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African Journal of Agricultural Research

Full Length Research Paper

"Climate change perception and adaptation strategy associated with farming techniques in Tamou district wester Niger" farmers

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The variability of climate parameters in most of agricultural areas in Niger represents a major risk for farmers. This work is aimed at analyzing farmer's perception and adaptation to climate change parameters in Tamou district. The study was conducted on seventy three (73) millet farmers from seven villages in Tamou, namely Allambaré, Bani Guiti, Guieme, Tollondi , Moli Haoussa and Welgorou. The sample was random and tables were built from the results of a brief survey of millet farmers. The results obtained showed that farmers appreciate changes in climae parameters through rainfall (92.6%) while 7.4% do not perceive this parameter as factor, wind (88.9%), and temperature (85.2%). The adaptations techniques include organic and mineral fertilizers (87%) adjustment of farming to rainfall calendar (96%), using improved seeds varieties (75%), crop diversification (49%), water and soil preservation techniques (34%) and rural migration (3.8%). These adaptations are identified in the rural community of Tamou by several factors: degree of experience importance in terms of agricultural practices, the supervision of extension agents, the number of workers per household, the property rights and a good annual income. These adaptations and determining factors must be taken into account by all stakeholders in decision making in Niger's agricultural policy to guarantee food securityfarmers.

Key words: Millet, farmers, climate parameters, perception, adaptation, Tamou.

INTRODUCTION

In the history of humanity, the need to understand the impact of climate variability has never been so great as in the 21st century, especially in the tropics where deforestation and species extinction are relatively more

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important and living conditions more precarious (Bush and Flenley, 2007). Disasters caused by phenomena related to climate change and disruption has a great impact on agriculture in developing countries (Frank et al., 2003).

Climatic factors are affected by temperature, wind, rain, and drought that people feel comfortable or not comfortable in the area because the planning and management are not well. Some of the studies show that there is the range of bioclimatic comfort zone which people feel comfortable with. Drought evaluation is very important as well as climatic ranges. Drought assessments give people an active scenario in the city to protect the damaging socioeconomic and politic problems.

Recent studies with drought stress using remote sensing shows monitoring of drought stress. It is envisaged that both current land uses and future potential future use will be affected by the negative consequences of possible sea-level rise. The morphological structure of low elevations suggests that the impacts of elevation can easily proceed to the interior (Cetin, 2015a, b; Cetin and Sevik, 2016; Cetin, 2016; Cetin et al., 2018a, b; Yucedag et al., 2018). The fourth report of the Intergovernmental Panel on Climate Change (IPCC, 2007) announces that poor communities will be the most vulnerable because of their low adaptive capacity and high reliance on resources that are very sensitive to climate change such as water resources and agricultural production systems. These variability risks and climate change, notwithstanding their global impact, it is poor regions like Africa and particularly West Africa which will suffer the most from climate change because of their high vulnerability.

Indeed, in this region, the gradual change in temperature and rainfall and the frequency of extreme weather hazards are expected to lead to crop losses, livestock death and other of productive assets. Such losses will not only threaten food production, but also access, stabilize and utilization of food resources (Hamani, 2007). The rural sector (agriculture, livestock, forestry) is the bedrock and driving force of the economy of Sahelian countries in general and Niger republic in particular.

Unfortunately, in Niger, in the last 30 years, the main climate factors (water, sunshine, temperature and wind) are highly variable and unpredictable, so that today, farmers cannot accurately predict the evolution of their activities even for a near future. This situation weakens the production systems and makes agricultural activity uncertain (Oumarou et al., 2016).

In Niger, climate change has been the subject of several national report, policies, strategies, research papers, articles, dissertations and thesis (Hamani, 2007; Oumarou et al., 2016; CNEDD, 2011; AGRHYMET, 2012). The most of these documents have highlighted the trends and risks that represent this phenomenon for the development of agriculture in Sahel in general, and in Niger in particular. These documents also highlighted a significant gap in the knowledge of the relations of strategies perception and the adaptation to climate change developed by local actors. This study aims to fill the gap of the work of partners at the local level with specific case of Niger.

MATERIALS AND METHODS

Zone of the study

The study was carried out in Tamou district (Figure 1). The study area is between 12° 28 ' N and 12° 50' N and 2° 06 'and 2° 24' S. The population is estimated at 89,782 inhabitants out of which 51.16% are men and 48.84% are women (INS 2014). The main ethnic groups are Fulani and Foulmangani. In this district, 80 to 90% of arable lands are sandy soils conducive to millet cultivation, lowlands (for example, the river basin and its tributaries) rich in organic minerals essential to sorghum cultivation. We also meet some lateritic plateau and hardened soils that are undergoing restoration, and are being exploited for agriculture and animal husbandary. It is one of the most watered region in Niger (500 to 700 millimeter of annual rainfall). Agriculture and livestock are the main economic activities of these populations. The choice of this study area is based on the fact that it constitutes a buffer zone for the "W " National park of Niger (Figure 1).

Sampling

Observation units are farms represented by the farm manager. A farm is a production unit characterized by unique management, and owned means of production (Yegbemey et al., 2014). A sample of 73 farmers was randomly selected in 7 villages using the random number table constructed from the results of a summary census of millet farmers. Table 1 shows the constitution of the study samples.

Data collection

Data collection was carried out through surveys conducted in the form of semi-structured interviews. At the village level, two types of surveys were carried out: focus group interviews and individual interviews. Focus group interviews were conducted with a group of farmers (key informants from the village) in each village. Therefore, during the surveys, interviews on the farmers' perception, the evolution of the main climate parameters (rainfall, temperature, wind) and climate change (causes, manifestations and impactsimpacts) were organized with a group of farmersfarmers (all categories combined). Individual surveys were conducted with farmers separately. For this purpose, the main data collected include human and socio-economic factors, the perception of

