlueTiparot' (ABBB) is reclassified as a triploid ABB (Jenny and Carreel, 1997; Horry et al., 1998). The difficulties associated with the use of whole plant or floral morphology has led researchers to develop other techniques for the correct identification of *Musa* species and cultivars.

Different communities refer to the same local cultivars by different names, and lack of clear clone identity in the crop has resulted in unnecessary duplication in cultivation, conservation and research (Onguso et al., 2004). To alleviate this problem, application of modern finger printing technology through DNA studies have been recommended for accurate selection of banana clones (Robinson, 1996).

As a more reliable alternative, various DNA fingerprinting techniques have been used to study the genetic diversity and taxonomy of cultivated bananas which include disozyme analysis (Bhat et al., 1992), restriction fragment length polymorphism (RFLP) (Bhat et al., 1994; Jarret et al., 1992: Kaemmer et al., 1992), rRNA spacer length heterogeneity (Lanaud et al., 1992), inter simple sequence repeat (ISSR) markers (Godwin et al., 1997), sequence tagged microsatellite sites (STMS) (Grapin et al., 1998; Kaemmer et al., 1997) and amplified fragment length polymorphism (AFLP) (Loh et al., 2000; Wong et al., 2001).

To make the collection useful for plant breeders, morphological and molecular characterization of the germplasms is necessary. The disadvantages of phenotype-based assays can be overcome by direct identification of genotypes with DNA-based markers (Nsabimana and Staden, 2007). Molecular markers have been employed in *Musa* genotypes to assess ploidy (Oselebe et al., 2006), phylogenetic relationships (Jain et al., 2007; Nsabimana and Staden, 2007; Uma et al., 2006) and genetic diversity because of somaclonal variation (Lakshmanan et al., 2007; Bairu et al., 2006; Ray et al., 2006) or mutation induction (Hautea et al., 2004; Finalet et al., 2000; Toruan-Mathius and Haris, 1999). Polymorphis generated by RAPD analysis has been used for fingerprinting and classification of the *Musa* genotypes. Linkage of RAPD markers to specific traits such as disease resistance has been possible (Damasco et al., 1996) and RAPD markers are usually preferred as the technique is simple, versatile, relatively inexpensive and able to detect minute differences (Pillay et al., 2000; Williams et al., 1990; Welsh and McClelland, 1990; Howell et al., 1994). RAPD based fingerprinting has been successfully applied to the characterization of diverse *Musa* germplasms (Bhat and Jarret, 1995; Onguso et al., 2004), analysis of *Musa* breeding populations (Crouch et al., 1999) and detection of somaclonal variants (Grajal-Martin et al., 1998). A proper classification of *Musa* clones and cultivars is important in assisting the selection of characters for banana breeding. This research work therefore describes the use of RAPD markers to evaluate the genetic diversity and relationships amongst sixteen different Mauritian *Musa* germplasms collected from different parts of India.

MATERIALS AND METHODS

Plant materials

The present study was made at the Department of Fruits and Orchard Management, Faculty of Horticulture, Bidhan Chandra KrishiViswavidyalaya, Nadia, West Bengal, India. Sixteen germplasms of banana collected from various states of India (Table 1) were studied for their genetic diversity and relationship. Young leaves from nursery grown randomly selected plant as well as its donor plants, were taken for genomic DNA isolation.

Plant DNA isolation

Fresh, green tender leaf samples were collected from the field and were immediately wrapped in aluminum foil and stored until the

Table 1. Banana genotypes used in the present study.

Germplasm	Genome	Sub group	Source
Sobri	AAB	Silk	Krishnaganj, Majhdia, Nadia, West Bengal
Nendran	AAB	Plantain	BRS, Kannara, Kerala
Krishnavazai	AAB	Pome	Horticultual Research and Developmental Farm, Chunchura, West Bengal
Malbhog	AAB	Silk	Pundibari, Coochbehar, West Bengal
Rasthali	AAB	Silk	TNAU, Tamil Nadu
Amritpani	AAB	Silk	ARS, Kovvur, Andhra Pradesh
Champa	AAB	Mysore	Bandel, Hooghly, West Bengal
Kalibhog	AAB	Silk	Horticulture Research Farm, Kalyani, West Bengal
Dudhsagar	AAB	Silk	AAU, Jorhat, Assam
Martaman	AAB	Silk	Ghoragancha, Nadia, West Bengal
Kanaibashi	AAB	Hatidat	Chakdaha, Nadia, West Bengal
Chang Monua	AAB	Silk	Baneswar, Coochbehar, West Bengal
Poovan	AAB	Mysore	TNAU, Tamil Nadu
Manohar	AAB	Athiakol	AAU, Jorhat, Assam
Bamandeshi	AAB	Pome	Senpara, Jalpaiguri, West Bengal
Alapan	AAB	Mysore	RAU, Pusa, Bihar

material reached the laboratory. The samples were washed thoroughly and dried with tissue paper. Approximately, 100 mg of laminae (with partially intact petioles) was taken and immediately transfer to precooled (-50°C) mortar following excision from the plant and the tissue stored at –50°C for at least 30 min. The leaf tissue was grinded as quickly as possible and DNA was extracted following the standard procedure of CTAB method with slight modification. The samples were then preserved in a refrigerator (4°C) and quantified accordingly.

Optimization of PCR primers

RAPD amplification was performed in 25 µl of reaction mixture that contained template DNA 2.0 µl, 10x PCR buffer 2.5 µl, 2.5 mMdNTPs 2.0µl, primer 1.0 µl, 2.5 mM $MgCl_2$ 2.0 µl, Taq polymerase enzyme 0.3 µl, and ddH_2O 13.0 µl. The PCR reaction mixture of 25 µl was then set for the reaction in PCR machine. The amplification was performed in a thermal cycler (Mastercycler, Eppendorff, AG22331, Germany). The gel was photographed under UV light transillumination (Gel Doc, Biotech, Yercaud, Salem, India). The 20 ng DNA sample and 30 µl polymerase enzyme was used.

Screening of primers

Altogether, 25 random decamer oligonucleotide primers (Eurofins Genomics, Bengaluru, India) were screened for the study (Table 2).

Agarose gel preparation and gel electrophoresis

Agarose (SRL, India, cat≠ 01441Q) was dissolved in 100 ml of IX TAE buffer which was then boiled in microwave oven (Godrej, GMG22B) for 3 min and allowed to cool to 60°C and solidified on gel tray (Genei^{TM}). In each PCR tube, 2.5 µl of 10x gel loading dye was added. The tube was spinned for 3 s in a centrifuge (Mastercycler, Eppendorff, AG22331, Germany) and loaded onto the agarose gel. The gel was run at constant 80 volts till the dye front moved a distance of about 2/3 from the loading point. The photograph was taken in Geldoc (Biotech, Yercaud,Salem, India).

Table 2. Primers used during the study, their sequence and annealing temperature.

S/N	Code	Primer sequence (5´–3´)
1	OPE 01	CCAGATGCAC
2	OPE 02	GAGACATGCC
3	OPE 03	AAGACCCCTC
4	OPE 04	CTTCACCCGA
5	OPE 05	TTATCGCCCC
6	OPE 06	GGTGACTGTG
7	OPE 07	GGACTGCAGA
8	OPE 08	ACGGCGTATG
9	OPE 09	AACGGTGACC
10	OPE 10	CCAAGCTTCC
11	OPE 11	CCAGTACTCC
12	OPE 12	TGAGCGGACA
13	OPE 13	CAGGCCCTTC
14	OPE 14	TGCCGAGCTG
15	OPE 15	AGTCAGCCAC
16	OPE 16	AATCGGGCTG
17	OPE 17	AGGGGTCTTG
18	OPE 18	GGTCCCTGAC
19	OPE 19	GTGACGTAGG
20	OPE 20	GTGATCGCAG
21	OPE 21	CAATCGCCGT
22	OPE 22	TCGGCGATAG
23	OPE 23	CAGCACCCAC
24	OPE 24	TTCCGAACCC
25	OPE 25	AGGTGACCGT

Annealing temperature (°C) = 27; 29°C for OPE 13, 14, 18 and 23.

Table 3. Production of total and polymorphic bands using specific primers.

Primer	Number of RAPD products (bands)		
	Total bands	Polymorphic	% polymorphism
OPE 01	2	2	100.0
OPE 02	5	2	40.0
OPE 03	0	0	0
OPE 04	3	3	100.0
OPE 05	0	0	0
OPE 06	2	1	50.0
OPE 07	3	3	100.0
OPE 08	0	0	0
OPE 09	3	3	100.0
OPE 10	0	0	0
OPE 11	0	0	0
OPE 12	4	2	50.0
OPE 13	0	0	0
OPE 14	3	2	66.67
OPE 15	0	0	0
OPE 16	5	4	80.0
OPE 17	0	0	0
OPE 18	0	0	0
OPE 19	5	3	60.0
OPE 20	3	3	100.0
OPE 21	3	2	66.67
OPE 22	0	0	0
OPE 23	0	0	0
OPE 24	4	3	75.0
OPE 25	1	1	100.0

1% agarose gel was used to perform this operation.

Statistical analysis

Cluster analysis was done to identify a smaller number of groups such that the genotypes residing in a particular group were more similar to each other than to genotypes belonging to other groups (Singh and Chowdhury, 1985). Grouping in the present study was done by Tocher method (Rao, 1952) with the help of Mahalanobis (1936) method. The dendrogram was generated using unweighted pair group arithmetic mean method (UPGMA) using NTSYS pc version 2.1 software for classifying under several clusters.

RESULTS AND DISCUSSION

Evaluation of parameters for PCR

Initially, three different parameters namely, Taq DNA polymerase concentration, $MgCl_2$ concentration and primer concentration were examined with fixed amount of *Musa* spp. template DNA (2.0 µl). Optimum concentration was selected based on clear banding pattern. It was observed that a combination of 1.0 µl of primer and 0.3 µl of Taq DNA polymerase gave the best results with addition of 2.0 µl $MgCl_2$ (25 mM) in the reaction mixture which improved the banding pattern.

Annealing temperature

In a separate set of experiments, a regime of two different annealing temperatures (27 and 29°C) were tested (Table 2) with four standardized concentration primers (OPE-01, OPE-02, OPE-04 and OPE-07) and Taq DNA polymerase with cultivars *of Musa* spp. A clear and prominent banding pattern was recorded at these two different annealing temperatures (Table 3), and it was observed that OPE 1, 4, 7, 9, 20 and 25 showed 100% polymorphism.

Template DNA

For optimization of template DNA, concentrations such as 1.0, 2.0 and 3.0 µl reaction mixtures were used. Among the different reaction mixtures, 2.0 µl performed best

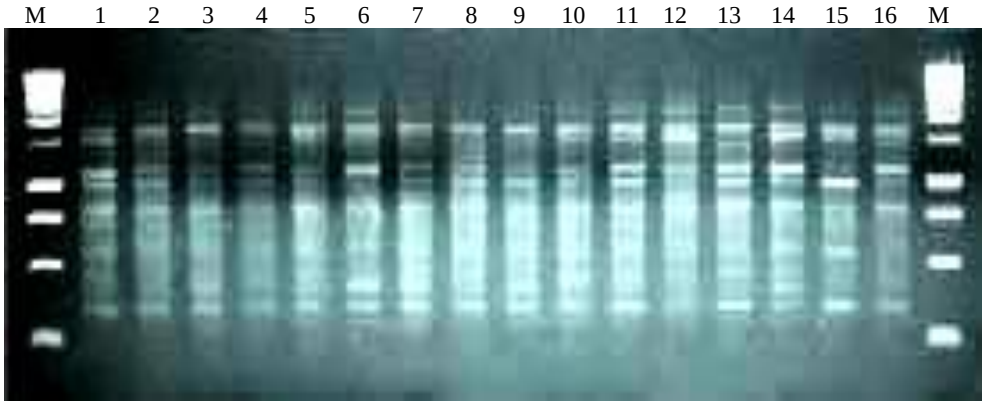

Figure 1. Banding pattern of banana germplasms with primer OPE-02.
Lane 1-16: RAPD profile of banana varieties (1) Sobri (2) Nendran (3) Krishna Vazai (4) Malbhog (5) Rasthali (6) Amritpani (7) Champa (8) Kalibhog (9) Dudhsagar (10) Martaman (11) Kanai Bansi (12) Chang Monua (13) Poovan (14) Manohar (15) Bamandeshi (16) Alapan. Lane M: DNA ladder.

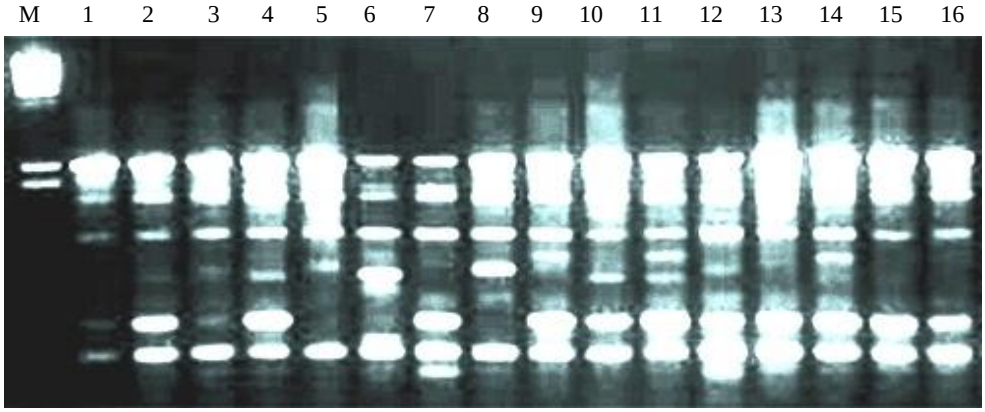

Figure 2. Banding pattern of banana germplasms with primer OPE-19.
Lane 1-16: RAPD profile of banana varieties (1) Sobri (2) Nendran (3) Krishna Vazai (4) Malbhog (5) Rasthali (6) Amritpani (7) Champa (8) Kalibhog (9) Dudhsagar (10) Martaman (11) Kanai Bansi (12) Chang Monua (13) Poovan (14) Manohar (15) Bamandeshi (16) Alapan. Lane M: DNA ladder.

amplification. Lower or higher concentrations either reduced amplification or produced smearing. Therefore, in the subsequent experiments 2.0 µl template DNA concentration was used. In all the aforementioned reactions, a control was set up, which contained all the constituents of reaction mixture except template DNA that was replaced by the exact amount of sdH_2O.

Screening of primers

As mentioned earlier, 25 random decamer (10 base pair) oligonuclieotide sequences (primers) were screened for sixteen germplasms of banana to study the robustness of amplification, reproducibility, and scorability of banding patterns. As a result from the 25 primers, 11 did not produce any polymorphic bands and 1 gave only one band. The RAPD primers showed that higher degree of polymorphism (Table 3 and Figures 1 to 3) were used to identify the genotypes under study and those showing no polymorphism were not suitable for study purpose.

Grouping of varieties into cluster

The dendrogram (Figure 4) was generated using unweighted pair group arithmetic mean method (UPGMA) using NTSYS pc version 2.1 software and resulted in eight major clusters. From the clustering pattern of the genotypes, it revealed that the 16 genotypes were

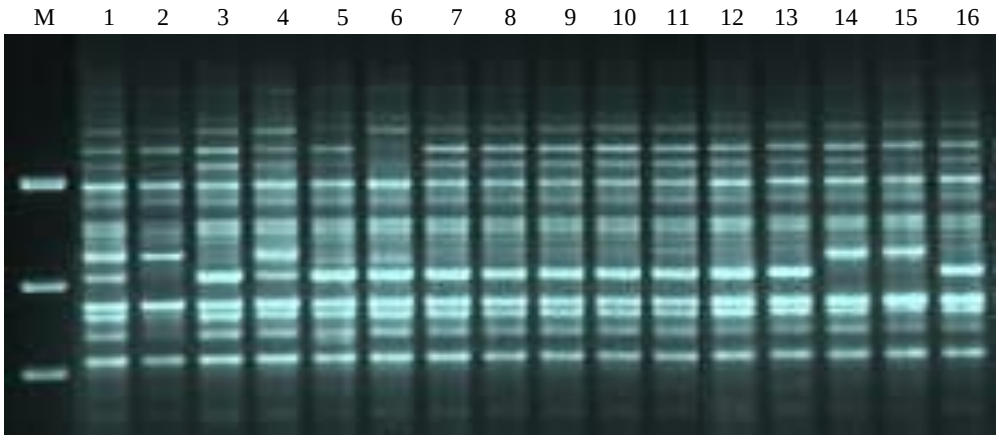

Figure 3. Banding pattern of banana germplasms with primer OPE-14.
Lane 1-16: RAPD profile of banana varieties (1) Sobri (2) Nendran (3) Krishna Vazai (4) Malbhog (5) Rasthali (6) Amritpani (7) Champa (8) Kalibhog (9) Dudhsagar (10) Martaman (11) Kanai Bansi (12) Chang Monua (13) Poovan (14) Manohar (15) Bamandeshi (16) Alapan. Lane M: DNA ladder.