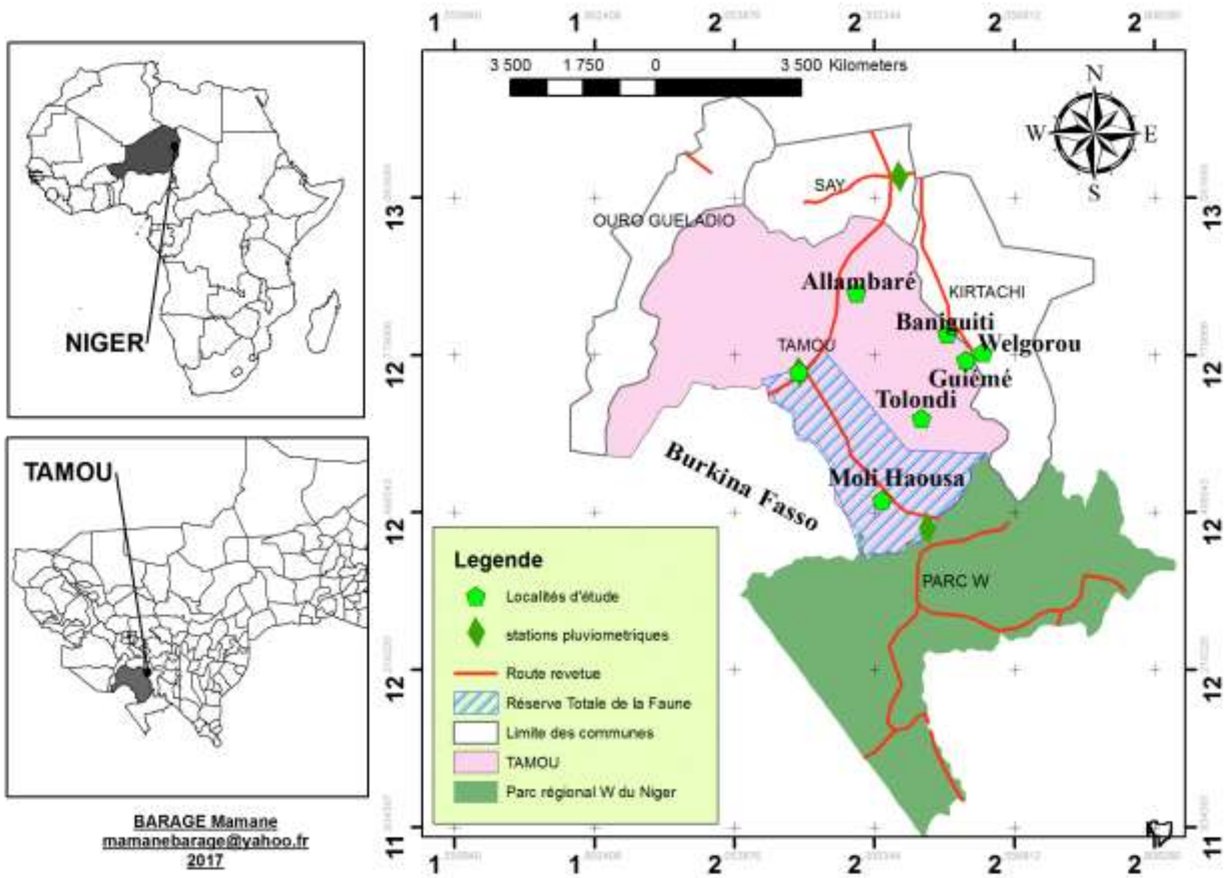

Figure 1. Presentation of the study area.

Table 1. Size of the study sample (field survey source).

Village	Registered farmers	Sample size	Sampling rate (%)
Tamou	158	15	10
Allambaré	148	14	10
Bani Guiti	95	11	10
Guiémé	91	9	10
Tollondi	122	12	10
Molli Haoussa	52	5	10
Welgorou	66	7	10
Total	**732**	**73**	**10**

changes in climate parameters, adaptation strategies, and their determinants. To evaluate data trend, the triangulation of information was done between data from focus groups and individual. For most part, the data from the focus groups made it possible to complete and deepen the data from the individual surveys. The farmers factors questions focused on sex, age, marital status, main and secondary activities, level of education, experience in agriculture, the farm size, mobilized means of production, number of available agricultural assets, average number of employees per day, cultivated area, level of income, and production of millet.

Data processing

On the basis of the response given, a farmer is considered to perceive climate change if and only if:

(1) He perceived at least one change of at least one climate parameter over the last ten years.
(2) He was able to identify the parameter (s) from which he perceived the change (s); and
(3) He was able to describe the change (s) he perceived.

In doing so, the variable "perception of climate change" was later treated as a dummy dichotomous variable considering the value of 1 if the farmer perceived climate change and the value 0 otherwise. Consequently, a farmer is considered to be adapting to climate change if and only if:

(1) He has adjusted his farming practices in order to adapt his production system to the previous change (s) he would have mentioned; and
(2) He has adopted at least one adaptation strategy. This variable was then treated as a dummy dichotomous variable taking the value of 1 if the farmer applies one of the strategies and the value 0 if otherwise.

Finally, an "external adaptation strategy consolidates the endogenous strategies" if and only if:

(1) The farmer has affirmed that it improves his technical performance of development and / or the diffusion of techniques adapted to the new conditions.
(2) It improves the profit of his activities, the complementarity and contribution in his initiatives
(3) It corrects the negative impacts felt in the conduct of the exploitation, and especially reinforces the profitability of the activities.

The collected data were processed using Microsoft EXCEL spreadsheet and statistical package for social sciences (SPSS) version 20.

RESULTS

Demographic and socio-economic factors of farmers

The demographic and socio-economic factors of farmers are summarized in Table 2. Ninety-six percent (96%) of the respondents are male, and 42% are illiterate. Most of those who have received formal education have a primary school certificate. For 94.3% of respondents, agriculture is the main activity and 5.7% are agro-pastoralists. In addition to agriculture, 49% of the farmers are engaged in a secondary activity between handicrafts, gathering, logging, livestock raising and petty trading. The organization of farmers of the study zone, especially the village development committees, was initiated by the projects and non-governmental organizations (NGOs) working in the area. These village structures promote access to inputs (fertilizers and cowpeas' pesticides), agricultural loans and training provided by extension agents in the locality to about 68% of farmers. Access to land is based on inheritance, purchase and, leasing. Access to land through these modes confers different property rights. For example, 86% of respondents said they own their land. The average number of farm workers per household is 4 and the experience in farming practices ranges from 12 to 60 years (Table 2).

Perception of changes in climatic parameters by farmers

The majority of farmers surveyed in the study area saw changes in climate parameters from 2004 to 2016. These changes mainly concern rainfall (92.6%), temperature (85.2%), wind frequency and duration (88.9%). These parameters are shown in Figure 2A. All farmers who have seen climate change have also experienced changes in rainfall (the decrease in rainfall amounts to 81%, and increasingly random and sporadic rainfall at 81% floods. Farmers then noted the seasonal shift with a tendency to reduce length of the rainy season (88%), a delay in the stabilization of the seasons (86%) and the increase frequency of floods and droughts (77%). These results revealed that rainfall trends affect the Western part of Niger and more specifically Tamou district. Concerning the temperature, 85.2% of respondents perceived thermal variations manifestation by the increament of the hot dry season (96%) or the increase in high temperature waves (88%). In addition, 96% of the farmers surveyed perceived other changes such as stronger winds (93%), dusty winds at the beginning of the raining season likely to cause damage to the seedlings, the eratic frequent strong winds likely to cause severe lodging of cereals during cultivation, etc. In addition, some farmers have noted changes such as the sharp decline of certain woody food plant species (*Parkia biglobosa*, *Adansonia digitata* and *Vitelaria paradoxa*) (Figure 2).

Perception of the causes and impacts of changing climate parameters

Perception of driven factors

According to interviewees, changes in climate parameters are linked to human factors (62%) and natural factors (100% of respondents). These perceptions

Table 2. Demographic and socio-economic factors of farmers.

Qualitative variable		Total	Percentage (%)	
Head of household	Man head of household	70	95.90	
	Female household head	3	4.10	
Marital status	Married	70	95.90	
	widow	3	4.10	
Level of education of the respondent	Literate	31	42.3	
	Koran	15	21.2	
	Primary	11	15.4	
	Secondary	4	5.8	
	Superior	10	13.5	
Main mode of access to the land	Heritage	70	95.90	
	Leasing	2	2.7	
	Purchase	1	1.3	
Annual income	50 000 à 100 000	42	57.7	
	200 000 à 500 000	31	42.3	
Main activities	Agriculture	69	94.3	
	Agro pastoralism	4	5.7	
Secondary activities	Craft	4	6.1	
	Cueillete	6	8.2	
	Wood exploitation	3	6.1	
	Other (small shops)	58	79.4	
Membership in an organization	Membre d'une organisation villageoise	68	93	
Contact with a technical office	-	37	50.6	
Quantitative variables	**Minimum**	**Maximum**	**Average**	**Standard deviation**
Age (year)	35	82	52	13
Household size	2	25	12	10
Area exploited (ha)	5	20	7	6
Annual millet production monetary value (thousand FCFA)	20	300	84	68
Experience in agricultural practices (year)	12	60	35	13
Number of farm assets	1	10	4	2

of the causes of changes in climate factors are recorded in Table 3.

Impacts perception of the impacts of changes in climate factors by farmers

Interviewees noted the degradation of soil quality (15%), loss of seedlings (30%), delay in crop growth (92%), production lost (37%) and emergence of new insects pests (14%) as the main immediate impacts of climate change. These different perceptions of farmers about the impacts of changes in climatic factors are shown in Table 4. The impacts of changes in climatic parameters were also seen through the analysis that growers make on their crop fields (Figure 3). For this purpose, they noted

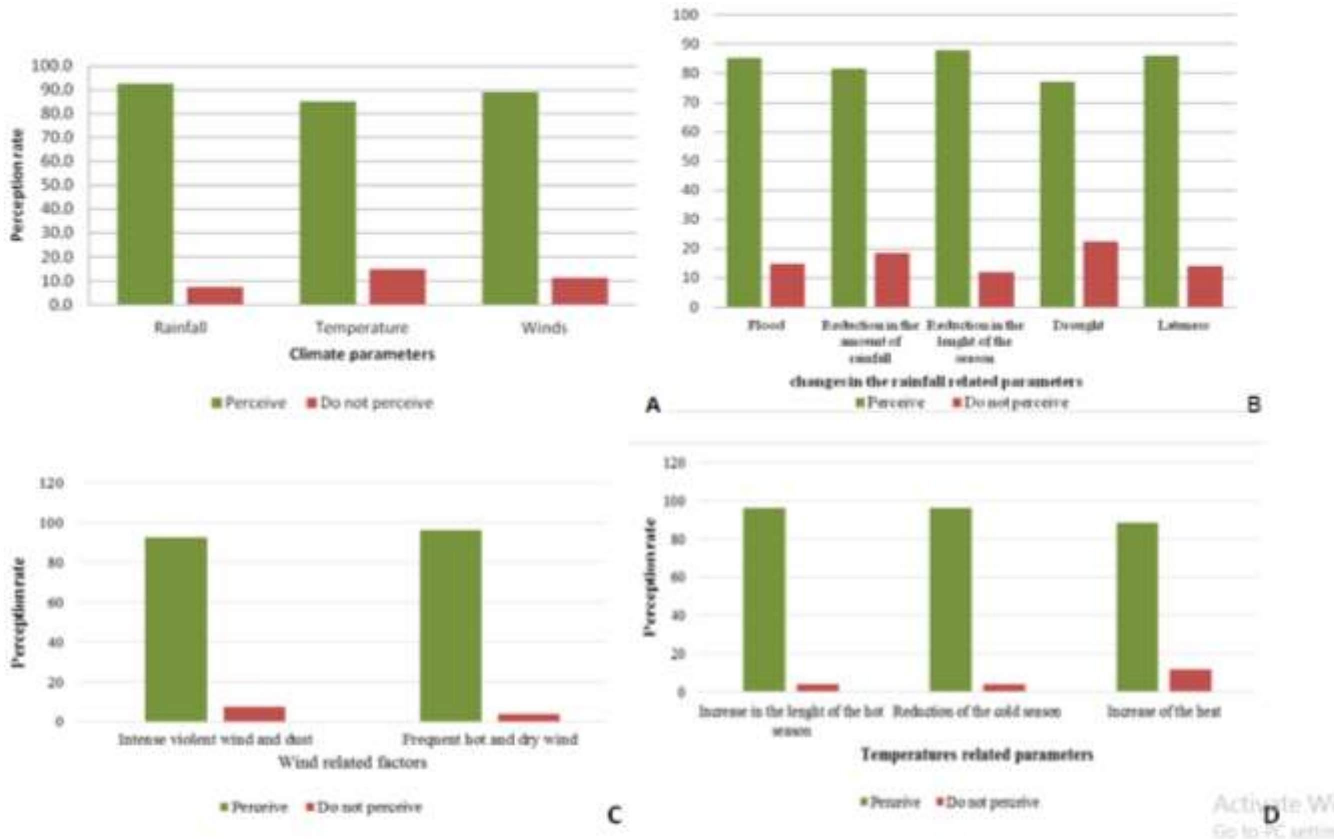

Figure 2. Perception of changes in climatic parameters by millet farmers.
(A) Change of climate parameters; (B) change in rainfall related parameters; (C) Change in wind related parameters; (D) Change in temperature related parameters.
Source: Field survey.

Table 3. Perception of the factors of changes in climateby farmers.

Type of factors	Perception rate (%)	Rate of non perception (%)
Human factors from the perspective of non-compliance with society's standards	62	38
Natural factors (related to climate change)	100	0

Table 4. Perception of the impacts of changes in climate factors by farmers.

Type of effect	Perception rate (%)	Rate of non-perception (%)
Degradation of soil quality	75	15
Loss of seedlings	70	30
Drying of crops (delay in crop growth)	8	92
Occurrence of new insect pests	86	14
Decrease in agricultural production	63	37

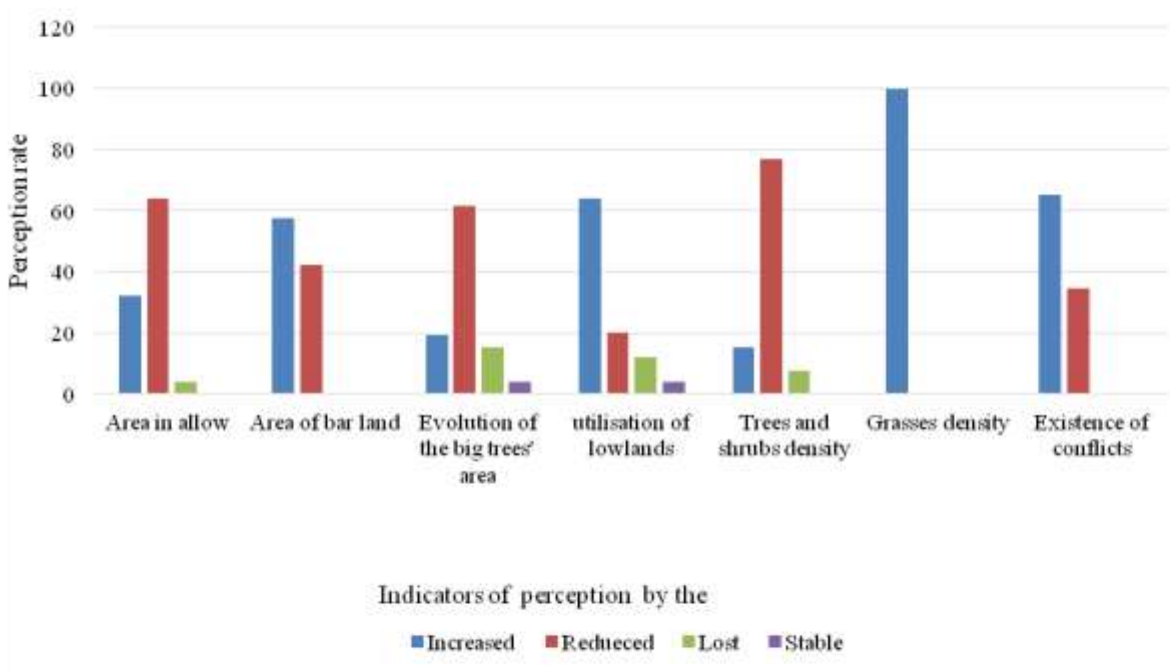

Figure 3. Perception of farmers on certain socio-environmental indicators of climate variability and change in the study area.
Source: Field survey.