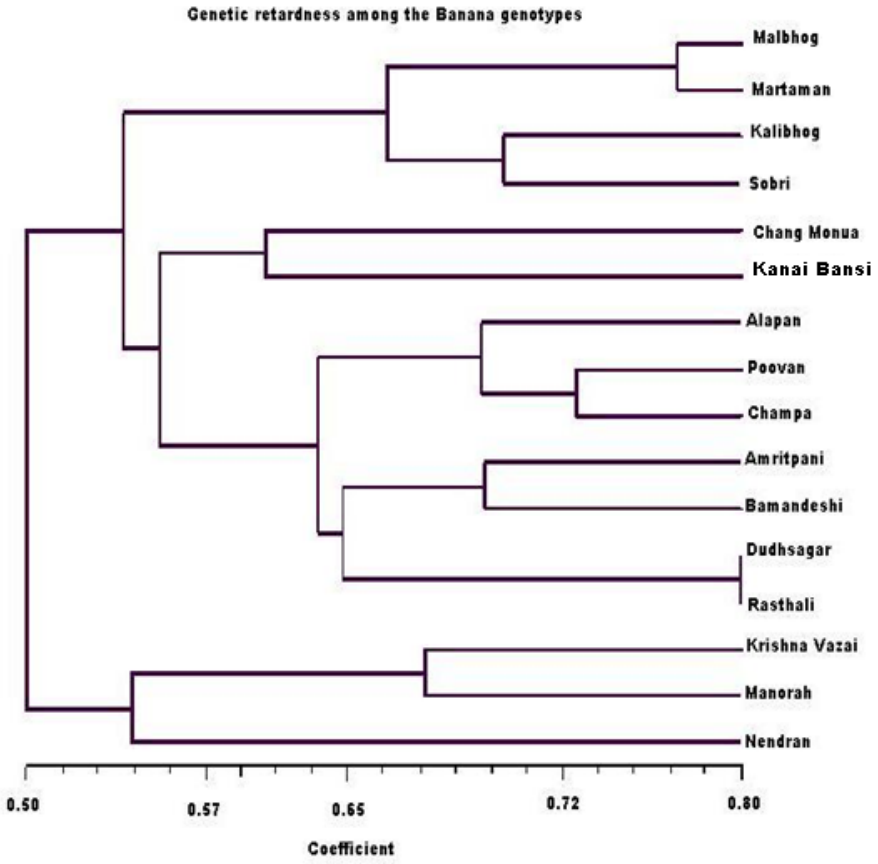

Figure 4. Genetic relationships among the 16 banana genotypes under study.

grouped into eight clusters that is, Malbhog, Martaman, Kalibhog and Sobri- Cluster-I; Alapan, Poovan and

Champa- Cluster II; Amritpani, Bamandeshi- Cluster III; Dudhsagar, Rasthali- Cluster IV; Krishna Vazai, Manohar- Cluster V; Chang Monua- Cluster VI; Kanai Bansi- Cluster VII and Nendran- Cluster VIII.

Analysis of polymorphism

The 25 random 10-mer primers yielded more than 327 scorable polymorphisms. However, the 10 decamer oligonucleotide primers produced reproducible bands (Table 3). They generated 46 amplification products of which 34 bands (73.91%) were polymorphic. The number of bands per primer varied between 1 to 5, with an average of 3.29 bands per primer. However, the range of polymorphic bands per primer was 1 to 4, with a mean of 2.43 polymorphic bands per primer (Table 3). The representative RAPD patterns generated by primers OPE-01, OPE-02, OPE-04, OPE-06, OPE-07, OPE-09, OPE-12, OPE-14, OPE-16, OPE-19, OPE-20, OPE-21, OPE-24 and OPE-25 are illustrated in this research article.

The number of individual samples considered in this study might not truly represent the total available diversity of *Musa* of this region; nevertheless, the percentage of polymorphic bands (73.91%) of RAPD marker in the species was higher than some other plants such as *Changium smyrnioides* (69%) (Fu et al., 2003), *Lactoris fernandeziana* (24.5%) (Brauner et al., 1992), *Cathaya argyrophylla* (32%) (Wang et al., 1996). The numerical value also suggested that the species genetic diversity was high and hence enables it to adapt to environmental variations.

The isolates of banana were subjected to PCR analysis where 14 universal primers resulted in robust and reproducible DNA fragment patterns. The selected primers generated numerous bands but, 327 distinct and reproducible were considered for analysis. Each primer showed polymorphic banding pattern. The genetic similarity between the isolates of banana was determined on the basis of Jaccard's similarity coefficient. The highest genetic similarity was observed between Dudhsagar and Rasthali isolates, ranging from 80%. Malbhog and Martaman showed 77% similarity, whereas Poovan and Champa depicted high level of similarity i.e, 74%. Amritpani and Bamandeshi showed 69% similarity. Krishna Vazai and Manohar showed 67% similarity which may be a potential of such a high degree of similarity. There was 65% similarity between the two sub clusters Malbhog and Martaman (familiar in West Bengal and also originated here) and Kalibhog and Sobri (also collected from West Bengal). There was 60% similarity depicted between Chang Monua and Kanai Bansi, both of them made a sub cluster (Figure 4). Kanai Bansi and Chang Monua gave just 60% similarity. The lowest degree of similarity was shown between Nendran and Chang Monua i.e. 50% (high degree of dissimilarity) which is in broad agreement with the geographical distribution of these two genotypes (These two were collected from various location, Nendran from Kerala and Chang Monua were collected from West Bengal) and moreover, this can be attributed to the broad genetic base in the origin of the species. This similarity coefficient values of banana in this study is higher or in the same range with respect to other reported species such as *Panax ginseng* (19.7 to 49.1%) (Um et al., 2001), *Poa trivialis* (7 to 74%) (Rajasekar et al., 2006), *Rhododendron* spp. (26.2-90.6%) (Lanying et al., 2008), *Lathyrus sativus* (13-66%) (Sedehi et al., 2008), Common bean (19 to 91%) (Tiwari et al., 2005), *Ensete ventricosum* (16 to 85%) (Birmeta et al., 2002).

The degree of polymorphism revealed in populations by amplification with arbitrary primers is extensive. The degree of polymorphism in this study might be due to the wide geographical origin of the genotypes. A dendrogram constructed based on shared fragments revealed broad existence of clusters. In general, the clustering concurs with the place of collection of different genotypes. It can also be opined that Dudhsagar and Rasthali are same genotypes as revealed by their maximum similarity coefficient. Essentially little morphological variation between then misleads us to treat them as different landraces.

The present study addressed the utility of RAPD markers in revealing genetic relationships at molecular level among landraces of *Musa* spp. of North Bengal and North Eastern, Southern and Eastern part of India. The RAPD polymorphism may be attributed to the outcome of a nucleotide change that alters the prime-binding site or an insertion or deletion within the amplified region (Williams et al., 1993). The RAPD markers were able to distinguish groups among the banana cultivars in different clusters. The polymorphism showed by RAPD has been problematic due to their dominance. As heterozygotes are not normally detectable, the results are not readily usable for computing Hardy Weinberg equilibrium or Nei's standard genetic distance (Lynch and Milligan, 1994). The level of polymorphism observed in the present study (Table 4) was moderately high, indicating a wide and diverse genetic base for the banana landraces in various parts of India. The 73.91% RAPD polymorphic bands suggest that banana landraces maintain a higher intra-specific genetic diversity which is very important for future breeding programme to generate good quality germplasms in the context of climate change and environmental hazards (Maji and Das, 2008; Maji, 2013; Singh et al., 2013; Soni et al., 2013a, b; Kumar et al., 2013). The conventional classification of banana genotypes into distinct genome combinations based on their morphological similarity is as follows *Musa acuminate* Colla or *Musa balbisiana* Colla. The difficulty faced in the identification of banana cultivars, which are mostly sterile, therefore highlights the need for a DNA marker system for classification (Loh et al., 2000). The

Table 4. Similarity index based on Nei's estimates of 16 cultivars of *Musa* spp.

	Sobri	Nendran	Krishna Vazai	Malbhog	Rasthali	Amritpani	Champa	Kalibhog	Dudhsagar	Martaman	Kanai Bansi	Chang Monua	Poovan	Manohar	Bamandesi	Alapan
Sobri	1															
Nendran	0.77	1														
Krishna Vazai	0.61	0.7	1													
Malbhog	0.61	0.55	0.6	1												
Rasthali	0.42	0.48	0.52	0.52	1											
Amritpani	0.48	0.62	0.52	0.52	0.6	1										
Champa	0.5	0.56	0.54	0.6	0.54	0.6	1									
Kalibhog	0.63	0.58	0.5	0.56	0.56	0.56	0.68	1								
Dudhsagar	0.54	0.48	0.59	0.67	0.52	0.46	0.65	0.68	1							
Martaman	0.62	0.63	0.61	0.48	0.61	0.54	0.67	0.69	0.6	1						
Kanai Bansi	0.58	0.71	0.7	0.65	0.55	0.62	0.68	0.54	0.57	0.56	1					
Chang Monua	0.41	0.46	0.57	0.57	0.65	0.5	0.64	0.6	0.8	0.65	0.52	1				
Poovan	0.56	0.5	0.54	0.61	0.69	0.54	0.67	0.63	0.6	0.62	0.52	0.52	1			
Manohar	0.45	0.45	0.6	0.54	0.54	0.48	0.71	0.57	0.65	0.61	0.56	0.64	0.74	1		
Bamandesi	0.46	0.39	0.36	0.67	0.5	0.58	0.52	0.48	0.5	0.4	0.48	0.41	0.59	0.46	1	
Alapan	0.44	0.38	0.41	0.48	0.48	0.41	0.44	0.41	0.48	0.38	0.46	0.33	0.71	0.56	0.61	1

DNA fingerprinting pattern would help in the identification of duplications among accessions in the field.

Conclusions

The present study clearly concluded that there was a wide genetic variation in the banana germplasms collected from various parts of India. 14 RAPD markers (OPE-01,OPE-02, OPE-04, OPE-06, OPE-07, OPE-09, OPE-12, OPE-14, OPE-16, OPE-19, OPE-20, OPE-21, OPE-24 and OPE-25) were best among the 25 markers screened. The RAPD markers were able to distinguish groups among the banana cultivars in eight different clusters among the selected genotypes namely Cluster I- Malbhog, Martaman, Kalibhog and Sobri; Cluster II- Alapan, Poovan and Champa; Cluster III-Amritpani, Bamandeshi; Cluster IV- Dudhsagar, Rasthali; Cluster V-Krishna Vazai, Manohar; Cluster VI- Chang Monua; Cluster VII- Kanai Bansi and Cluster VIII-Nendran.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Authors would like to express their sincere thanks to Dr. J. Tarafdar for availing the molecular lab facility and Mr. Govind, Research Scholar, Babasaheb Bhimrao Ambedkar University, Uttar Pradesh, India for preparing the manuscript.

REFERENCES

Anonymous (2014). FAO Database. http://www.fao.org/faostat/en/#data/CC

Anonymous (2015). NHB Database. pp. 34-40. http://nhb.gov.in/area-pro/NHB_Database_2015.pdf

Bairu MW, Fennell CW, Van Staden J (2006). The effect of plant growth regulators on somaclonal variation in Cavendish banana (*Musa* AAA cv. 'Zelig'). Sci. Hort. 108:347-351.

Bhat KV, Bhat SR, Chandel KPS (1992). Survey of isozyme polymorphism for clonal identification in *Musa*Esterase, acid phosphatase and catalase. J. Hort. Sci.67:501-507.

Bhat KV, Jarret RL, Liu ZW (1994). RFLP characterization of Indian *Musa* germplasm for clonal identification and classification. Euphytica. 80:95-103.

Bhat KV, Jarret RL (1995). Random amplified polymorphic DNA and genetic diversity in Indian *Musa* germplasm. Genet. Res. Crop Sci. 42:107-118.

Bhat KV, Jarret RL, Chandel KPS, Lakhanpaul S, Ali S (1995).