the increase of denuded areas in the culture beds (68%), the decrease in the density of ligneous (77%) and herbaceous (68%). Parallel to this more precarious environmental context, 41% of farmers questioned noted the increase in conflicts related to the management of natural resources and the over-exploitation of lowlands in favorable areas (Table 4).

Adaptation to changes in climate parameters by farmers

To address the adverse impacts of climate change, 96% of farmers in rural Tamou district have developed various adaptation strategies, either individually or collectively, based on endogenous knowledge. Individual strategies are grouped into six groups (Figure 4). In fact, the adjustment of the farming calendar, the use of improved seeds, the diversification of crops, the practice of water and soil conservation / soil defense and restoration techniques, especially the Zai by digging small holes of water while placing the excavated sand in a circular arc downstream of the hole so as to capture the rainwater to the benefits of sown plants and the organic and mineral fertilizers are used respectively by 96, 75, 49, 34 and 87% of respondents respectively. Almost all the farmers surveyed (96%) do not migrate; for the minority who migrate, respondents say they give up millet farming and try their chances in big cities like Say, Niamey, Maradi and Cotonou. As for the collective strategies, it is essentially the prayers that are made on great occasion of gatherings such as Friday prayers, marriages and naming ceremonies to implore the mercy of God.

Determinants of farmer's adaptation to changes in climate parameters

The determinants of the different strategies developed by farmers to cope with changes in climate parameters are numerous. Among these factors, being a member of farmers' organization, experience in farming practices, a good level of training, access to technical information, the number of active farmers, the right of ownership and a good annual income are of great importance. In the field of agriculture, 31.4% of farmers benefited from supervision, compared to 68.6%. With regard to livestock farming, 50% of the farmers questioned said they benefit

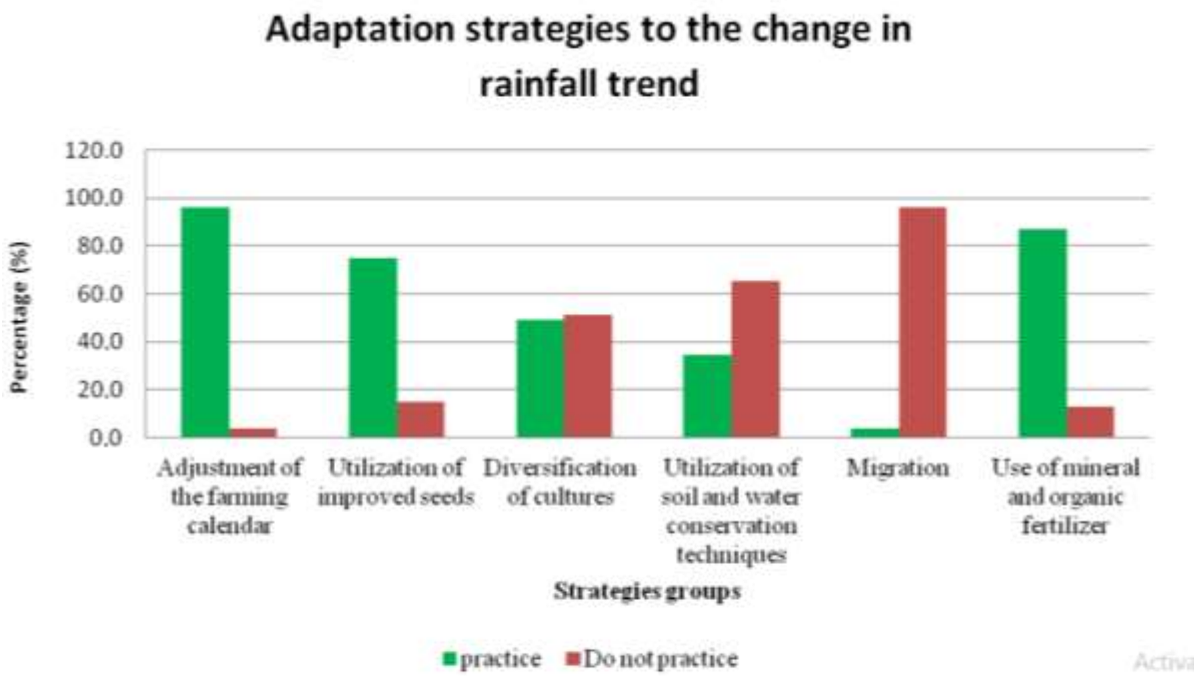

Figure 4. Groups of adaptation strategies developed by farmers.
Source: Field survey.

from the supervision of technical services and development partners operating in the area. As for environmental management, only 22% of the farmers questioned claimed to have benefited from supervision. The different coaching received by farmers were tremendous in the choice of farmers' adaptation strategies to changes in climate parameters are presented in Figure 5.

DISCUSSION

Demographic and socio-economic factors of farmers

In the rural Tamou district, agriculture is the main economic activity of the population (94.3% of farmers). Production is in rainfed crops for some crops and irrigated for others. Apart from agriculture, other secondary activities such as breeding, crafts, logging and gathering are practiced. Concerning the farms of the zone, they have an average size of 12 people, higher than the national average which is of 7 people. The low income of the majority of farmers reflects the level of poverty in which the population is staggering. This poverty is rooted in the poor yield of agricultural activities. In addition, land ownership methods in the area are by inheritance, gift, purchase and loan or lease. Inheritance is the most popular form of tenancy in all types of farms. The small sizes areas cultivated also reflect a land problem that is rooted in the population increment and the massive influx of people from other parts of the country into the area. The age range of the farmers surveyed is between 35 and 82 years old. Three-quarters of the farmers surveyed are over 45 years old. More than 40% are illiterate and the 60% are divided between primary level, Koranic school and literacy classes.

Perception of farmers on changes in climatic parameters

The results obtained by this study revealed that the farmers interviewed in the study area have a real perception of the changes in climate parameters that have occurred in recent years. Firstly, farmers affirmed that rainfall varies from year to another year. The rains start late to stop early with the presence of drought periods. In addition, the rain becomes more and more random, and often in small quantities, though, some intense storms may cause floods. They also noted that

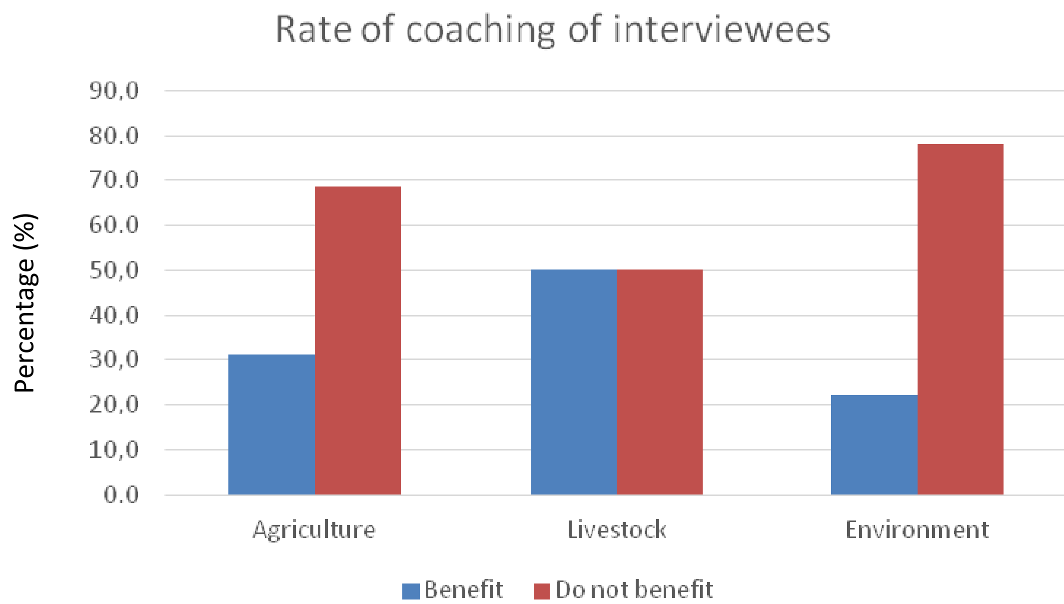

Figure 5. Management rate of millet farmers.
Source: Field survey.

the general trend of the rainy season period shows a reduction. Thus, rainfall disturbances affect the rural Tamou district which often has an impacts on agricultural production. Secondly, the farmers pointed out that the temperatures are rising and that is causing an increase of the heat which finds its sources in the changes of the extremes of temperatures characterized by long warm periods. On the other hand, the reduction of the duration of the cold period was also noted. Finally, farmers perceive warmer, drier, more violent winds, accompanied by dust and deflation, which represents a serious problem in the growth of the first seedling. The results of this study confirms those of Djenontin (2010) and Dedjan (2010), who observed in Northern part Benin republic that local people perceived climate change in their environment through the delay in the start of rainfall, drought spelts during the rainy season, poor spatial distribution of rainfall, strong winds, and excessive heat. These results are also in line with those obtained by the AGRHYMET Regional Center (2012), Lona (2011) and CNEDD (2009) in central Niger and those of Gnanglé et al. (2011) in Benin, Yegbemey et al. (2014) and Doumbia et al. (2013). The results of this study confirmed those of Gado (2012) and Oumarou et al. (2016), who observed in central Niger that local people perceived climate change in their environment through violent storms, irregular rainfall, recurrate droughts, disruption in the duration of the different seasons of the year, sowing periods, gradual disappearance of biodiversity, decrease in crops yields and the modification of the grasses. The results also corroborated those of Maddison (2007) and Mertz et al. (2009), which reveal that people are aware of climate variability and identify wind, lack and excessive rainfall as the most noticeable factors.

Causes of changes in climate parameters

From the perception of the people who were questioned, the perceived climate changes seem to be attributed either to divine causes that is to say natural, or to upheavals of social norms. The issue of population growth and pressure on the environment is never indexed as such. However, this could have an important role since the investigations showed that the population of the rural Tamou district has as main activities like agriculture, the livestock, and the wood exploitation. In addition, according to INS (2014), this population is estimated at 89,782 inhabitants, out of which 51.16% are men and 48.84% are women, whereas it was formerly only 33,788 inhabitants (RENACOM, 2004). The rainfall varies over 30 years (1980 to 2010) between 1200 mm and 600 mm with an average of 710.5 mm. These different causes have already been highlighted by AGRHYMET (2012), CNEDD (2009) and Oumarou et al. (2016).

Impacts of climate change

The impacts of climate change are also perceived by the farmers interviewed. They are expressed by lower soil quality. This decline in fertility could be explained by erosion and nutrient removal through continuous cultivation over years without input from the farmers. The lost of seedlings could be associated with rainfall scarcity, the deflation, the silting and the heat. The lost of production and the delay in crop growth could be caused by insufficient rainfall, decline in soil fertility, insects, wind, and heat. This findings corroborates the results of AGRHYMET (2012) and AMOUKOU (2011) in other growing areas in Niger.

Adaptation of farmers to changes in climate parameters

Facing the real threat posed by changes in climate parameters, the farmers interviewed developed a variety of strategies, some of which fall within the framework of prevention, others of mitigation or management of the impacts of climate disturbances.

The objective of the strategies for crop diversification and adjustment of the farming calendar (the use of short period varieties) is to reduce the length of the cropping period. In fact, by reducing the time of crops development so as to align with the period of the highest frequency of rainfall, these strategies could help in minimizing the risk of significant drougth occurrence in the area. The most used techniques of soils and water conservation techniques are Zaï and half-moons with the aim of increasing the cropping area superficies and mostly the farms yield. Thanks to development projects carried out by the government and its partners most of these strategies have been introduced in the area.

In the sector of rural development including agriculture, environmental management, and livestock breeding, the farmers affirmed that, agents of decentralized services, non-governmental organizations (NGOs), and projects, have successessfully triggered a dynamic of sustainable self-management of the natural resources of their lands. The perceptible impacts of this supervision are the reduction of certain harmful activities such as clearing of new farmlands, the excessive cutting of wood, the picking of certain of woody food species, etc.

In addition, the new activities practiced relate to the practice of assisted natural regeneration, market gardening and prayers which are customary practices that well characterize African societies. These findings confirm the results of Gnanglé et al. () in Benin, Doumbia et al. (2013), Amoukou (2009), Yegbemey et al. (2014) and Oumarou et al. (2016). Farmers developing no adaptation strategy mentioned, among other things, the lack of information on adaptation strategies and financial constraints as the main barriers to adaptation. These results are corroborated by the observations of Deressa et al. (2009).

Determinants of producer adaptation to changes in climate parameters

According to the results obtained at the scale of the rural Tamou district, several factors influence millet farmers in the perception of changes in climate parameters and adaptation strategies developed. Indeed, some are specific to farmers and others are favored by the environment. This diversity may be related to a specific context of the farmer or its particular factors.

It is in order of importance agricultural practices experience coaching of the extension agents, the number of agricultural assets per household, property rights and a good annual income. This result shows that the most experienced farmers perceive climate change faster and adapt more quickly. Indeed, the number of years spent in the practices of agricultural activity allows the producer to master the entire production process and the factors that influence the various stages of this process. These results confirmed the results obtained by Maddison (2007), Derressa et al. (2009), Gbetibouo (2009) and Oumarou et al. (2016) who came to the conclusion that experience in agriculture is a key determinant of the farmer’s level of perception and adaptation to climate change. It should also be noted that the experience increases the farmer’s ability to diversify crops, adjust the farming calendar and use more effective land use and management techniques to address climate change.