DNA fingerprinting in *Musa* cultivars with oligodeoxyribonucleotide probes specific for the simple repeat motifs. Genet. Anal.12:45-51.
Birmeta G, Nybom H, Bekele E (2002). RAPD analysis of genetic diversity among clones of the Ethiopian crop plant Enseteventricosum. Euphytica 124:315-325.
Brauner S, Crawford DJ, Stuessy TF (1992). Ribosomal DNA and RAPD variation in the rare plant family Lactoridaceae. Am. J. Bot. 79:1436-1439.
Cheesman EE (1948). Classification of bananas IIIc *Musa paradisiaca* Linn. And *Musa sapientum* Linn.Kew Bull. 2:146-153.
Crouch HK, Crouch JH, Jarret RL, Cregan PB, Ortiz R (1998). Segregation of microsatellite loci from haploid and diploid gametes in *Musa*. Crop Sci. 38:211-217.
Crouch JH, Crouch HK, Constandt H, Van Gysel A, Breyne P, Van Montagu M, Jarret RL, Ortiz R (1999). Comparison of PCR-based molecular marker analyses of *Musa* breeding populations. Mol. Breed. 5:233-244.
Damasco OP, Graham GC, Henry RJ, Adkins SW, Smith MK, Godwin ID (1996). Random amplified polymorphic DNA (RAPD) detection of dwarf off-types in micropropagated Cavendish (*Musa* spp. AAA) bananas. Plant Cell Reports. 16:118-123.
Finalet J, Peteira B, Leon O, Ventura JCM, Lopez J (2000). Application of RAPD markers to the differentiation of *Musa* spp. mutants.Revista de Proteccion Vegetal. 15:60-63.
Fu C, Qiu Y, Kong H (2003). RAPD analysis for genetic diversity in Changiumsmyrnioides (Apeaceae) and endangered plant. Botanical Bull. Academic Sinica. 44:13-18.
Godwin ID, Aitken EAB, Smith LW (1997). Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis.18:1524-1528.
Grajal-Martin M, Siverio-Grillo G, Marrero-Dominguez A (1998). The use of randomly amplified polymorphic DNA (RAPD) for the study of genetic diversity and somaclonal variation in *Musa*. Acta Hort. 490:445-454.
Grapin A, Noyer JL, Carreel F, Dambler D, Baurens FC, Lanaud C, Lagoda PJL (1998). Diploid *Musa acuminate* genetic diversity assayed with sequence tagged microsatellite sites. Electrophoresis 19:1374-1528.
Hautea DM, Molina GC, Balatero CH, Coronado NB, Perez EB, Alvarez MTH, Canama AO, Akuba RH, Quilloy RB, Frankie RB, Caspillo CS (2004). Analysis of induced mutants of Philippine with molecular markers, in: Jain, S.M., Swennen, R. (Eds.). Banana Improvement: Cellular, Molecular Biology and Induced Mutations.
Horry JP, Dolozel J, Dolezelova M, Lysak MA (1998). 'Do natural A X B tetraploid bananas exist?'. Infomusa. 7:5-6.
Howell EC, Newbury HJ, Swennen RL, Withers LA, Ford-Lloyd BV (1994). The use of RAPD for identifying and classifying *Musa* germplasm. Genome 37:328-332.
Jain PK, Saini ML, Pathak H, Gupta VK (2007). Analysis of genetic variation in different banana (Musa species) variety using random amplified polymorphic DNAs (RAPDs). Afr. J. Biotechnol. 6:1987-1989.
Jarret RL, Gawel NJ, Wittermore A 1992. RFLP- based phylogeny of *Musa* species in Papua New Guinea. Theor. Appl. Genet. 84:579-584.
Jenny C, Carreel F (1997). Revision on banana taxonomy: 'KlueTiparot' (*Musa* spp.) reclassified as a triploid. Fruits. 52:83-91.
Kaemmer D, Afza R, Weising K, Kahl G, Novak FJ (1992). Oligonucleotide and amplification fingerprinting of wild species and cultivars of banana (*Musa* spp.).Biotec. 10:1030-1035.
Kaemmer D, Fischer D, Jarret RL, Baurens FC, Grapin A, Dambier D, Noyer JL, Lanaud C, Kahl G, Lagoda PJP (1997). Molecular breeding in the genus *Musa*: a strong case for STMS marker technology. Euphytica 96:49-63.
Kumar D, Ram RB, Kumar S, Maji S, Kumar M (2013). Variability and physio-chemical studies in snap melon (*Cucumismelo*var. *Momordica*). The Asian J. Hort. 8:751-753.
Lakshmanan V, Sreedhar RV, Bhagyalakshmi N (2007). Molecular analysis of genetic stability in long-term micropropagated shoots of banana using RAPD and ISSR markers. Elec. J. Biotechnol. 10:17-22.
Lanaud C, Tezenas du Montcel H, Jolivot MP, Glaszmann JC, Gonzalez De Leon D (1992). Variation in ribosomal gene spacer length among wild and cultivated bananas. Heredity 68:148-156.
Lanying Z, Yongqing W, Li Z (2008). Genetic diversity and relationship of Rhododendron species based on RAPD analysis.American-Eurasian J. Agric. Environ. Sci. 3:626-631.
Loh JP, Kiew K, Set O, Gan LH, Gan YY (2000). Amplified fragment length polymorphism fingerprinting of 16 banana cultivars (*Musa* cvs.). Mol. Phylogenet. 17:360-366.
Lynch M, Milligan BG (1994). Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3:91-99.
Mahalanobis PC (1936). On the generalized distance in statistics. Proceeding of National Institute of Sci. (India) 2:49-55.
Maji S, Das BC (2008). Quality improvement of guava: an organic approach. J. Asian Hort. 4(3):191-195.
Maji S (2013).Different Farming Options to Reduce Pesticide Load in Crop and Environment, in: Chattopadhyay, T., Bhowmik, B. (Eds.) Green Chemistry and sustainable Agriculture Practices: a Step towards a Better Future, E-Publishing Inc., Council for MS Academic pp. 45-65.
Nsabimana A, Van Staden J (2007). Assessment of genetic diversity of highland bananas from the National Banana Germplasm Collection at Rubona, Rwanda using RAPD markers.Sci. Hort. 113:293-299.
Onguso JK, Kahangi ME, Ndiritu WD, Mizutani F (2004). Genetic Characterization of cultivated bananas and plantains in Kenya by RAPD markers. Sci. Hort. 99:9-20.
Oselebe HO, Tenkouano A, Pillay M, Obi IU, Uguru MI (2006). Ploidy and genome segregation in *Musa* breeding populations assessed by flow cytometry and randomly amplified polymorphic DNA markers. J. Am. Soc. Hortic. Sci.131, 780-786.
Pillay M, Nwakanma DC, Tenkouano A (2000). Identification of RAPD markers linked to A and B genome sequences in *Musa* L. Genome. 43:763-767.
Rajasekar S, Fei S, Christians NE (2006). Analysis of genetic diversity in rough bluegrass determined by RAPD markers. Crop Sci. 46:162-167.
Rao CR (1952). Advanced Statistical Methods in Biometric Reaserch.John Willey and sons, New York.
Ray T, Dutta I, Saha P, Das S, Roy SC (2006). Genetic stability of three economically important micropropagated banana (*Musa* spp.) cultivars of lower Indo-Gangetic plains, as assessed by RAPD and ISSR markers.Plant Cell Tiss. Org. Cul. 85:11-18.
Robinson JC (1996). Bananas and Plantains.*Crop production Science in Horticulture*. 5- Willingford, U.K., CAB International.
Sedehi AAV, Solooki M, Arzani A, Ghanbari A, Lotfi A, Lmamjomeh AA, Bahrami S (2008). Comparative ananlysis of genetic diversity among grass pea landraces as detected by random, semi random and morphological markers. Asian J. Plant Sci. 7:454-460.
Simmonds NW, Shepherd K (1955). Taxonomy and origins of cultivated bananas. J. Linn. Soc. Bot. 55:302-312.
Singh P, Kumar S, Maji S, Singh A (2013). Genetic variability, heritability and genetic advance in cauliflower (*Brassica oleracea* var. *botrytis* L.). Int. J. Plant Sci. 8:179-182.
Singh RK, Chowdhury BD (1985). Biometrical methods in quantitative genetic analysis.Kalyani Publishers pp. 224-252.
Soni S, Kumar S, Maji S (2013a). Correlation and path coefficient analysis studies for the yield and its traits in cabbage (*Brassica oleracea*var *Capitata* L.). Annals Hort. 6:331-336.
Soni S, Kumar S, Maji S, Kumar A (2013b). Heritability and genetic advance in Cabbage (*Brassica oleracea*var. Capitata L.) under Lucknow condition.HortFlora Res. Spectrum 2:274-276.
Tiwari M, Singh NK, Rathore M, Kumar N (2005). RAPD markers in the analysis of genetic diversity among common bean germplasm from central Himalaya.Genet.Resour. Crop Evol. 52:315-324.
Toruan-Mathius N, Haris N (1999). Induction of genetic variation of banana cv. Nangka by gamma Co-60 irradiation and fusaric acids. Menara Perkebunan 67:13-22.
Um JY, Chung HS, Kim MS, Na HJ, Kwon HJ, Kim JJ, Lee SJ, Lim JP, Do KR, Hwang WJ, Lyu YS, An NH, Kim HM (2001). Molecular authentication of Panax ginseng species by RAPD analysis and PCR-RFLP. Biol. Pharmaceut. Bull. 24:872-875.
Uma S, Siva SA, Saraswathi MS, Manickavasagam M, Durai M, Durai P, Selvarajan R, Sathiamoorthy S (2006). Variation and intraspecific

relationships in Indian wild *Musa balbisiana* (BB) population as evidenced by random amplified polymorphic DNA. Genet. Resour. Crop Evol. 53:349-355.
Wang XQ, Zou YP, Zhang DM, Hong DY (1996). RAPD analysis for genetic polymorphisms in *Cathaya argyrophylla* Sci. China 26:437-441.
Welsh J, McClelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res.18:7213-7218.
Williams JGK, Hanafey MK, Rafalski JA, Tingey SV (1993). Genetic analysis using random amplified polymorphic DNA markers. Methods Enzymol. 218:704-740.
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers as useful genetic markers. Nucleic Acids Res.18:6531-6535.
Wong C, Kiew R, Loh JP, Gan LH, Set O, Lee SK, Lum S, Gan YY (2001). Genetic diversity of the wild banana *Musa acuminate* Colla in Malaysia as evidenced by AFLP. Ann. Bot. 88:1017-1025.

academicJournals

Vol. 13(5), pp. 258-271, 1 February, 2018
DOI: 10.5897/AJAR2015.9825
Article Number: 4A3EC8E55874
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Screening of wheat germplasm for seed associated fungi in geographical areas of Pakistan

Attiq-ur-Rahman[1], Riffat Tahira[2], Aqleem Abbas[3*], Shahid Iqbal[4], Israr Ahmad[1] and Waseem Ahmad[1]

[1]Department of Plant Breeding and Genetics, The University of Agriculture, Peshawar, Pakistan.
[2]Plant Genetic Resources Institute, National Agriculture Research Center, Islamabad, Pakistan.
[3]Department of Plant Pathology, The University of Agriculture, Peshawar, Pakistan.
[4]Department of Agronomy, Poonch Rawalkot University, Pakistan.

Received 18 April, 2015; Accepted 12 November, 2015

One hundred wheat (*Triticum aestivum* L.) accessions were selected on the basis of different geographical areas of Pakistan. Isolation and identification of seed born fungi were conducted according to standard blotter test and a total of five major seed borne fungi including *Alternaria alternata, Aspergillus niger*, *Fusarium* species, *Drechslera* species and *Phytophthora* species were isolated from the wheat seeds. The frequency of occurrence of these five seed born fungi was 49, 46, 42, 35, and 16%, respectively. Infection percentage varied from 0 to 90% in all 100 wheat accessions. Among the accessions, the highest infection (100%) of seed born fungi was recorded in 011185 and 011757 accessions while the lowest infection (10%) was recorded in 011415 accessions. Moreover, in accessions collected from Gilgit Baltistan and Azad Jammu Kashmir. *Alternaria niger* and *Alternaria fusarium* were dominant, while in Khyber Pakhtunkhawa province, *A. niger* was prevalent followed by *A. alternata.* In the case of Baluchistan province, the dominant seed born fungi was *A. alternata* followed by *Drechslera* spp. Similarly, in case of Punjab, the occurrence of *A. alternata, Drechslera* spp., *Fusarium* spp., and *A. niger* associated with seeds were similar. For accession collected from Sindh province, the dominant seed born fungi was *A. niger* and *Drechslera* spp. However, the *Phytophthora* spp. infection of wheat seeds accession of Baluchistan was the highest followed by wheat seeds accession collected from Gilgit Baltistan and AJK, Kpk and Punjab, whereas wheat seeds accessions collected from Sindh province were found to be free from *Phytophthora* spp.

Key words: Bread wheat, screening, wheat germplasm, seed born fungi.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple foods among agricultural crops since it constitutes the basis for human nutrition and is of enormous economic importance worldwide. Wheat is used mainly for human consumption and supports nearly 35% of the world population (Schuster et al., 2009). It supplies a

*Corresponding author. E-mail: aqlpath@gmail.com.

large fraction of the dietary protein, total food supply. It is also a principal source of carbohydrates and proteins both for human beings and animals (Ali et al., 2013).

In Pakistan, wheat (*T. aestivum* L.) crop is considered as the best cereal since it ranks the first among the cultivated cereals in the country and occupies about 66% of the annual food crop area (Ansari et al., 2006). Wheat contributes 10.1% to the value added in agriculture and 2.2% to gross domestic product (GDP). Area under wheat increased to 8693 thousand hectares in 2012 to 2013, from 8650 thousand hectares showing an increase of 0.5% over last year's area. In Pakistan, the production of wheat crop stood at 24.2 million tonnes during 2012 to 2013, against the target of 25.5 million tonnes which is 5.1% decrease while an increase of 3.2% over the last year production of 23.5 million tonnes has been witnessed. The yield per hectare in 2012 to 2013 stood at 2787 (kg/ha) posted a positive growth of 2.7% as compared to negative 4.2% growth last year (PBS, 2013).

Wheat is stored for a period of time before it can be marketed or used as feed or seed. The length of time cereal can be safely stored will depend on the condition it was harvested and the type of storage facility being utilized. Conditioning of grain has the single purpose of preserving the quality of grain. Low moisture content and temperature have been shown to be essential for successful storage of grain for a long period of time (Chaudhary et al., 2000).

The quality seed of improved wheat varieties is also considered as most important input for obtaining optimum production. Only the good seed can give an economic benefit to the wheat grower. It can maintain the quality of production, which fetches higher value in the market. Therefore, availability of healthy and pure seed should be confirmed, otherwise most of the seed-borne diseases of wheat could become responsible for heavy losses (Marwat et al., 2002).

Wheat crop is subjected to a number of diseases, which reduces its overall production to a great extent, because wheat plants in all stages of growth and in all natural environments are subject to various mechanical, physiologic and biological stresses that interfere with their normal growth and development. Biotic hazards, insects, viruses, fungi, nematodes, bacteria and weeds are primary hazards to wheat production (Ahmad et al., 2003).

The actual number of wheat diseases is unknown, but nearly 200 have been reported from the wheat-growing countries in the world. Over 100 infectious diseases caused by pathogens and by weeds are transmissible from plant to plant. Amongst these, about 50 are generally seed-borne. In Pakistan, 50 diseases are reported to occur which have great financial repercussions (Iftikhar et al., 1991). The rusts are considered the most destructive, but the problem of seed-borne diseases is also of great importance and could not be neglected. Smut, bunt, blight and root rot are some important seed-borne diseases, which are perpetuated through seeds and cause considerable losses to crops under favorable conditions (Dawson and Bateman, 2001). Seed-borne diseases have been found to affect the growth and productivity of crop plants and especially seed-borne fungi are important from the economic point of view as they render losses in a number of ways. Numerous examples exist in agriculture literature for the international spread of plant diseases as a result of importation of seeds that were infected or contaminated with pathogens (Clear and Patrick, 1993). The study of seed-borne pathogens is necessary to determine seed health and to improve germination potential of seed which finally leads to increase of the crop production (Bishaw et al., 2013).

Fungi are the principal pathogen organisms associated with crop seeds. A complex of seed-borne fungi including genera of *Tiletia, Ustilago, Bipolaris, Fusarium, Alternaria, Drechslera, Stemphylium, Curvularia, Cladosporium, Rhizopus, Aspergillus* and *Penicillium* have been convincingly reported as the most frequent seed-borne fungi of wheat throughout the world (Kumar et al., 2008). The present study was conducted to isolate the wheat seed associated fungi from seeds collected from different geographical locations of Pakistan in order to know the importance of seed borne pathogens and their effects on wheat crop in these locations.

MATERIALS AND METHODS

Collection of seed samples

Wheat seeds (100 accessions) were obtained from the National Agriculture Research Center (NARC) gene bank, Islamabad. The accessions were selected on the basis of different geographical areas of Pakistan (Table 1a, b, c, d and e).

Isolation of seed associated fungi

For isolation of fungi associated with wheat seed, blotter test was used. Initially 90 mm size discs of blotting paper were moistened with autoclaved distilled water and placed at the bottom of 90 mm sterilized Petri plates. The seed were surface sterilized with 5% hypochlorite solution followed by a rinse with autoclaved water. Ten seeds of each wheat accession were placed at equal distance in separate Petri plates using a sterilized pair of forceps. The lids of the Petri plates ware held in place with parafilm. The plates were incubated at 27°C for a period of 5 to 7 days under 12 h alternating cycle of light and darkness. After the incubation period, fungi growing out from seeds were examined, identified and their percentage frequency (PF) and relative abundance of infected seeds were calculated by the following formula:

Frequency of occurrence (%) = No. of seeds on which a fungal species occurs/Total No. of seeds × 100

Purification of cultures

Pure cultures were obtained after repeated sub-culturing of fungi appearing on seeds on Potato Dextrose Agar (PDA) plates.

Table 1. Wheat accessions collected from Gilgit and Azad Kashmir.

S/N	Accession	District	Town
a. Gilgit and Azad Kashmir			
01	011451	Rawalakot	Ghel
02	011484	Gilgit	Jutial
03	011489	Gilgit	Hunza
04	011602	Gilgit	Gitch
05	011620	Gilgit	Ghizer
06	011768	Gilgit	Austor
07	011765	Skardu	Sarri
08	012104	Muzaffarabad	Kahala
09	012285	Diamer	Jal village
10	011443	Bagh	Bagh
11	011480	Skardu	Hussainabad
12	011813	Gilgit	Juglote
13	011797	Chilas	Governer farm
14	012271	Chilas	Chilas
15	011487	Rawalakot	Ghel
16	011485	Rawalakot	Palandari
17	011766	Skardu	Keris
18	011588	Baltistan	Sordas
19	025912	Gilgit	Sheesh kot
20	011619	Gilgit	Tero
b. Khyber Pakhtunkhwa			
01	012012	Abbottabad	Tarhanagala
02	012021	Mansehra	College Durai
03	012040	Sawat	Char bagh
04	012035	Sawat	Bihar
05	012065	Dir	Shingerdar
06	012066	Malakand	Batkhaila
07	012069	Mardan	Rashakai
08	012072	Nowsehra	Pubbai
09	012208	Chitral	Nohedes
10	012268	Kohistan	Dalowndassu
11	018926	Lakkimarwat	Basit sultan abad
12	018925	Tank	Patthan colony
13	018921	D.I.khan	Hathala
14	018934	Bannu	Domel
15	018936	Karak	Jahangiribanda
16	011513	Para chinar	Zaran
17	012231	Haripur	Hawalian
18	012244	Buner	Guswanda
19	018786	Mansehra	Battal
20	018898	Chitral	Ayun
c. Balochistan			
01	011542	Quetta	Baleli
02	011548	Pinhin	Gawal
03	011771	Chagai	Ahmad wal
04	011781	Kalat	Rodingo
05	011752	Khuzdar	Surgaz
06	011759	Kechb	Asiaabadtump

Table 1. Contd.

07	012151	Loralai	Loralai
08	012144	Ziarat	Zandra
09	012155	Bolan	Kalpor
10	013189	Qilasaifullah	Bakacheena
11	011200	Pishin	Barozai
12	011185	Kharan	Karan
13	011232	Sibi	Sabi
14	011266	Qilaabdullah	Kili haji babari
15	011285	Pinhin	Pinhin
16	011535	Quetta	Marghat
17	011521	Awaran	Jibri
18	011758	Kech	Churbak
19	011757	Panjgur	AandayDaz
20	012180	Chagai	Noshki
d. Sindh			
01	011452	Hydderabad	Sebhat
02	011524	Nawabshah	Near river bank
03	011525	Dadu	Sherm.dawtch
04	011526	Thatta	Haji qasim
05	018730	Shikarpur	Haji khan wastil
06	018728	Larkana	Shahdadkot
07	018731	Jacobabad	Goth ghulammuhammad
08	018712	Ghotki	Ubaro/chimni
09	018716	Sukkar	Jahan khan
10	011378	Hyderabad	Mori
11	011383	NaushahroFiro	Haji esaboughi
12	011386	Sukkar	Nasirabad
13	018726	Larkana	Channagoth
14	018715	Ghotki	Panuaqil
15	018717	Larkana	Geaja
16	018736	Jacobabad	Bakhshapur
17	018719	Larkana	Mauta
18	018713	Ghotki	Mir purmathel
19	018733	Jacobabad	Goth ghulam Muhammad
20	018714	Ghotki	Ghotki
e. Punjab			
01	010715	Islamabad	-
02	010734	Islamabad	-
03	010759	Islamabad	-
04	010947	Faisalabad	-
05	010982	Faisalabad	-
06	011491	Okara	RenalaKhurad
07	011784	Attock	FatehJhang
08	011786	Chakwal	Dhoklalkahn
09	012096	Rawalpindi	M. khan
10	018702	Mainwali	Shahazkhel
11	018711	Khushab	Sakesar
12	018900	Jhelum	Suhan
13	018904	Sheikhupura	Kotlakahlune
14	018908	Layyah	Islamabad walipul

Table 1. Contd.

15	018910	D.G Khan	Haibatkot
16	011415	Bhakkar	-
17	018911	D.G Khan	Dao Mor
18	018969	Chakwal	Mureed Air Base
19	018948	Attock	Chakki
20	018949	Attock	Thatti S.I wala

Figure 1. Appearance of seeds associated fungi on wheat seeds.

Identification of fungi

The pure cultures of fungi were identified on the basis of spore morphology and colony characteristics were examined using a stereo-binocular microscope (Barnett and Hunter, 1992).

RESULTS

Identification of seed born fungi associated with wheat seeds

After incubation, fungi infection appeared on the wheat seeds as shown in Figure 1. Pure culture of seed born fungi is as shown in Figures 3, 4, 5, 6 and 7.

Frequency of fungi in wheat seeds

The frequency of each fungus in wheat seeds is presented in Figure 2: *Alternaria alternata* (49%), *Aspergillus niger* (46%), *Fusarium* species (42%) *Drechslera* species (35%), and *Phytophthora* species (16%).

Isolation of fungi from wheat seeds

In total, five fungi including both saprophytic as well as pathogenic were isolated from wheat seeds (Table 2). Fungi isolated from wheat seeds were *A. alternata*, *A. niger, Fusarium* spp., *Drechslera* spp., and *Phytopthra* spp. Infection percentage varied from 0 to 90% in all the accessions tested with accessions no. 011185 and 011757 showing the highest 90% infection, three accessions numbers 011487, 011200 and 011232 showed 70% infection, 012285 showed 60% infection, two accessions numbers 011381 and 011565 showed 50% infection. Six accessions numbers 010947, 011443, 011758, 012069, 012144, and 011542 showed infection of 40%. Seventeen accessions 012065, 011619, 012104, 012180, 018714, 011521, 018731, 011378, 011561, 011588, 011781, 011771, 011752, 011602, 012271, 012268, and 012072 showed infection of 30%. Eighteen accessions 012040, 018715, 011620, 011489, 018925, 010982, 011386,012151, 011285, 018898, 010715, 011766, 012021, 011524, 018900, 012066, 011765, and 012036 showed infection of 20%. Eighteen accessions 018726, 011451, 011383, 018936, 018719, 018926, 012208, 018904, 018908, 011232, 018728, 018910, 018948, 012012, 011520, 011797, 011525, and 011415 showed 10% infection.

Occurrence of fungal species in relation to geographical areas of Pakistan

Table 3 reveals the occurrence of fungal species in wheat seed accessions in relation to different geographical

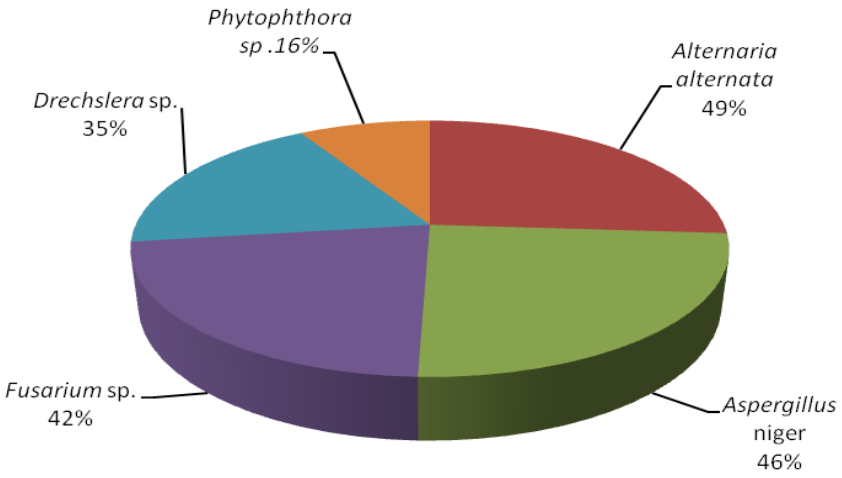

Figure 2. Frequency of occurrence of seed associated fungi isolated from wheat.

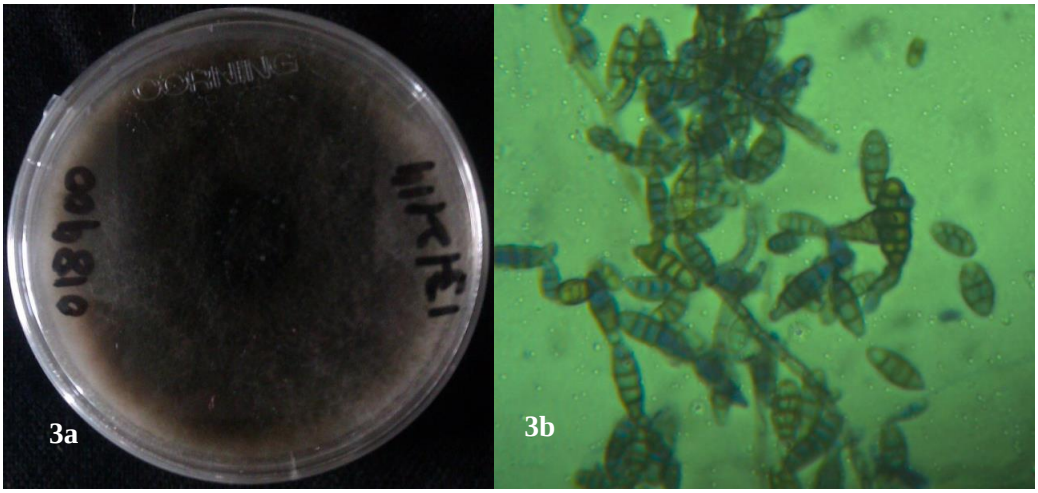

Figure 3. Morphological characteristics of *Alternaria alternate*: (a) Pure culture, (b) Microphotograph showing conidia at 40× magnification.

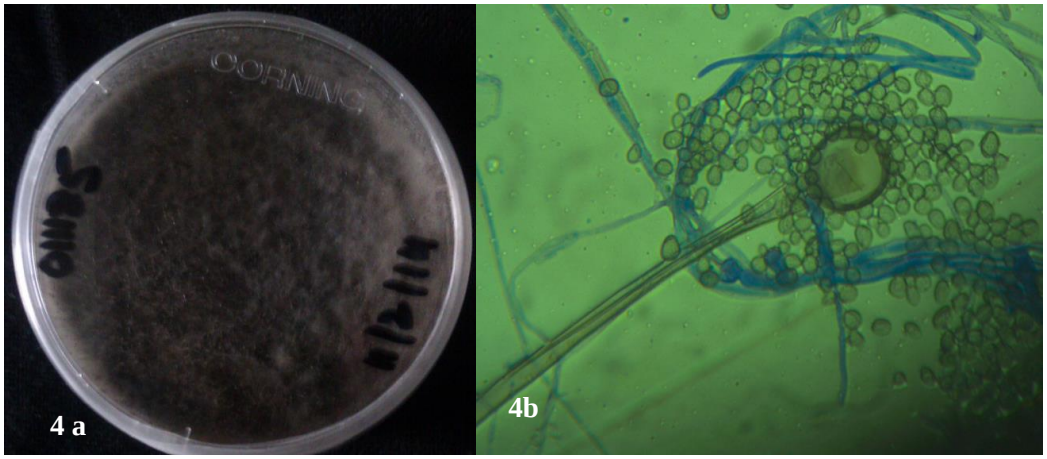

Figure 4. Morphological characteristics of *Aspergillus niger*: (A) Pure culture, (B) Microphotograph showing conidia at 40× magnification.

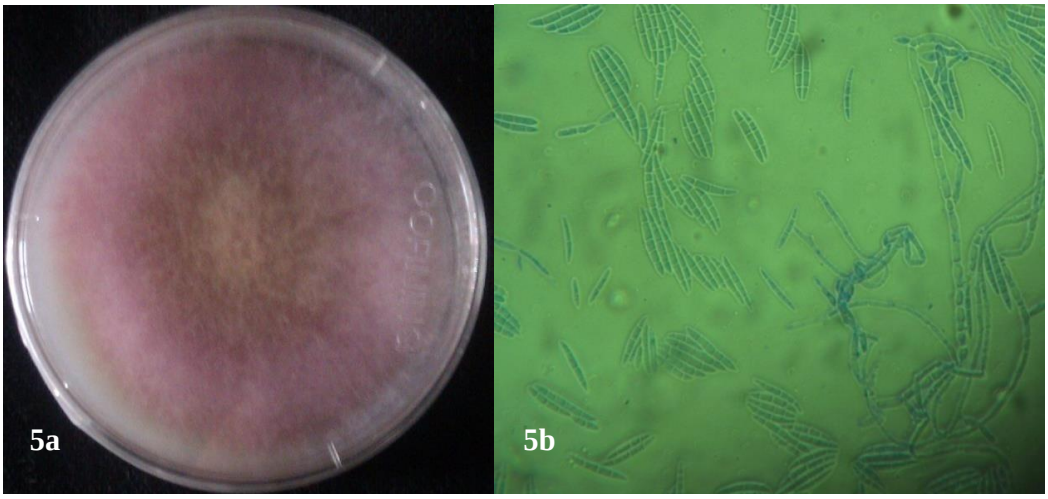

Figure 5. Morphological characteristics of *Fusarium* spp.: (a) Pure culture, (b) Microphotograph showing conidia at 40× magnification.

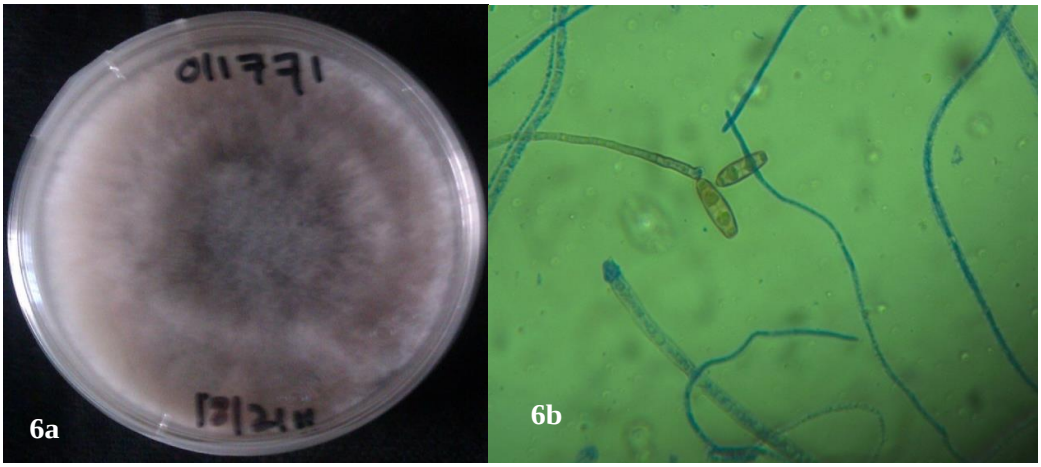

Figure 6. Morphological characteristics of *Drechlera* spp.: (a) Pure culture, (b) Microphotograph showing conidia at 40× magnification.

areas of Pakistan. In the case of Gilgit Baltistan and AJK, from a total amount of 200 tested seeds, 9 were found to be infected with *A. alternata,* 10 with *A. niger* and *Fusarium* spp., 9 with *Drechslera* spp., 5 with *Phytopthora* spp. whereas the remaining seeds were free from seed born fungi. In case of wheat accessions for kpk, out of 200 tested seeds, 7 seeds were found to be infected with *A. alternata,* 8 seeds were found to be infected with *A. niger*, 5 were found to be infected with *Fusarium* spp., 3 were found to be infected with *Drechslera* spp. and 2 were found to infected with *Phytopthora* spp. whereas the rest of the seeds were found to free from seed born fungi. In case wheat accession for Baluchistan province, out of total 200 tested seeds, 15 were found to be severely infected with *A. alternata*, 13 seeds were recorded to be infected with *A. niger,* 12 seeds were found to be infected with *Fusarium* spp., 14 seeds were found to be infected with *Drechslera* spp. and 6 seeds were infected with *Phytopthora* spp. In case of wheat accessions from seeds, out of total 200 tested seeds, 2 seeds were infected with *A. alternata*, 6 seeds were found to be infected with *A. niger,* 5 seeds were infected with *Fusarium* spp., 6 seeds were found to be infected with *Drechslera* spp. and no seeds were found to be infected with *Phytopthora* spp. whereas the rest of the seeds were free from seed born fungal infections. In case of wheat accession collected from Punjab province, out of 200 tested seeds, 3 seeds exhibited infection of *A. alternata,* 3 seeds were found to be infected with *A. niger,* 3 seeds

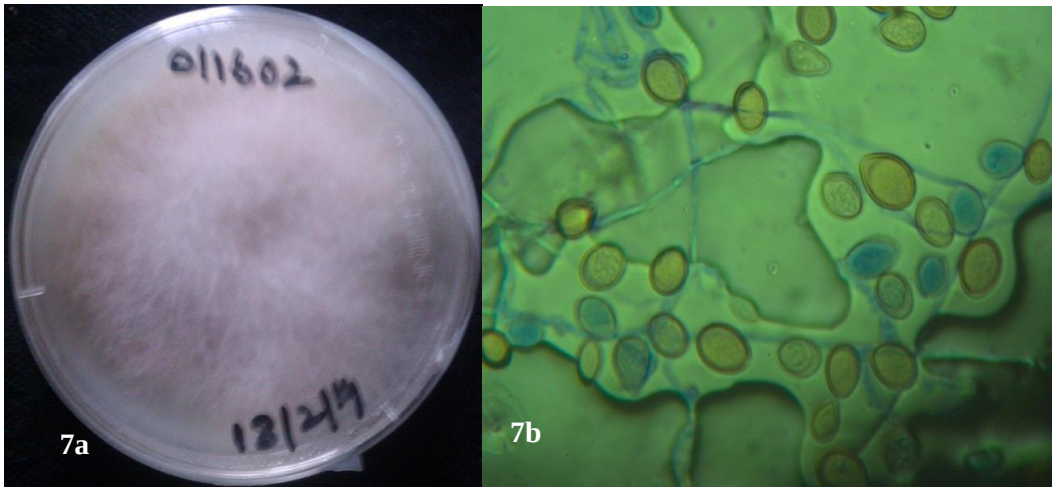

Figure 7. Morphological characteristics of *Phytopthora* spp.: (a) Pure culture, (b) Microphotograph showing conidia at 40X magnification.

Table 2. Frequency of occurrence of seed associated fungi from wheat.

S/N	Accession No.	*A. alternata*	*A. niger*	*Fusarium* spp.	*Drechslera* spp.	*Phypthera* spp.	Total	Frequency (%)
1	012096	-	-	-	-	-	-	-
2	010947	2	1	1	-	-	4	40
3	012040	1	1	-	-	-	2	20
4	011480	-	-	-	-	-	-	-
5	011487	-	2	3	1	1	7	70
6	012065	2	-	-	1	-	3	30
7	011443	1	1	2	-	-	4	40
8	018715	-	-	1	1	-	2	20
9	011185	2	2	3	1	1	9	90
10	011619	-	1	-	1	1	3	30
11	018786	-	-	-	-	-	-	-
12	011620	1	-	1	-	-	2	20
13	011489	-	1	-	-	1	2	20
14	018726	-	-	-	1	-	1	10
15	018713	-	-	-	-	-	-	-
16	018717	-	-	-	-	-	-	-
17	011451	-	1	-	-	-	1	10
18	010759	-	-	-	-	-	-	-
19	010734	1	-	-	-	-	-	-
20	011786	-	-	-	-	-	-	-
21	011758	1	-	2	1	-	4	40
22	018768	-	-	-	-	-	-	
23	012104	1	-	-	1	1	3	30
24	011383	-	1	-	-	-	1	10
25	011757	2	3	1	2	1	9	90
26	018730	-	-	-	-	-	-	-
27	012180	1	-	1	1	-	3	30
28	011200	2	1	1	2	1	7	70
29	018925	-	1	-	1	-	2	20
30	018736	-	-	-	-	-	-	-

Table 2. Contd.