Through farmers' organizations, farmers are enlightened on the current climate variability as well as the present and future consequences on agricultural activities and the environment. They build relationships among themselves that serve as channels for sharing experiences that can trigger climate change adaptation initiatives. This result also shows that the contact between the farmer and the extension agent seems to be more beneficial, because the farmer obtains from the latter information on the evolution of the climate, the innovative agricultural techniques in progress and technical advice so as to facilitate adaptation to changes in climate factors. For example, sowing periods are widely discussed with extension agents. The importance of extension in the perception and adaptation of farmers to climate change had been already highlighted in the work of Maddison (2007), Gbetibouo (2009) and Deressa et al. (2011). Other studies such as those conducted by Oumarou et al. (2015) and Yegbemey et al. (2014) have also shown that extension is an important motivating factor in the use of best practices for sustainable management of the environment.

Conclusion

Millet farmers in the rural Tamou district perceive the change in climate parameters (rainfall, temperature and wind) and develop various strategies to adapt to them. For these farmers, climate change is reflected in rainfall and temperature disruptions, high winds, and the lost of some species of trees and animals species. The causes of these changes are human and natural. The impacts of changes in climate parameters are dominated by a decrease in soil fertility, lost of seedlings, delay in crop growth and lost of production. The main adaptation strategies developed as a response to the change in climate parameters are crop diversification, the adjustment of cropping practices and the farming calendar. The main factors favoring the use of adaptation strategies are supervision of extension agents, the number of farm workers per household, property rights, experience in farming practices and a good annual income. In addition, the various adaptation strategies developed by farmers and their determinants, such as holding regular public awareness, training, exchange and knowledge-sharing sessions on future climate conditions and adaptation to climate change could constitute potential tools which could be used by all stakeholders and directed towards the farmers' organizations of through the extension services. These findings would be taken into account by the government, researchers and policy/decision makers in the development of agricultural policies in general and millet production in particular.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Cowpea response to nutrient application in Burkina Faso and Niger

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Cowpea (*Vigna unguiculata* (L.) Walp) is important in semi-arid West Africa. Yields are low due to inadequate water and nutrient availability and other constraints. Grain and fodder yield responses to nutrient application were determined from 21 site-years of research conducted in the Sahel and Sudan Savanna. The incomplete factorial treatment arrangement varied by country but included: Four levels each of P and K in 7.5 and 10.0 kg ha^{-1} increments, respectively; Mg-S-Zn-B package (Mg-S-Zn-B); and comparable with and without manure treatments. Yield increases due to P application always occurred with curvilinear to plateau or linear responses. The overall mean grain yield increase was 0.35 Mg ha^{-1} and 47% due to application of 22.5 kg ha^{-1} P. Application of K resulted in a linear negative effect in 2014 and positive effect in 2015 for on-station trials in Niger, but had no effect in Burkina Faso and for on-farm trials in Niger. Yield was not affected by Mg-S-Zn-B in Burkina Faso but was increased by a mean of 0.085 Mg ha^{-1} in Niger. Manure application resulted in a mean yield increase of 0.1 Mg ha^{-1} in Niger but only with fertilizer P applied, and had no effect in Burkina Faso. Cowpea grain and fodder yields were responsive to fertilizer P up to 22.5 kg ha^{-1} but little affected by other applied nutrients.

Key words: Fertilizer, fodder, grain, manure, micronutrient, phosphorus, secondary nutrient, Sahel, Sudan Savanna.

INTRODUCTION

Cowpea (*Vigna unguiculata*) is an important food and cash pulse crop of semi-arid areas of Africa including in the Sahel and Sudan Savanna of West Africa because of its tolerance to soil water deficits and low nutrient availability. National mean yields vary with 2013 estimates of 0.28 Mg ha^{-1} for Niger and 0.78 Mg ha^{-1} for Nigeria (FAO, 2017). Cowpea production is often on soil of low clay and organic C contents, with low nutrient availability.

Cowpea is often responsive to applied manure and fertilizer. Adeoye et al. (2011) reported increased yields with cattle and poultry manure application. Cowpea is efficient in fixation of atmospheric N but Dugje et al.

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Table 1. Site information and soil test properties for the 0 to 0.2 m depth at the research sites in Burkina Faso and Niger.

Property	------------------ Niger ------------------				Burkina Faso	
	Bengou	Maradi	Magaria	Konni	Boni	Dori
Latitude	11.979	13.459	12.975	13.825	11.541	14.042
Longitude	3.558	7.104	8.917	5.289	-3.436	-0.136
Elevation	170	360	400	270	325	280
Soil type	Luvisol	--------- Arenosol --------			Luvisol	Arenosol
pH-H_2O	6.6	5.6	5.6	6.9	5.2	6.2

(2009) found that cowpea was often responsive to 15 kg N ha^{-1} applied at planting. Agboola (1978) reported mean cowpea grain yield increases of 0 and 0.24 Mg ha^{-1} due to 10 kg ha^{-1} N without and with P uniformly applied, respectively. However, Abayomi et al. (2008) reported that application of 40 kg N ha^{-1} depressed flowering and reduced grain yield, but increased vegetative growth. Yield was increased by a mean of 0.19 Mg ha^{-1} due to 10 kg ha^{-1} P applied (Magani and Kuchinda, 2009; Ndor et al., 2012). Abayomi et al. (2008) evaluated the effects of NPK fertilizer application and recommended the application of 150 ha^{-1} NPK fertilizer to supply 30-15-15 kg N-P-K ha^{-1} for cowpea. In consideration of available information, the International Institute of Tropical Agriculture recommended 30, 14 and 12.5 kg ha^{-1} N, P and K, respectively, for cowpea production (Dugje et al., 2009). The profit potential for fertilizer application to the pearl millet-cowpea intercrop was greater than with application to either of the sole crops (Maman et al., 2017a).

Crop-nutrient response information is important fertilizer use decisions aimed at high farmer profitability but such information is scarce for cowpea in West Africa. Field studies included 21 trials conducted in Burkina Faso and Niger to determine cowpea yield responses to fertilizer N, P, K, Mg-S-Zn-B, and manure. The hypotheses were that cowpea would be most responsive to applied P with occasional responses to other nutrients and that nutrient response would be enhanced with manure application.

MATERIALS AND METHODS

Experimental sites

Cowpea trials were conducted in the Sahel during the 2014 and 2015 on-station at Tarna-Maradi, Magaria, and Birnin Konni in Niger, and at Dori, Burkina Faso, and on-farm in Niger near Maradi and Magaria in 2014 and 2015, Birnin Konni in 2015 (Table 1). The trials at Boni Burkina Faso were conducted on-farm. The soil was Arenosol for the Sahel sites (Jones et al., 2013). Trials were conducted in the Sudan Savanna with Luvisol soil at Boni, Burkina Faso, and Bengou, Niger in 2014 and 2015. The rainfall pattern was mono-modal with >700 mm for Bengou and Boni and <600 mm for the Sahel locations (Figure 1).

Composite soil samples of 0 to 0.2 m depth were collected by block before land preparation and treatment application. The samples were air-dried, sieved to a 2-mm diameter and sent to the World Agroforestry Center Soil-Plant Spectral Diagnostic Laboratory in Nairobi, Kenya. The analyses were for particle size distribution, pH, organic C and N, exchangeable bases, S, Zn, Cu, Fe, Mn, and B (https://www.worldagroforestry.org/sd/landhealth/soil-plant-spectral-diagnostics-laboratory/sops).

Soil properties ranged from 5.2 to 6.6 pH, 1.1 to 7.6 g kg^{-1} soil organic C, 11 to 40 mg kg^{-1} Mehlich-3 P, and 41 to 94 mg kg^{-1} Mehlich-3 K (Table 1).

Experimental design and treatments

The experimental design was a randomised complete block design with three replications. Plot size was 4.8 m × 6.0 m with access alleys separating blocks. There were 16 treatments in Niger and 11 treatments in Burkina Faso (Table 2). The treatment structure was an incomplete factorial. There were four P levels with increments of 7.5 kg ha^{-1} with 0 and 20 kg ha^{-1} K uniformly applied and four K levels with 10 kg ha^{-1} increments with 15 kg ha^{-1} P uniformly applied. The Mg-S-Zn-B was applied with 15 kg ha^{-1} P and 20 kg ha^{-1} K. In Niger, treatments T_1 to T_{12} had 2.5 Mg ha^{-1} manure broadcast applied and four additional treatments (T_{13} to T_{16}) had no manure application. Treatments T_1 to T_{11} and T_{13} to T_{16} had 10 kg ha^{-1} N uniformly applied. Treatment T_{12} had no N applied. The five on-farm trials had the same design and treatment structure as for the on-station trials in Niger, but the on-farm trials had one complete block per field across five or six fields of different farmers. In Burkina Faso, adjacent experiments were conducted with and without 5 Mg ha^{-1} of manure broadcast applied.

The nutrient sources were triple super phosphate, potassium chloride, and urea. The Mg-S-Zn-B treatment had rates of 10-15-2.5-0.5 kg ha^{-1} with nutrients supplied from $MgSO_4$ as kieserite with 15% Mg and 22% S, zinc sulfate monohydrate with 34% Zn and 18% S, and granular borax with 14.5% B. Nutrient rates were specified in elemental form. The fertilizers were point applied and incorporated 0.05 to 0.1 m from the plants at 7 to 10 days after seedling emergence.

In Niger, the mean manure nutrient concentrations for N, P, K, Mg, S, Zn and B, respectively, were 10.9, 1.1, 7.3, 2.3, 0.8, 0.02 and 0.05 kg Mg–1. The mean manure pH was 8.5 and the C:N ratio was 18.6. In Burkina Faso, the mean manure N, P, and K contents, respectively, were 12, 4, and 21 kg Mg–1, and the C:N ratio was 17. The manure used in Niger was from penned goats and sheep. The manure used in Burkina Faso was a compost of cattle excrement mixed with unconsumed crop stover.

Management practices

The experimental sites were ploughed and harrowed. The variety planted was *cv* IT99K573-1-1 which has a semi-erected growth

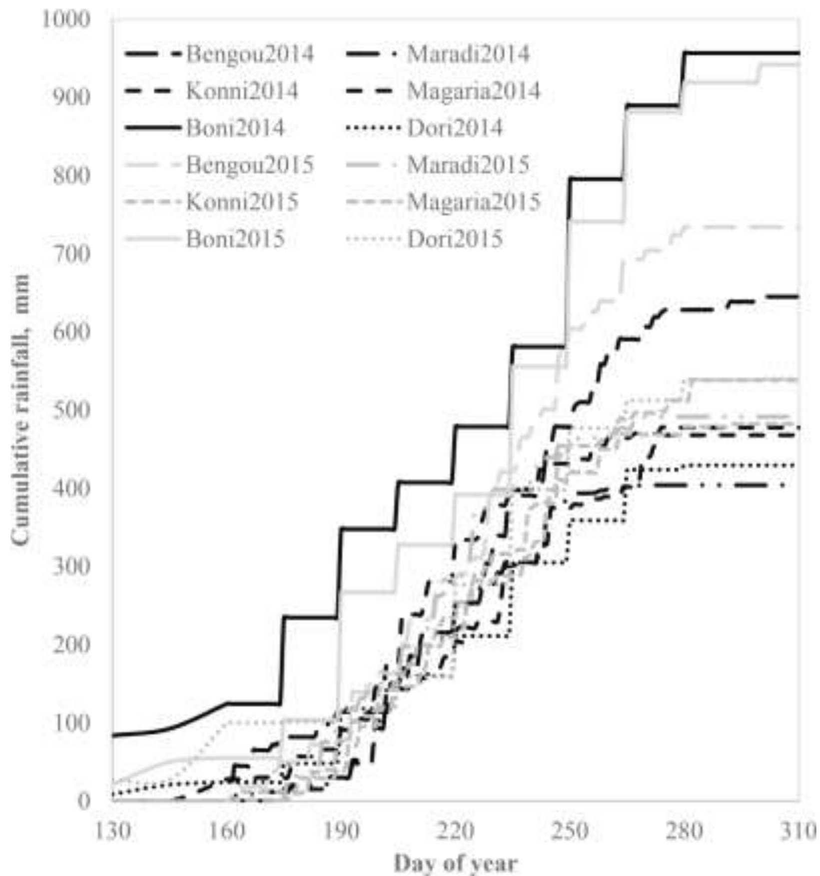

Figure 1. Cumulative rainfall for four research sites in Niger (a) and Burkina Faso (b) in 2014 and 2015.

Table 2. Nutrient rate (kg ha^{-1}) treatments (T) for cowpea response trials conducted in Niger and Burkina Faso.

T	N-P-K, Niger	T	N-P-K, Burkina Faso
1	10-0-0†	1	0-0-0
2	10-7.5-0	2	0-7.5-0
3	10-15-0	3	0-15-0
4	10-22.5-0	4	0-22.5-0
5	10-0-20	5	0-0-20
6	10-7.5-20	6	0-7.5-20
7	10-15-20	7	0-15-20
8	10-22.5-20	8	0-22.5-20
9	10-15-10	9	0-15-10
10	10-15-30	10	0-15-30
11	10-15-20-D	11	0-15-20-D
12	0-15-20		
13	10-0-0‡		
14	10-7.5-0		
15	10-15-0		
16	10-15-20		

†The nutrient rate (kg ha^{-1}) treatments refer to: N-P-K, and D, the diagnostic treatment. The diagnostic treatment contained 15 S, 10 Mg, 2.5 Zn and 0.5 B kg ha^{-1} combined with N, P, and K rates comparable to treatment 7. ‡ Treatments 1-12 in Niger had 2.5 Mg ha^{-1} manure applied and treatments 13-16 had no manure applied. Side-by-side trials in Burkina Faso had 0 or 5 Mg ha^{-1} manure applied in 2014 and were repeated on the same plots in 2015 with no more manure application.

habit with 70 to 80 days to maturity, a grain yield potential of 1.5 Mg ha^{-1} and good fodder production potential (Baoua et al., 2012). The seeds were treated with fungicide Apron Star 42 W of Syngenta {thiamethoxam [3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine], mefenoxam{(R,S)-2-[(2,6-dimethylphenyl)-methoxyacetylamino]-propionic acid methyl ester}, and difenoconazole [1-((2-(2-chloro-4-(4-chlorophenoxy)= phenyl)-4-methyl-1,3-dioxolan-2- yl)methyl)-1H-1,2,4-triazole] with 20, 20 and 2 g kg^{-1} a.i.; 5 g kg^{-1} of seed}. Seed was manually sown at 50-mm depth with 0.8 × 0.3 m spacing. The seedlings were thinned to 2 plant $hill^{-1}$ after the first weeding. Manual hand-hoe weeding was done at 3 and 6 weeks after sowing. The trials were sprayed at flowering and pod formation for protection against a spectrum of insect pests including *Maruca testulalis* and pod sucking coreid species with cypermethrine {[cyano-(3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate} plus dimethoate (2-dimethoxyphosphinothioylsulfanyl-N-methylacetamide) at 1 L ha^{-1}.