31	012069	2	1	-	-	1	4	40
32	018714	-	1	1	1	-	3	30
33	018712	-	-	-	-	-	-	-
34	018936	-	-	1	-	-	1	10
35	011484	-	-	-	-	-	-	-
36	011521	-	1	1	-	1	3	30
37	010982	1	-	1	-	-	2	20
38	011381	1	2	1	-	1	5	50
39	018731	-	1	1	1	-	3	30
40	011759	-	-	-	-	-	-	-
41	011386	1	-	-	1	-	2	20
42	011378	-	2	-	1	-	3	30
43	011284	-	-	-	-	-	-	-
44	018719	-	-	1	-	-	1	10
45	018934	-	-	-	-	-	-	-
46	018926	1	-	-	-	-	1	10
47	018700	-	-	-	-	-	-	-
48	012151	-	1	-	1	-	2	20
49	011565	2	1	-	1	1	5	50
50	011561	1	-	2	1	-	3	30
51	018769	-	-	-	-	-	-	-
52	011588	-	1	1	1	-	3	30
53	011485	-	-	-	-	-	-	-
54	018921	-	-	-	-	-	-	-
55	011781	1	-	1	-	1	3	30
56	018733	-	-	-	-	-	-	-
57	011285	-	1	-	1	-	2	20
58	012144	2	-	1	-	1	4	40
59	018716	-	-	-	-	-	-	-
60	012155	-	-	-	-	-	-	-
61	011542	1	2	-	1	-	4	40
62	012208	-	-	1	-	-	1	10
63	011771	2	-	1	-	-	3	30
64	018728	-	-	-	-	-	-	-
65	011752	-	2	-	1	-	3	30
66	018969	-	-	-	-	-	-	-
67	018898	1	-	-	-	1	2	20
68	018904	-	1	-	-	-	1	10
69	011602	1	-	-	2	-	3	30
70	010715	-	1	-	1	-	2	20
71	018911	-	-	-	-	-	-	-
72	011491	-	-	-	-	-	-	-
73	018908	-	-	1	-	-	1	10
74	011784	-	-	-	-	-	-	-
75	011232	2	2	1	1	1	7	70
76	012244	-	1	-	-	-	1	10
77	018949	-	-	-	-	-	-	-
78	011766	1	-	1	-	-	2	20
79	012021	-	1	1	-	-	2	20
80	018728	-	-	-	1	-	1	10
81	011524	1	-	1	-	-	2	20
82	018910	-	-	-	1	-	1	10

Table 2. Contd.

83	012285	2	2	1	-	1	6	60
84	012271	1	1	-	1	-	3	30
85	018948	-	1	-	-	-	1	10
86	012268	2	-	1	-	-	3	30
87	018711	-	-	-	-	-	-	-
88	018900	1	-	1	-	-	2	20
89	012012	-	1	-	-	-	1	10
90	018702	-	-	-	-	-	-	-
91	011513	-	-	-	-	-	-	-
92	012072	1	1	-	1	-	3	30
93	012066	-	1	1	-	-	2	20
94	011520	-	-	-	1	-	1	10
95	011813	-	-	-	-	-	-	-
96	011797	-	-	1	-	-	1	10
97	011525	1	-	-	-	-	1	10
98	011415	-	1	-	-	-	1	10
99	011765	1	-	-	1	-	2	20
100	011768	-	-	-	-	-	-	-
101	012036	1	-	1	-	-	2	20
Total		49	46	42	35	16	-	-

Table 3. Occurrence of fungal species in relation to geographical areas of Pakistan.

S/N	Accession	District	*A. alternata*	*A. niger*	*Fusarium* spp.	*Drechslera* spp.	*Phytopthora* spp.
Gilgit Baltistan and AJK							
01	011451	Rawalakot	-	1	-	-	-
02	011484	Gilgit	-	-	-	-	-
03	011489	Gilgit	-	1	-	-	-
04	011602	Gilgit	1	-	-	2	-
05	011620	Gilgit	1	-	1	-	-
06	011768	Gilgit	-	-	-	-	-
07	011765	Skardu	1	-	-	1	-
08	012104	Muzaffarabad	1	-	-	1	1
09	012285	Diamer	2	2	1	-	1
10	011443	Bagh	1	1	2	1	1
11	011480	Skardu	-	-	-	-	-
12	011813	Gilgit	-	-	-	-	-
13	011797	Chilas	-	-	1	-	-
14	012271	Chilas	1	1	-	1	-
15	011487	Rawalakot	-	2	3	1	1
16	011485	Rawalakot	-	-	-	-	-
17	011766	Skardu	1	-	1	-	-
18	011588	Baltistan	-	1	1	1	-
19	025912	Gilgit					
20	011619	Gilgit	-	1	-	1	1
Total tested seeds = 200			9	10	10	9	5
Khyber Pakhtunkhwa							
01	012012	Abbottabad	-	1	-	-	-

Table 3. Contd.

02	012021	Mansehra	-	1	1	-	-
03	012040	Sawat	1	1	-	-	-
04	012035	Sawat	-	-	-	-	-
05	012065	Dir	1	-	-	1	-
06	012066	Malakand	-	1	1	-	-
07	012069	Mardan	1	1	-	-	1
08	012072	Nowsehra	1	1	-	1	-
09	012208	Chitral	-	-	1	-	-
10	012268	Kohistan	1	-	1	-	-
11	018926	Lakkimarwat	1	-	-	-	-
12	018925	Tank	-	1	-	1	-
13	018921	D.I.khan	-	-	-	-	-
14	018934	Bannu	-	-	-	-	-
15	018936	Karak	-	-	1	-	-
16	011513	Para chinar	-	-	-	-	-
17	012231	Haripur	-	-	-	-	-
18	012244	Buner	-	1	-	-	-
19	018786	Mansehra	-	-	-	-	-
20	018898	Chitral	1	-	-	-	1
	Total tested seeds=200		7	8	5	3	2
Balochistan							
01	011542	Quetta	1	1	-	1	1
02	011548	Pinhin	-	-	-	-	-
03	011771	Chagai	2	-	1	-	-
04	011781	Kalat	1	-	1	1	-
05	011752	Khuzdar	-	1	-	1	-
06	011759	Kechb	-	-	-	-	-
07	012151	Loralai	-	1	-	1	-
08	012144	Ziarat	1	-	1	1	-
09	012155	Bolan	-	-	-	-	-
10	013189	Qilasaifullah	-	-	-	-	-
11	011200	Pishin	2	1	1	2	1
12	011185	Kharan	2	2	3	1	1
13	011232	Sibi	2	2	1	1	1
14	011266	Qilaabdullah	-	-	-	-	-
15	011285	Pinhin	-	1	-	1	-
16	011535	Quetta	-	-	-	-	-
17	011521	Awaran	-	1	1	-	1
18	011758	Kech	1	-	1	1	-
19	011757	Panjgur	2	3	1	2	1
20	012180	Chagai	1	-	1	1	-
	Total tested seeds= 200		15	13	12	14	6
Sindh							
01	011452	Hydderabad	-	-	-	-	-
02	011524	Nawabshah	-	1	1	-	-
03	011525	Dadu	1	-	-	-	-
04	011526	Thatta	-	-	-	-	-
05	018730	Shikarpur	-	-	-	-	-
06	018728	Larkana	-	-	-	-	-
07	018731	Jacobabad	-	1	1	1	-

Table 3. Contd.

08	018712	Ghotki	-	-	-	-	-
09	018716	Sukkar	-	-	-	-	-
10	011378	Hyderabad	-	2	-	1	-
11	011383	NaushahroFiro	-	1	-	-	-
12	011386	Sukkar	1	-	-	1	-
13	018726	Larkana	-	-	-	1	-
14	018715	Ghotki	-	-	1	1	-
15	018717	Larkana	-	-	-	-	-
16	018736	Jacobabad	-	-	-	-	-
17	018719	Larkana	-	-	1	-	-
18	018713	Ghotki	-	-	-	-	-
19	018733	Jacobabad	-	-	-	-	-
20	018714	Ghotki	-	1	1	1	-
	Total tested seeds= 200		2	6	5	6	0
Punjab							
01	010715	Islamabad	-	1	-	1	-
02	010734	Islamabad	-	-	-	-	-
03	010759	Islamabad	-	-	-	-	-
04	010947	Faisalabad	-	-	-	-	-
05	010982	Faisalabad	1	-	1	-	-
06	011491	Okara	-	-	-	-	-
07	011784	Attock	-	-	-	-	-
08	011786	Chakwal	-	-	-	-	-
09	012096	Rawalpindi	-	-	-	-	-
10	018702	Mainwali	-	-	-	-	-
11	018711	Khushab	-	-	-	-	-
12	018900	Jhelum	1	-	1	-	-
13	018904	Sheikhupura	1	-	-	1	1
14	018908	Layyah	-	-	1	-	-
15	018910	D.G Khan	-	-	-	1	-
16	011415	Bhakkar	-	1	-	-	-
17	018911	D.G Khan	-	-	-	-	-
18	018969	Chakwal	-	-	-	-	-
19	018948	Attock	-	1	-	-	-
20	018949	Attock	-	-	-	-	-
Total tested seeds = 200			**3**	**3**	**3**	**3**	**1**

were found to be infected with *Fusarium* spp., 3 were infected with *Drechslera* spp. and 1 was found to be infected with *Phytophthora.*

Identification of fungi

The isolated fungi were identified on the basis of spore morphology and colony characteristics. Some features on the basis of which fungi were identified are as follows:

A. alternata

The fungus *A. alternata* was identified as it produced woolly or powdery chains of dark brown conidia of uneven shapes and lengths. The colony colour was dark brown (Figure 3a). The mycelium was abundant and variable in colour, usually light olive green to dark brown. Hyphae were thick, septate, dark brown and branched, conodiophores were erect and simple with septate conidia (Figure 3b).

A. niger (van Tieghem)

Colony of *A. niger* on seed grew slowly, consisting of a compact to fairly loose white to faintly yellow basal mycelium, which bears abundant erect and usually

crowded conidial structures (Figure 4a). Conidiophores arise directly from the seeds coat and are smooth, hyaline or faintly brownish near the apex (Figure 4b).

Fusarium spp. *(Sacc)*

Fusarium spp. had a rapid growth on Potato dextrose agar PDA. The texture of colony was flat to wooly and pink in colour (Figure 5a). Conidia were 2 or more celled, curved, thick-walled, smooth, and canoe-shaped (Figure 5b)

Drechslera halodes (Ito) and Phytopthora spp.

Colony on PDA was dark brown (Figure 6a). Conidiophores were thick, septate, cylindrical, and paler toward the apex and were simple. Conidia were straight and slightly curved and thick walled (Figure 6b) and *Phytopthora* (Figure 7a and b).

DISCUSSION

Seed borne fungal pathogens transmit most of the major disease of wheat crop and reduce seed quality, nutrient contents, germination capacity as well as seedling collapse, which consequently reduce crop yield (Mushtaq and Hashmi, 2005). Over the last two decades, various studies have been carried out to identify seed born fungal pathogen of wheat crop throughout the world. For example in Canada, 35 fungal genera and 59 seed born fungi exist in association with wheat seeds. From Pakistan, Khan (1992) reported 17 genera and 45 species of seed born fungal pathogens associated with wheat seeds. In this study, the results show that a total of 5 major fungal pathogen including *A. alternate*, *A. niger*, *Fusarium* spp., *Drechslera* spp. and *Phytophthora* spp. were identified and isolated from the seeds of wheat crop. Zare et al. (2006) who determined the fungi species and infection rates as 15% *Fusarium culmorum,* 13.1% *Fusarium graminearum,* 4.5% *Drechslera* spp*.,* 24.2% *A. alternate* and 5% *A. niger* in harvested wheat loads in different provinces of Iran. In the present study, the lowest infection rate of seed to *Pytophthora* was determined in seed accession collected from Sindh province and the highest infection rate was reported from Baluchistan province. The results also show that infection percentage of five major seed borne pathogens varied from 0 to 90% in all accessions collected from different geographical areas of Pakistan. Moreover, the frequencies of five mentioned major fungus were higher in wheat seed accessions collected from Baluchistan and Gilgit Baltistan and AJK as compared to other geographical regions of Pakistan. It was found that among the seed born fungi frequency, *A. alternata* in all accessions was the highest as compared to other fungi associated with wheat seeds. Rajput et al. (2005) tested on hundred and twenty sample of wheat seeds for the presence of fungal seed borne pathogens collected from wheat growing of Sindh. From twelve wheat varieties five seed borne fungi that are *A. niger, Alternaria tenuis, Fusarium moniliformie, Stemphylium herbarum* and *Curvuluria lunata* were isolated. Same experiment is performed by Babadost (1997) who detected some species of *Fusarium* fungus in wheat seeds collected from cereal fields in the North West of Iran. In the results, *A. alternata* was the most frequent fungi associated with the wheat seeds. The presence of weakly pathogenic or saprophytic fungi such as *Helminthosporium, Curvularia, Stemphylium, Rhizopus, Cladosporium, Aspergillus, Penicillium, Alternaria, Gonatobotrys* and *Nigrospora* has been reported from wheat seeds (Habib et al., 2011). Saberi et al. (2004) also isolated seed-borne fungi (*Aspergillus* species, *Alternaria* spp., *Cladosporium* species, *Penicillium* species and *Ulocladium* spp.) associated with wheat grains in Markazi province. Hussain et al. (2013) reported *Bipolaris sorokiniana* (11.125%), *Aspergillus flavus* (9.825%), *A. alternata* (7.15%) and *A. niger* (6.225%) associated with wheat seeds. It is apparent from the present research that all the accessions of wheat crop tested were contaminated by fungi. Knowing the major contribution of wheat crop in world food, its production must be enhanced to meet the nutritional requirements of a rising human populations (Oerke and Dehne, 2004). Certified and healthy seeds of wheat crop are significant input for crop production and consequently reduction of yield loss caused by these seed born fungi and is a main way to contribute to the food security in the world. Moreover, seed born fungi can be easily controlled through treatment of seeds using fungicides and biological compounds. Further, using standard storage facilities for preserving wheat seeds and developing resistant germplasm to reduce the infection level caused by these seed-borne fungi below damage threshold have been recommended (Clark et al., 2004).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmad J, Marwat MI, Ahmad HK (2003). Effect of herbicides and row spacing on different traits of wheat (*Triticum aestivum* L.). Pakistan J. Weed Sci. Res. 9(1-2):33-40.

Ali S, Shumaila U, Zahida N, Naseem Z, Saima N, Ammara Y, Tehseen Y (2013). Nutritional evaluation and stabilization studies of wheat germaplasm. Pak. J. Food sci. 23:148-152.

Ansari MA, Meman HR, Tunio SD, Keerio SA (2006). Effect of planting pattern on growth and yield of wheat. Pak. J. Agric. Agril. Eng. Vet. Sci. 22(2):22-24.

Babadost M (1997). Species of Fusarium in seeds and wheat plants in West Azarbayjan and Ardabil. Iran. J. Plant Pathol. 31:88-100.

Bishaw Z, Struik PC, Van Gastel AJG (2013). Farmer's seed sources

and seed quality: 2. Seed health. Int. J. Plant Prod. 7:637-657.
Chaudhary MA, Ali A, Siddique MA, Sohail R (2000). Growth and yield response of wheat to different seed rates and wild oat (*Avena fatua*) competition durations. Pak. J. Agric. Sci. 37:152-154.
Clark B, Cockerell V, Thomas J (2004). Wheat seed health and seedborne diseases-A guide. HGCA, Caledonia House, London.
Clear RM, Patrick SK (1993). Prevalence of some seedborne fungi on soft white winter wheat seed from Ontario, Canada. Can. Plant Dis. Surv. 73:143-149.
Dawson, WAJM, Bateman GI (2001). Fungal communities on roots of wheat and barley and effect on seed treatment containing fluquiconazol applied to control take-all. Plant Pathol. 50:5-82.
Habib A, Sahi ST, Javed N, Ahmad S (2011). Prevalence of seed-borne fungi on wheat during storage and its impact on seed germination. Pak. J. Phytopathol. 23(1):42-47.
Hussain M, Muhammad UG, Muhammad IH, Raza M (2013). Seed borne mycoflora of some commercial wheat cultivars in Punjab, Pakistan. Int. J. Pathol. 2:2.
Iftikhar, S, Ahmad I, Aslam M (1991). Seed born diseases of wheat in Pakistan and their control. Pak. Agric. Res. Council Islamabad 11(6):14-18.
Khan SAJ (1992). Studies on fungi causing seed-borne diseases of wheat and rice and their control. PhD dissertation, University of Karachi, Karachi, Pakistan. Ph.D. Thesis, Department of Botany/ University of Karachi, Pakistan 261pp.
Kumar A, Singh US, Kumar J, Garg GK (2008). Application of molecular and immune diagnostic tools for detection, surveillance and quarantine regulation of Karnal bunt (*Tilletia indica*) of wheat. Food Agric. Immunol. 19:293-311.
Marwat MI, Ahmad HK, Khan HH, Khan A (2002). Integrated weed management in wheat. 1. Weed density, dry weed biomass, absolute growth rate and grain yield. J. Weed Sci. Res. 8(1-2):81-93.
Mushtaq SNM, Hashmi MH (2005). Seed-borne mycoflora of Sunflower (*Helianthus annuus* L.). Pak. J. Bot. 37(2):451-457.
Oerke EC, Dehne HW (2004). Safeguarding production-losses in major crops and the role of crop protection. Crop Prot. 23:275-285.
PBS (2013). Pakistan Bureau of statistics, Ministry of National Food Security and Research (Economic survey of Pakistan, report).
Rajput MA, Pathan MA, Lodhi AM, Shah GS, Khanzada KA (2005). Studies on seed-borne fungi of wheat in sindh province and their effect on seed germination. Pak. J. Bot. 37(1):181-185.
Saberi R, Javan-Nikkhah M, Heidarian R, Hosseni S, Soleimani P (2004). Detection of fungal infectious agent of wheat grains in storepits of Markazi province. Iran. Agric. Appl. Biol. Sci. 67:418-423.
Schuster I, Vieira ESN, da Silva GC, de Assis Franco F, Marchioro VS (2009). Genetic variability in Brazilian wheat cultivars assessed by microsatellite markers. Genet. Mol. Biol. 32(3):557-563.
Zare L, Harsini M, Hosseini-bai S, Motieshare B (2006). Contamination of seed harvested wheat loads to a number of seed-borne fungal pathogen. In Proceeding 17th Iranian Plant Protection Congress. P 24.

academicJournals

Vol. 13(5), pp. 272-280, 1 February, 2018
DOI: 10.5897/AJAR2017.12896
Article Number: 08563B955883
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Gas exchange and yield of Prata-type banana plants with fertilizer sources for organic management

Pedro Ricardo Rocha Marques[1*], Sergio Luiz Rodrigues Donato[1], Abel Rebouças São José[2] and Raul Castro Carriello Rosa[3]

[1]Instituto Federal de Educação Ciência e Tecnologia Baiano, Campus Guanambi, Caixa Postal 009, Distrito de Ceraima, CEP 46430‑000 Guanambi, Bahia, Brazil.
[2]Universidade Estadual do Sudoeste da Bahia, Campus Vitória da Conquista, DFZ, Estrada do Bem Querer, km 4, Caixa Postal 95, CEP 45083-900, Vitória da Conquista, Bahia, Brazil.
[3]Embrapa Agrobiologia, Rodovia BR 465, km 7, Ecologia, CEP 23891-000. Seropédica, RJ. Brazil.

Received 20 November, 2017; Accepted 4 January, 2018

The aim of this paper is to evaluate gas exchanges and yield of Prata-type banana plants subjected to fertilizer sources for organic management in soil with improved fertility. Two cultivars ('Dwarf-Prata' and 'BRS Platina'), 5 K_2O rates (0, 200, 400, 600, and 800 kg ha^{-1} $year^{-1}$) supplied by cattle manure and Naturalplus® rock powder, and 2 evaluation times (8:00 am and 2:00 pm) were arranged in a randomized complete block design with 15 replicates. As for production, a randomized block design with 4 cycles and 3 replicates was used. In fertile soils, gas exchanges are little influenced by fertilization. Fertilization with increasing application rates determines a quadratic variation in stomatal conductance and internal CO_2 concentration in 'Dwarf-Prata' and a linear variation for leaf temperature and instantaneous water-use efficiency in 'BRS Platina'. Quantum efficiency of photosynthesis is higher at 8:00 am, whereas at 2:00 pm, for leaf temperature. Photosynthesis and leaf transpiration directly correlate with stomatal conductance, instantaneous water-use efficiency, and leaf temperature, while instantaneous water-use efficiency and photosynthesis inversely associate with transpiration and leaf temperature. Cattle manure and rock powder fertilizations do not increase yield in banana plants grown in soils with improved fertility.

Key words: *Musa* species, manure, rock powder, productivity, physiological variables.

INTRODUCTION

Bananas are grown in a variety of climatic zones, such as the semiarid tropics and under different abiotic stresses, which limit their production (Donato et al., 2016). Improving production under these conditions requires, besides plant breeding, studies on management practices that enable higher resilience across the soil-water-plant-atmosphere interactions. Among these practices, natural and organic fertilizations increase

*Corresponding author. E-mail: pedro.marques@ifbaiano.edu.br.

diversity and biological activity and promote suppression of pathogens (Geense et al., 2015).

'BRS Platina', which is derived from 'Dwarf Prata' through hybridization, exhibits comparative advantages, such as resistance to Yellow Sigatoka Leaf Spot and Panama Disease, as well as high-quality fruits that are very similar to those of its genitor. According to Silva et al. (2011), a safe recommendation of a new cultivar requires its evaluation in different environments, regions, and repetitions of production cycles, aiming at the identification of cultivars, as it allows establishing a genotypic variation of physiologic responses under local conditions (Turner et al., 2007). These variations allow extrapolation of these results to be used in the management of specific production systems and to properly manage cultivars under similar conditions (Arantes et al., 2016).

Rock powder or rock dust, a low-cost fertilizer in which there are K, P, Ca, and other nutrients, is used to replenish or to fertilize several types of soils (Harley and Gilkes, 2000). It slowly releases nutrients into the soil, particularly in soils with high pH; furthermore, its use in combination with cattle manure, and other organic materials, enhances its efficiency (Osterroht, 2003).

Various studies on organic fertilization in banana plants (Damatto Júnior et al., 2011; Moniem et al., 2008; Ribeiro et al., 2013) verified the possibility of using these sources; however, studies on physiological characteristics in fertilizer-related trials are needed as the aforementioned studies are predominantly on Cavendish cultivars (Robinson and Gálan Saúco, 2012) even though there are some available results for Prata-type cultivars (Arantes et al., 2016), where cultivars and irrigation systems were studied.

Fertilizations that are based on organic materials might enable nutritional improvements in banana plants. Therefore, this paper aimed to evaluate gas exchanges and yield of 'Dwarf-Prata' and 'BRS Platina' banana plants subjected to fertilizer sources for organic management in soils with improved fertility.

MATERIALS AND METHODS

The experiment was carried out in area at Federal Institute of Bahia (IF Baiano), campus Guanambi, BA. Originally, the soil was classified as a typical dystrophic yellow-red Latosol (Oxysol), weak A horizon, medium texture, located at latitude 14°17'27''S, longitude 42°46'53''W, altitude of 537 m, average annual rainfall and temperature of 680 mm and 26°C, respectively (Aw climate-Köppen), whose weather variables recorded over the experimental period are as shown in Figure 1.

The experiment was established with micro-propagated plantlets in a spacing of 2.5 × 2.0 m with crop practices consonant with the recommendations of Rodrigues et al. (2015). The area was sub-soiled, plowed, harrowed, furrowed, and the fertilizers were incorporated into the planting hole, according to the treatment.

The irrigation method was micro-sprinkler with Netafim^{TM} emitters (pressure compensating model, Netafim Israel, KibutzHatzerim, Israel), 130 L h^{-1} flow rate, wetted diameter of 7.4 m, with both laterals and emitters spaced out in 5 m apart. The irrigations were performed based on the crop evapotranspiration, through to the product of the reference evapotranspiration (ETo), calculated by the modified Penman-Monteith method, and the crop coefficient, which varied with the phenological stage in the first cycle and became a fixed value of 1.4 from the flowering, in accordance with Coelho et al. (2012).

The treatments were 2 cultivars ('Dwarf-Prata' and 'BRS Platina'), 5 K_2O rates (0, 200, 400, 600, and 800 kg ha^{-1} $year^{-1}$) supplied by cattle manure and Naturalplus®rock powder, and 2 times for gas exchanges evaluation (8:00 am and 2:00 pm) were arranged in a randomized complete block design, in a 2×5×2 factorial experiment, with 15 replicates. As for production, a randomized block design was used, with 4 production cycles, in a 2×5×4 factorial experiment and 3 replicates. The experimental plots consisted of 20 plants, from which the 6 plants in the middle of the plot were the measurement plants. Before the onset of the experiment, soil samples were collected from each experimental block, whose chemical attributes exhibit high fertility (Table 1) due to man-made modifications.

In average, the manure used, on a dry basis (65°C), contained 16.72% of moisture, 63.73 g kg^{-1} of organic matter, and the following macronutrients (g kg^{-1}): Ca = 1.7; Mg = 0.2; K = 2.5; N = 5.2; S = 2.3 (EPA 3051 / APHA 3120B) and P (APHA 4500-PC); and micronutrients (mg kg^{-1}): B = 2.1; Cu = 45.2; Zn = 200.5; Mn = 391.8; and Fe = 1.932.4 (EPA 3051 / APHA 3120B). pH was 7.42 (Official Method, OM), and the density, 0.38 g cm^{-3}. The Naturalplus®rock powder (natural fertilizer), which was from a soil in Ipirá-Bahia State, Brazil, produced by Terra Produtiva Mineradora Ltd., contains 30.0 g kg^{-1} of K_2O (total), 10.0 g kg^{-1} of P_2O_5, 52.0 g kg^{-1} of CaO, 30.0 g kg^{-1} of MgO, 63.0 g kg^{-1} of Fe_2O_3, 1.5 g kg^{-1} of MnO, 630 g kg^{-1} of SiO_2, 69 mg kg^{-1} of Zn (ICP95A – Lithium metaborate fusion – ICP OES), 127 mg kg^{-1} of Cu and 5 mg kg^{-1} of OM (IMS95A – Lithium metaborate fusion – ICP MS).

The rates were set on the basis of the maximum recommendation for N found in the literature (Souto et al., 1997), 700 kg ha^{-1} $year^{-1}$ of N. From this rate, 5 rates were defined, with an interval of 175 kg ha^{-1} $year^{-1}$ from one rate to another, down to a fertilizer rate of 0 (700, 525, 350, 175, and 0 kg ha^{-1} $year^{-1}$ of N). The maximum K_2O rate was fixed at 800 kg ha^{-1} $year^{-1}$ with intervals of 200 kg ha^{-1} $year^{-1}$ from one rate to another (800, 600, 400, 200, and 0 kg ha^{-1} $year^{-1}$ of K_2O). the N/K_2O ratio was fixed at 1.7/1. The manure rate (160 Mg $ha^{-1}year^{-1}$) was defined to meet the demand of 700 kg ha^{-1} $year^{-1}$ of N. The amount of K_2O (405 kg ha^{-1} $year^{-1}$) was calculated on the basis of the manure rate. Based on the K_2O content in the rock powder, the rock powder rate was calculated (13 Mg ha^{-1} $year^{-1}$) to supply 395 kg ha^{-1} $year^{-1}$ of K_2O to complement the requirement of 800 kg ha^{-1} $year^{-1}$ of K_2O. All fertilizer rates were split into 6 applications, every 60 days, distributed to 2,000 plants ha^{-1}. Additionally, 10 g of zinc sulfate and 10 g of boric acid were applied to each mother plant/sucker via rhizome (Rodrigues et al., 2015). Concerning the second cycle, copper sulfate (3 g/mother plant-sucker) split into 3 rates, and 30 g of magnesium sulfate.

Always, 3rd or 4th leaf was measured, in case the 3rd leaf is of difficult access or damaged by the wind, from tip to base (Arantes et al., 2016), the incident radiation on leaf (O_{leaf}) expressed in µmol; leaf temperature (T_{leaf}), °C; internal CO_2 concentration (C_i), µmol CO_2 mol^{-1}, stomatal conductance (g_s), mol H_2O $m^{-2}s^{-1}$, transpiration (E), mmol H_2O $m^{-2}s^{-1}$, net photosynthesis (A), µmol CO_2 $m^{-2}s^{-1}$, instantaneous water-use efficiency (A/E), µmol CO_2 $m^{-2}s^{-1}$/mmol H_2O $m^{-2}s^{-1}$, carboxylation efficiency (A/C_i), quantum efficiency or photochemistry of photosynthesis (A/Q_{leaf}), µmol CO_2 $m^{-2}s^{-1}$/µmol photons $m^{-2}s^{-1}$. These measurements were performed by anLcpro+® Portable Photosynthesis System (ADC BioScientific Limited, UK) infrared gas analyzer (IRGA), always with the radiation shield facing the sun, with ambient temperature and irradiance, and airflow of 200 ml min^{-1}.

For the statistical analysis, the following procedure was adopted;

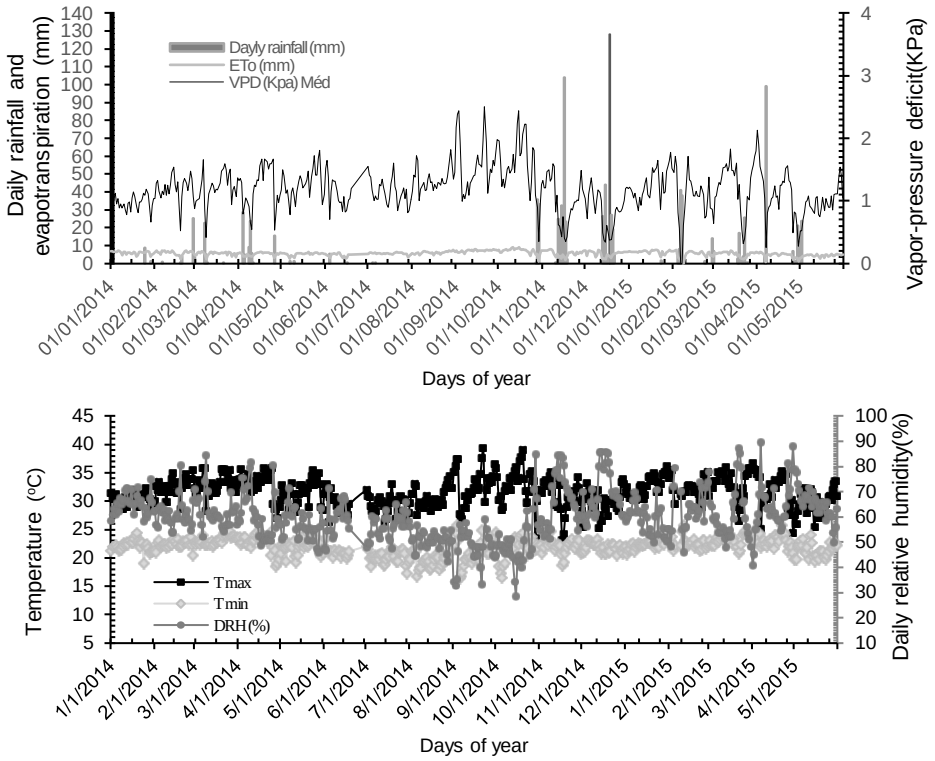

Figure 1. A: Rainfall (mm), reference evapotranspiration (ETo-mm), and vapor-pressure deficit (VPD-Kpa); B: Maximum and minimum temperatures (°C) and relative humidity (%) recorded at a weather station located at IF Baiano campus Guanambi in the period from January, 2014 to May, 2015.

for physiological characteristics, a 2×5×2 factorial design was used; 2 cultivars, 5 cattle manure rates + rock powder, and 2 times for gas exchange readings, with 15 replicates (evaluation season was: from January, 2014-flowering of 2nd cycle, 510 days after transplanting to May, 2015-flowering of the 4th cycle, 900 days after transplanting: periods of higher physiologic maturity and production); as for yield of the bunches and hands, a 2×5×4 factorial design, with 4 production cycles. The data were subjected to analysis of variance and proceeded to split the interactions according to their significance. For the interactions and independent effect of K_2O rates, regression analysis was performed. In the absence of interactions, the means for cultivars and times were compared to one another by the omnibus F test. As for cycles, Tukey's test was done at 5% of significance level. Moreover, correlation studies were done across the variables of gas exchanges.

RESULTS AND DISCUSSION

Gas exchanges of the 3rd leaf of Prata-type banana plants had three-way interactions, considering cultivar, fertilizer rate, and time with stomatal conductance (Figure 2), and two-way interactions, considering cultivar and fertilizer rate with leaf temperature, instantaneous water-use efficiency, and transpiration (Figure 2). Leaf temperature, transpiration, and quantum efficiency of the photosynthesis in Prata-type banana plants varied with evaluation time, regardless of the cultivar or fertilizer rate (Table 2). The associations between gas exchanges in Prata-type banana plants were moderated and positive for $A \times g_s$ and $A \times A/E$e, and moderated and negative for $A \times T_{leaf}$ (Figure 4). The correlations $A/E \times T_{leaf}$, $A/E \times E$, and $E \times T_{leaf}$ were of high magnitude and negative, medium magnitude and negative, and medium magnitude and positive, respectively; and are as shown in Figure 4. The bunch and hand weights interacted with cultivar and fertilizer rate and varied with evaluation season in an independent manner.

Few physiological variables were influenced by the treatments. This is probably due to the high soil fertility at the beginning of the experiment, whose average P and K contents before planting were 468.33 and 493 mg dm^{-3}, respectively. Also, doing the physiological readings at specific times contributed to this outcome as these readings vary with the atmospheric conditions and soil moisture at the time of measurement, which might not reflect the conditions imposed by the treatments (Santos et al., 2013; Arantes et al., 2016). The 'Dwarf-Prata' cultivar, at 8:00 am exhibited a decreasing quadratic behavior for g_s rates (Figure 2). The model estimates a minimum value of 0.59 mol H_2O $m^{-2}s^{-1}$ for g_s when 401.13 kg $ha^{-1}year^{-1}$ is applied and rates of 0.78 and 0.80 mmol

Table 1. Chemical attributes of the soil from the blocks (B1, B2, and B3), before planting, at the depths of 0-20 and 20-40 cm.

Dep.	Chemical composition																				
	pH[1]	OM[2]	P[3]	K[3]	Na[3]	Ca[4]	Mg[4]	Al[4]	H+Al[5]	SB	t	T	V	m	B[6]	Cu[3]	Fe[3]	Mn[3]	Zn[3]	S[7]	Prem[8]	EC
Cm	dag kg^{-1}		mg dm^{-3}			….....cmol$_c$dm^{-}.....							..%...		..... mg dm^{3}							
BL1,0-20	7.2	1.2	463.7	439	0.1	4.3	1.8	0.0	0.8	7.4	7.4	8.1	91	0	0.7	2.1	19.4	47.7	42.4	-	44.7	1.3
BL2, 0-20	7.6	1.5	502.6	520	0.1	5.1	1.6	0.0	0.8	8.1	8.1	8.9	91	0	1.2	2.0	18.0	46.7	51.8	-	43.3	1.5
BL3, 0-20	7.5	1.0	438.7	520	0.1	4.3	1.6	0.0	0.8	7.4	7.4	8.1	91	0	0.9	2.6	29.4	45.1	28.3	-	42.8	1.6
BL1, 20-40	7.2	0.2	233.4	359	0.1	3.3	1.3	0.0	0.8	5.6	5.6	6.4	88	0	1.0	1.1	25.6	28.3	9.5	-	43.8	1.1
BL2, 20-40	7.4	0.2	294.3	439	0.1	3.9	1.0	0.0	0.8	6.2	6.2	6.9	89	0	0.9	1.3	19.9	26.5	10.7	-	43.6	1.4
BL3, 20-40	7.4	0.1	159.5	318	0.1	3.4	1.1	0.0	0.7	5.4	5.4	6.1	89	0	1.1	1.2	35.0	28.4	6.0	-	39.3	1.3

Dep.: Depth of the soil layer; BL1: block 1; BL2: block 2; BL3: block 3; [1]pH in water; [2]colorimetry; [3]Mehlich-1 extraction; [4]KCl 1 mol L^{-1}; [5]pH SMP; [6]$BaCl_2$ extractor; [7]Extractor: $Ca(H_2PO_4)_2$, 500 mg^{-1}L of P in HOAc 2 mol L^{-1}; [8]Equilibrium solution of P; OM, organic matter; SB, sum-of-bases; t, effective cation exchange capacity; T, cation exchange capacity at pH 7; V, base saturation; m, aluminum saturation; Prem, remaining phosphorus; EC, electric conductivity. Dag kg^{-1} = %; mg dm^{-1} = ppm; cmol dm^{-3} = meq 100cm^{-3}.

H_2O m^{-2}s^{-1} in the absence of fertilization and when the maximum fertilizer rate is applied, respectively. As for 2:00 pm, the behavior was inverse, rising at first, up to a maximum conductance of 0.57 mol H_2O m^{2}s^{1} when 315.47 kg ha^{-1}year^{-1} of K_2O is applied, followed by a decrease, down to 0.4206 mol H_2O m^{-2}s^{-1} for the maximum fertilizer rate. Regarding 'BRS Platina' cultivar, at the two reading times, the average of g_s rates were 0.64 and 0.52 mol H_2O m^{-2}s^{-1}, respectively, without adjusting the curve.

The amounts of N and K_2O supplied by cattle manure and rock powder contributed toward explaining the g_s rates at both reading times. The large quantity of nitrogen and potassium supplied by manure and rock powder could have promoted a better osmoregulation of the stomata opening of leaf cells. This improvement is due to a better cooling of the plant as organic compounds in the soil enhance the capacity of water storage in the soil and, consequently, a better diffusive flux of nutrients (Malavolta et al., 1997).

Melo et al. (2009), using N and K rates in fertigation, verified values of up to 0.51 mol H_2O m^{-2}s^{-1} of g_s for K_2O rates of 580 kg ha^{-1}year^{-1} and lower values when the amount of K_2O is reduced and N is increased. This evidences the importance of the correct calibration of N and K rates for stomatal osmoregulation of the banana plant and the excess of N and absence of K reduce g_s. Even though the N/K_2O ratio was fixed at 1.7/1 herein, the amount of N applied was much increased. Other factors, such as the increase in vapor-pressure deficit (VPD), have also an influence on the rates of g_s in leaves and, consequently, on the stomatal opening and closure, mainly in periods of higher stress, that is, 2:00 pm (Turner et al., 2007; Mahouachi, 2009; Dzomeku et al., 2016).

Under harsher conditions, the stomata close because of the uptake of K by the guard cells, which is induced by the production of ABA in the roots and, afterwards, by extruding malate by guard cells (Shimazaki et al., 2007). This is caused by the increase in VPD, which is a consequence of low relative humidity, irradiation, and high air temperature on the leaves (Ekanayake et al., 1994; Turner et al., 2007; Donato et al., 2016). Under seasons with more weather stress, such as September and October, the maximum ambient temperature over the period reached up to 39.2°C, relative humidity, from 30 to 35%, which characterizes a condition of high VDP, with recorded values above 2.5 kPa (Figure 2) and the incident radiation on the leaf reached up to 1.998 µmol photons m^{-2} s^{-1}.

These weather conditions are above what is considered to be optimum for the photosynthesis of the banana plant (Robison and Galán Saúco, 2012), and the decrease in photosynthetic rates might occur because of the stomatal closure or enzymatic problems, leading to decreases in carboxylation efficiency (Arantes et al., 2016). Conversely, under milder conditions, potassium accumulates in cell guards; as a result, their osmotic potential increases, and in addition to water, their turgidity is also increased, which favors the stomatal opening; therefore, the positive quadratic behavior at 8:00 am is

$$\hat{Y}(Dwarf - Prata - 8{:}00a.m) = 0.7842 - 0.0009436^{**}x + 0.0000011^{**}x^2, r^2 = 0.60$$
$$\hat{Y}(BRS\ Platina - 2{:}00p.m) = 0.64$$
$$\hat{Y}(Dwarf - Prata - 2{:}00p.m) = 0.5061 - 0.0003980x + 0.00000063^{**}x^2, r^2 = 0.63$$
$$\hat{Y}(BRS\ Platina - 2{:}00p.m) = 0.52$$

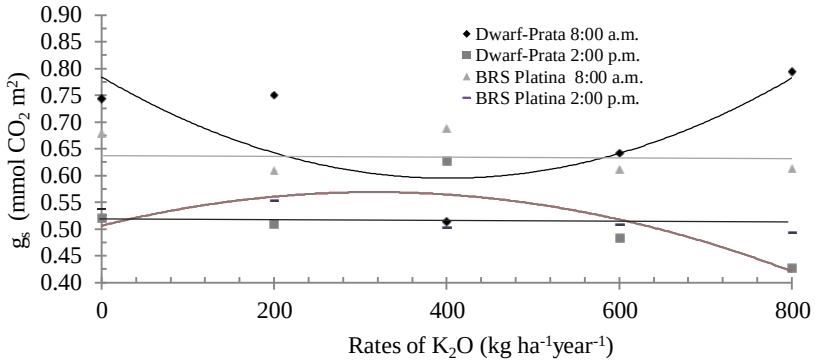

Figure 2. Stomatal conductance (g_s) at 8 am and at 2 pm in 'Dwarf-Prata' and 'BRS Platina' cultivars as a function of K_2O rates (kg $ha^{-1}year^{-1}$) supplied by cattle manure and rock powder. *Significant at 1%, by t test.

Table 2. Leaf temperature, transpiration, stomatal conductance, and quantum efficiency of photosynthesis measured at 8:00 a. m. and 2:00 p. m. on the third leaf of 'Dwarf-Prata' and 'BRS Platina' banana plants, fertilized with cattle manure and rock powder from January 2014 and May 2015.

Variable	8 am	2 pm	CV (%)
T_{Leaf} (°C)	32.34[b]	38.56[a]	7.18
E (mmol H_2O $m^{-2}s^{-1}$)	5.96[b]	8.77[a]	26.10
A/Q_{leaf} (µmol CO_2 $m^{-2}s^{-1}$/µmol photons $m^{-2}s^{-1}$)	0.0189[a]	0.0148[b]	58.08

Means followed by same letters, lowercase in the lines, do not differ from one another by Tukey's test at 5% of significance level.

explained. Leaf temperature (T_{leaf}) linearly increased ($p<0.05$) for 'BRS Platina' cultivar. There was an increase of 0.003043°C for each kg of K_2O applied, adding up to an increase of 8.1% for the maximum rate in comparison with no fertilized treatments. As for the 'Dwarf-Prata' cultivar, the model was not adjusted. The average T_{leaf} was 35.35°C (Figure 3 A). However, as the application of manure and rock powder maintained the soil moisture, as well as supplying K, which, together, helped in maintaining the stomatal activity for a longer time; thus, the cooling of leaves was favored, which decreased the possibility of thermal damage on the leaves.

Al-Busaidi (2015) verified that plants fertilized with cattle manure had lower electrolyte loss in leaf tissues as a function of the increase in temperature, which means that there were fewer changes than in conventional management; such fact can be attributed to the compost's capacity of supplying nutrients over a long time, such as Ca, important in cell membranes, and K, in the osmotic regulation. Besides, the osmotic regulation, K is associated with folding of the 2 halves of the lamina downwards; thus, reducing the stress caused by temperature and sunlight (Soto Ballestero, 2008). From a physiological standpoint, as leaf temperature increases, both instantaneous water-use efficiency and internal CO_2 concentration decrease. This is observed in Figure 3B and C, respectively. The air temperature (Figure 1), which also affects the T_{leaf} (Donato et al., 2017), could have affected the variables A/E and C_i; nevertheless, under these conditions, there would be a lower stomatal activity, which, perhaps, did not occurred right away because the average stomatal conductance was kept high (above 0.50 mol H_2O m^{-2} s^{-1}), as shown in Figure 2. The maintenance of the soil moisture, promoted by the crop residue left on the ground and by fertilizers applied every 60 days, might justify the high values of soil moisture.

The instantaneous water-use efficiency (A/E) of the 'BRS Platina' cultivar linearly decreased ($p<0.05$) as a function of K_2O rates supplied by cattle manure and rock powder. The decrease was of -0.00115 µmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1} for each kg of K_2O applied. As for 'Dwarf-Prata' cultivar, a model was not adjusted; its average was 3.57 µmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1} (Figure 3B). The increase in leaf temperature, as observed in Figure 3A, caused a decrease in A/E

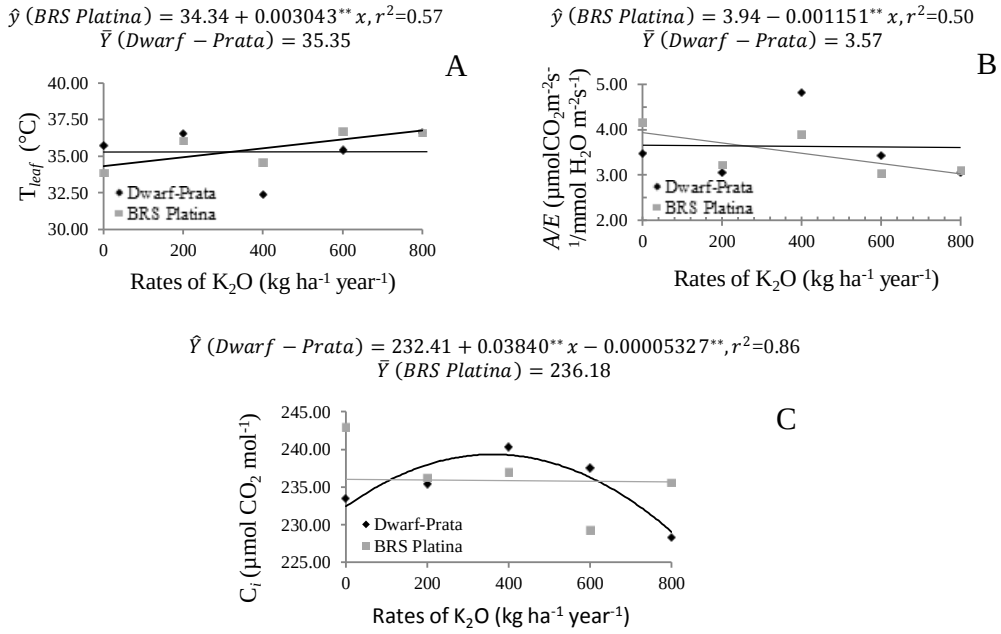

Figure 3. A: Leaf temperature (T_{leaf}), B: instantaneous water-use efficiency (*A/E*); and C: internal CO_2 concentration (C_i) of 'Dwarf-Prata' and 'BRS Platina' cultivars as a function of K_2O rates supplied by cattle manure and rock powder. *Significant at 5%, **Significant at 1%, by t-test.

(Donato et al., 2016), even when using a proper irrigation depth and a higher application of organic fertilizer. The fact that the experiment was conducted under semiarid conditions where the increases in temperature and background radiation are common and persistent in certain periods of the year, contributed much more to the lower *A/E* rates as carboxylation efficiency of rubisco decreases, which was a consequence of the increase in transpiration due to high temperatures (Arantes et al., 2016; Donato et al., 2017).

In banana orchards, where the management of crop residue in combination with the application of organic fertilizers contributes to higher rooting and enhanced chemical, physical, and biological attributes of the soil (Donato et al., 2016), there is an alleviation of conditions with excess of sunlight and high temperatures. As a consequence, the supply of K and N from manure and rock powder is essential as this supply is related to the water and osmotic regulation of the plant and protects against abiotic factors (Shimazaki et al., 2007). Melo et al. (2009) indicate that the increase in potassium fertilization provides the banana plant with lower N/K ratios and contributes to the maintenance of *A/E*. The internal CO_2 concentration (C_i) adjusted an increasing quadratic model for 'Dwarf-Prata' cultivar (Figure 3C) as a function of K_2O rates supplied by cattle manure and rock powder. A maximum C_i of 239.34 µmol CO_2 mol^{-1} was observed for an application of 360.42 kg ha^{-1} year^{-1}, and from this rate on, the C_i decreased. As for 'BRS Platina' cultivar, there was no adjustment to the curve with regard to the observed phenomenon; its internal CO_2 concentration was 236.18 µmol CO_2 mol^{-1}.

Melo et al. (2009) recorded increases in C_i as the stomatal opening rate increases. This is due to the fact that during gas exchange processes, stomata regulate the C_i and keep it relatively constant; during the stomatal closure process, the stomata restrain the uptake of CO_2 and, consequently, the photosynthetic activity (Farquhar and Sharkey, 1982).

The growing presence of N and K_2O as a function of fertilizer rates might have influenced the initial increase in C_i of 'Dwarf-Prata' cultivar, followed by a fall in the cultivars since these nutrients are related to the opening and closure of stomata. The rise in leaf temperature caused by the weather (Figure 1) could also have influenced the decrease in C_i since the T_{leaf} has influence on opening and closing stomata, on functioning of enzymes, and on transpiration (Turner et al., 2007; Donato et al., 2016).

The T_{leaf}, *E,* and A/Q_{leaf} rates differed from one another by the F-test ($p<0.05$) between 8:00 am and 2:00 pm, regardless of the cultivar and K_2O rates (Table 2). At 2:00 pm, the T_{leaf} and *E* rates were higher and the opposite effect occurred at 8:00 am for A/Q_{leaf} rates. The T_{leaf} and *E* exhibited, respectively, an increase of 16.13 and 32.34% between 8:00 am and 2 pm. Donato et al. (2016) and Arantes et al. (2016) observed higher rates for these same variables, at 2 pm as well. The increment in T_{leaf} is linked to the increase in ambient temperature between the period from 8:00 am to 2:00 pm, as ascertained for the 'Dwarf-Prata' clone called 'Gorutuba' (Donato et al., 2017). The temperature also influences the *E* as it is

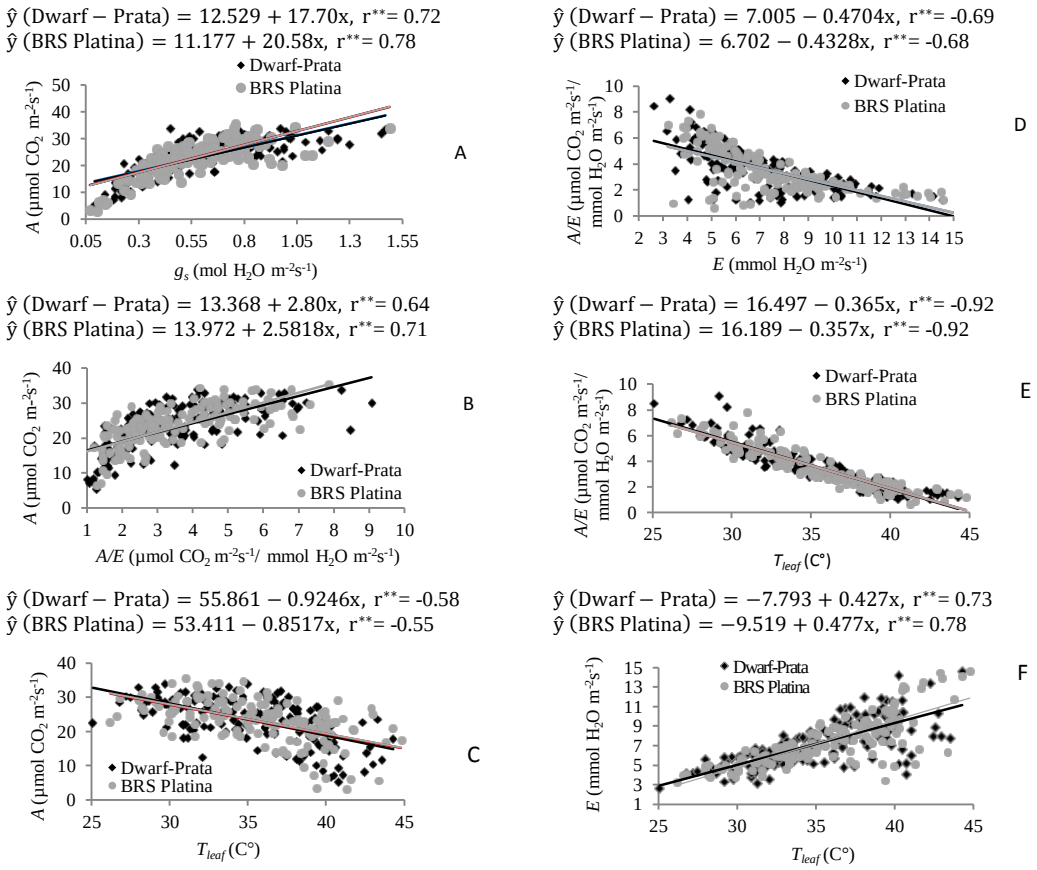

Figure 4. Correlations of photosynthesis (*A*) with stomatal conductance (g_s), water-use efficiency (*A/E*), and leaf temperature (T_{leaf}) (A, B, C); correlations of *A/E* with leaf transpiration rates (*E*) and T_{leaf} (D, E); and correlation between *E* and T_{leaf} (F) in 'Dwarf-Prata' and 'BRS Platina' cultivars of banana plants, from January, 2014 to March-May, 2015, fertilized with cattle manure and rock powder.

affected by the two temperatures (Donato et al., 2016). The reduction in quantum efficiency of the photosynthesis (Table 2) between the 2 times is because of the increase in vapor-deficit pressure in the afternoon and the change in the radiation quality, with wave lengths more suitable for photosynthesis at the beginning of the morning in comparison with the afternoon.

The VPD is influenced by the rise in ambient temperature and sunlight, and reduction in relative humidity, which naturally occur over the day and peak at 2 pm; this is verified by observing the other variables, T_{leaf} and *E* (Table 2). In addition to the VPD, the incident radiation on the leaf at 2 pm, in combination with the increase in leaf temperature, could have partially restrained the rubisco, which led to the decrease in CO_2 fixation, which culminated, therefore, in reducing the A/Q_{leaf}. There is a direct relationship between *A* and g_s (Figure 4A), whose correlation coefficients were 0.72 and 0.78 for 'Dwarf-Prata' and 'BRS Platina' and 0.64 and 0.71 for *A* and *A/E* (Figure 4B) for the two cultivars, respectively. Moreover, the correlation between *A* and T_{leaf} (Figure 4C) occurred; however, in an inverse way and of moderate magnitude, with coefficients of 0.58 and -0.55 for 'Dwarf-Prata' and 'BRS Platina' cultivars, respectively, which agrees with Arantes et al. (2016).

As for the correlation $A \times g_s$ of 'Dwarf-Prata' and 'BRS Platina', respectively, there are increases of 17.70 and 20.58 units in *A* for each increment in g_s. Similarly, for $A \times A/E$, the cultivars has increases of 2.80 and 2.58 units in *A* for each increment in *A/E*. As for the correlation $A \times T_{leaf}$, there were decreases of 0.9246 and 0.8517 units in *A* for each increment in T_{leaf}. Correlation studies, with inverse relationship, were also performed for $A/E \times E$ (Figure 4D), whose correlation coefficients were -0.69 and -0.68 for both cultivars; $A/E \times T_{leaf}$ (Figure 4E), whose correlation coefficients were -0.92 for both cultivars and direct association for $E \times T_{leaf}$ (Figure 4F) with coefficients of 0.73 and 0.78 for 'Dwarf-Prata' and 'BRS Platina', respectively.

As for the correlation $A/E \times E$, there are decreases of -0.4704 and -0.4328 units of *A/E* for each increment in *E* for 'Dwarf-Prata' and 'BRS Platina', respectively. As for $A/E \times T_{leaf}$, the cultivars had decreases of 0.3658 and 0.3574 units in *A/E* for each increment in T_{leaf}. In the

Table 3. Yield of bunches and hands assessed in Prata-type banana plants fertilized as a function of K_2O rates supplied by cattle manure and rock powder in Guanambi – BA.

Characteristic	Cycle I	Cycle II	Cycle III	Cycle IV	CV (%)
Bunch weight (t ha^{-1})	24.11^{C}	34.21^{B}	39.23^{A}	33.95^{B}	11.42
Hands weight (t ha^{-1})	21.31^{C}	30.38^{B}	35.48^{A}	30.39^{B}	11.98

Means followed by same letters, uppercase in the line, do not differ from one another by Tukey's test at 5% of significance level.

correlation $E \times T_{leaf}$, there were increases of 0.427 and 0.477 units in E for each increment in T_{leaf}. Figure 4D, E, and F demonstrates that the increasing T_{leaf}, which depends on the air temperature (Donato et al., 2016; 2017), limits the enzymatic activity of rubisco, which decreases the photosynthesis, though, the transpiration rate increases, leading to a reduction in *A/E*, even under favorable conditions of water availability and fertile soil (Donato et al., 2016). Arantes et al. (2016), who studied 'Dwarf-Prata', 'BRS Platina', and other cultivars, verified correlation between $A/E \times T_{leaf}$ and $E \times T_{leaf}$ that were similar to those found in this paper. There was a difference across means ($p<0.05$) for bunch and hand weights when the effect of the cycle in isolation was observed (Table 3). The 3rd cycle was the most productive, with mean value of 39.23 t ha^{-1} of bunches and 35.48 t ha^{-1} of hands. The yield averages are corrected for the real yield, which was obtained by multiplying the population effectively harvested (76%) from the density at planting due to losses caused by the wind or other factors that are not related to the treatments.

Donato et al., (2015) reported that well-managed banana orchards that were planted in soils with improved fertility exhibit yields above 40 t ha^{-1} cycle^{-1} (high yield > 32 t ha^{-1} cyle^{-1}). Silva and Simão (2015) recorded similar bunch weight when carrying out trials with K fertilization; though, with lower overall yield due to the lower population (1,235 plants ha^{-1}). Damatto Junior et al. (2011), with organic fertilization, reported an increase in bunch weight from the 1st to the 2nd cycle, with lower values than those herein, followed by a reduction in the 3rd cycle, reduction of which was claimed to be due to nutrient depletion of the soil.

Conclusions

Under high-fertility conditions, the gas exchanges in Prata-type banana plants are little influenced by the application of increasing K_2O rates supplied by cattle manure and rock powder. The application of increasing K_2O rates supplied by cattle manure and rock powder determines a quadratic variation in stomatal conductance (8 am and 2 pm) and internal CO_2 concentration in 'Dwarf-Prata' cultivar, and a linear variation for leaf temperature and instantaneous water-use efficiency in 'BRS Platina' cultivar. The quantum efficiency of photosynthesis is higher at 8 am, whereas the temperature and leaf transpiration are higher at 2 pm, regardless of the cultivar or fertilizer rate. The photosynthesis and leaf transpiration rates direct correlate with stomatal conductance, instantaneous water-use efficiency, and leaf temperature; whereas the instantaneous water-use efficiency and photosynthesis are inversely associated with the transpiration and leaf temperature. Organic fertilization with cattle manure and rock powder does not increase yield in Prata-type banana plants in soils with improved fertility.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Al-busaidi KTS (2015). Heat tolerance of Dwarf Cavendish banana (Musa AAA cv. Malindi) plants. Afr. J. Agric. Res. 10(14):1780-1784.

Arantes AM, Donato SLR, Siqueira DL, Amorim EP, Rodrigues Filho VA (2016). Chlorophyll index for real-time prediction of nutritional status of 'Prata' banana. Rev. Bras. Eng. Agríc. Ambient. 20:99-106.

Coelho EF, Donato SLR, Oliveira PM, Cruz AJS (2012). Relações hídricas II: evapotranspiração e coeficiente de cultura. In: COELHO, E. F. (Org.). Irrigação da bananeira. 1ª ed. v. único. Brasília: Embrapa pp. 85-117.

Damatto Junior ER, Villas Bôas RL, Leonel S, Nomura ES, Fuzitani EJ (2011). Crescimento e produção de bananeira prata-anã adubada com composto orgânico durante cinco safras. Rev. Bras. Frutic. pp. 713-721.

Donato SLR, Arantes AM, Rodrigues Filho VA, Rodrigues MGV, Rodrigues FE (2017). Aspectos de ecofisiologia e estratégias de manejo da bananeira. In: Fruticultura Tropical: diversificação e consolidação. Zucoloto M, Bonomo R. (org.). Alegre: Caufes pp. 57-73.

Donato SLR, Coelho EF, Marques PRR, Arantes AM (2016) . Considerações ecológicas, fisiológicas e de manejo. In: Ferreira CF, Silva SOE, Amorin EP, Santos-Serejo JA. Embrapa Mandioca E Fruticultura. O agronegócio da banana. Brasília, DF: Embrapa 03:45-110.

Donato SLR, Rodrigues MGV, Souza I (2015). Considerações sobre índices técnicos e econômicos de produção para a cultura da bananeira. Inform. Agropecu. 36(288):116-126.

Dzomeku BM, Sarkordie-Addo J, Darkey SK, Bam RKJ, Wünsche J, Staver C (2016). Responses of leaf stomatal parameters to induced water stress and its relationship with stomatal conductance in false horn plantain. Int. J. Plant Soil Sci. 12(2):1-14.

Ekanayake IJ, Ortiz R, Vuylsteke DR (1994). Influence of leaf age, soil moisture, VPD and time of day on leaf conductance of various Musa genotypes in a humid forest-moist savanna transition site. Ann. Bot. London 74:173-178.

Farquhar GD, Sharkey TD (1982). Stomatal conductance and photosynthesis. Ann. Rev. Plant Physiol. Palo Alto 33:317-345.
Geense P, Pattison AB, Kukulies TL, Scholberg JMS, Molina AB (2015). Can Changes in Soil Properties in Organic Banana Production Suppress Fusarium Wilt? Nat. Resour. 6:181-195.
Harley AD, Gilkes RJ (2000). Factors influencing the release of plant nutrient elements from silicate rock powders: a geological overview. Nutr. Cycl. Agroecosyst. 56:11-36.
Mahouachi J (2009). Changes in nutrient concentrations and leaf gas exchange parameters in banana plantlets under gradual oil moisture depletion. Scient. Hortic. Amsterdam 120(4):460-466.
Melo AS, Silva Júnior CD, Fernandes PD, Sobral LF, Brito MEB, Dantas JDM (2009). Alterações das características fisiológicas da bananeira sob condições de fertirrigação. Ciênc. Rural 39:3.
Moniem EAE, Abd-Allah ASE, Ahmed MA (2008). The Combined Effect of Some Organic Manures, Mineral N Fertilizers and Algal Cells Extract on Yield and Fruit Quality of Williams Banana Plants. Am. Eur. J. Agric. Environ. Sci. Dokki Giza Egypt 4(4):417-426.
Osterroht M (2013). Rochagem para quê? In: Oliveira JP. Rochagem-I: adubação com rochas silicatadas moídas. Agroecol. 3:12-15.
Ribeiro LR, Oliveira LM, Silva SOE, Borges AL (2013). Avaliação de cultivares de bananeira em sistema de cultivo convencional e orgânico. Rev. Bras. Frutic. 35(2):508-517.
Robinson JC, Gálan Saúco V (2012). Plátanos y bananos. 2nd ed. España: Ediciones Mundi-prensa 321p.
Rodrigues MGV, Donato SLR, Lichtemberg LA, Dias MSC (2015). Implantação e manejo do bananal. Inform. Agropecu. 36(288):27-44.
Santos MR, Martinez MA, Donato SLR (2013). Gas exchanges of Tommy Atkins mango trees under different irrigation treatments. Biosci. J. 29:1141-1153.
Shimazaki KI, Doi M, Assmann SM, Kinoshita T (2007). Light regulation of stomatal movement. Ann. Rev. Plant Biol. 58:219-247.
Silva JTA, Simão FR (2015). Produção, nutrição e incidência do mal do Panamá em bananeira 'Prata-Anã' adubada com potássio. Pesqui. Agropecu. Bras. 50:807-813.
Silva SO, Matos AP, Cordeiro ZJM, Lima MJC, Amorim EP (2011). Avaliação de genótipos tretraploides de bananeiras cultivadas em área infestada pelo agente causal do mal-do-panamá. Rev. Bras. Frutific. 33(1):137-143.
Soto Ballestero M (2008). Bananos: técnicas de producción, poscosecha y comercialización. In: Escuela de Agricultura de la Région Tropical Húmeda. Guácimo, Costa Rica.
Souto RF, Rodrigues MGV, Alvarenga CD, Silva JTA, Maeno P, Gonzaga V (1997). Sistema de Produção para a Cultura da Banana Prata-Anã no Norte de Minas. Belo Horizonte: EPAMIG. 32p.
Turner DW, Fortescue JA, Thomas DS (2007). Environmental physiology of the bananas (*Musa* spp.). Braz. J. Plant Physiol. Londrina 19:463-484.

African Journal of Agricultural Research

Related Journals Published by Academic Journals

- *African Journal of Environmental Science & Technology*
- *Biotechnology & Molecular Biology Reviews*
- *African Journal of Biochemistry Research*
- *African Journal of Microbiology Research*
- *African Journal of Pure & Applied Chemistry*
- *African Journal of Food Science*
- *African Journal of Biotechnology*
- *African Journal of Pharmacy & Pharmacology*
- *African Journal of Plant Science*
- *Journal of Medicinal Plant Research*
- *International Journal of Physical Sciences*
- *Scientific Research and Essays*

academicJournals