Data collection and statistical analysis

Upper fully expanded leaves of the treatment with 15 kg ha^{-1} P and 20 kg ha^{-1} K were sampled at flowering in Niger and analyzed for N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn, B, and Mo at the laboratory of the World Agroforestry Centre in Nairobi. This treatment was sampled to prevent suppression of expression of other nutrients deficiencies by low foliar P and K concentrations. In both countries, grain and fodder yield were determined from harvest of the two central rows for 4-m length. Harvest was by uprooting the plants from the two inner rows, removing the pods and air-drying before shelling. The harvested grain was weighed and air-dried grain yield calculated. The grain plus fodder yield was also determined on a grain value equivalent basis assuming that the value of one kilogram fodder was equivalent to the value of 0.25 kg grain.

The analysis of variance (ANOVA) was combined across within countries with years, locations, and replications as random variables and treatments as fixed variables with Statistix 10 Software (Tallahasse FL). The on-farm trials in Niger were each considered a site-year and the ANOVA was combined across site-year. In Burkina Faso, manure was a factor in the overall ANOVA. If there were significant treatment effects, overall or for location x year, contrast tests were applied to evaluate effects of N, K and Mg-S-Zn-B. Contrast tests were also applied to manure effects in Niger. The effects of P and its interactions were further analyzed with the sub-set of the first eight treatments for P rates (Table 2).

The analyses were for cowpea grain yield and for grain plus fodder yield expressed as grain yield equivalent. The grain yield equivalent was calculated assuming the value of food to be 25% of grain value. Therefore, the grain yield equivalent of grain plus fodder yield was the sum of the grain yields plus ¼ the fodder yield (Mg ha^{-1}).

Crop response to increasing nutrient rates is most typically curvilinear to plateau and the fitting of response data to an asymptotic regression function was first attempted where Yield (Mg ha^{-1}) = $a – bc^r$, with *a* being yield at the plateau due to the application of that nutrient, *b* being the maximum gain in yield due to the application of that nutrient, *c* being a curvature coefficient, and *r* being the nutrient rate (Wortmann et al., 2017). When the asymptotic function failed to give a realistic convergence, linear functions were attempted.

The economically optimum rates (EOR) of nutrient application, or the rate of maximum net profit per ha, were calculated for nutrient use cost to farmgate cowpea grain value ratios (CP) ranging from 3 to 12 kg kg^{-1}. The agronomic efficiency (AE) was calculated as the gain in grain yield per kg of P or K applied (kg kg^{-1}). The profit to cost ratio (PCR), or the ratio of net return due to the nutrient application relative to the cost of that nutrient application, was calculated for different CP and nutrient rates.

RESULTS

In Niger, the mean cowpea yields were 0.49, 0.78 and 0.67 Mg ha^{-1}, respectively, in 2014 and 2015, and for the on-farm trials. Fodder yield added an average of 60% to the value of the cowpea harvest. The year × location × treatment and the P × K interactions did not affect grain and fodder yields in the on-station trials but treatment, year, location, and year × location and year × treatment interaction effects were significant (Table 3). The year × P interaction occurred due to a small but curvilinear grain and grain plus fodder yield response to P in 2014, but linear and greater responses in 2015 (Table 4; Figure 2). The P rate effect was linear for the on-farm trials. For the on-station trials, grain and grain plus fodder yield response to K was negative in 2014, but grain yield response to K was positive in 2015. There was no K effect on yields for the on-farm trials. Grain and grain plus fodder were increased with Mg-S-Zn-B by 10% and by 19% with 10 kg ha^{-1} N for the on-station trials conducted in 2015 but not in 2014. Grain yield was also increased with Mg-S-Zn-B by 25% for the on-farm trials. There was a response to manure application only in 2015, but only with fertilizer P applied. Manure did not affect yield in the on-farm trials. The response to Mg-S-Zn-B could havbeen due to any of these nutrients. The foliar sample results indicate some cases of low N, P and K even though P and K were applied to the sampled plots (Table 5). Other foliar nutrient levels were above the critical values used for interpretation except for borderline S concentrations for some samples.

In Burkina Faso, the mean cowpea yields were 1.76 Mg ha^{-1} at Boni and 0.7 Mg ha^{-1} at Dori. Grain yield was affected by the treatment × location interaction but not by other interactions (Table 3). Grain yield had a greater and curvilinear response to P at Boni, averaged over the two years and two manure rates, compared with Dori where there was a linear response (Table 4; Figure 2). Grain yield was not affected by K, Mg-S-Zn-B, and manure application.

The effects of P were often linear with an EOR of at least 22.5 kg ha^{-1} (Table 4; Figure 2). For the linear responses at 22.5 kg ha^{-1} P, the AE ranged from 10 kg kg^{-1} at Dori to 25 kg kg^{-1} for grain plus fodder for 2015 in Niger. The corresponding ranges of PCR for CP = 12 and 3, respectively, were -0.2 to 2.3 \$ $\$^{-1}$ at Dori and 1.1 to 7.3 \$ $\$^{-1}$ for 2015 grain plus fodder in Niger. At Boni which had a curvilinear to plateau response, the EOR of P ranged from 22 to >30 kg ha^{-1} for CP = 12 to 3 and 6. The response to P in Niger in 2014 was also curvilinear to plateau with EOR of P ranging from to 4 to >30 kg ha^{-1} for grain alone and from 9 to 24 kg ha^{-1} for grain plus fodder with CP = 12 and 3, respectively. For Boni, AE at

Table 3. ANOVA results for cowpea yield response to applied nutrients in Niger for grain and grain plus fodder on a grain value equivalent basis with fodder value 0.25 kg kg^{-1} of grain.

On-station trials				On-farm trials			
Source	**df**	**Grain**	**Grain+fodder**	**Source**	**df**	**Grain**	**Grain+fodder**
Year (Y)	1	***†	**				
Location (L)	3	***	***	SY	4	***	***
YxL	3	***	***				
Treatment (T)	15	***	***	T	15	***	***
LxT	45	ns	ns	SYxT	60	ns	ns
YxT	15	***	***				
YxLxT	45	ns	ns				
Error	240				270		

† ns, not significant; ** and *** indicate significant effects at P ≤ 0.01 and 0.001, respectively.

Table 4. The P and K rate (kg ha^{-1}), N, Mg-S-Zn-B†, and manure effects on cowpea yields (Mg ha^{-1}), and P and K response coefficients, in Niger and Burkina Faso.

	Niger						Burkina Faso	
P rate	**Grain yield**			**Grain+fodder yield**			**Grain yield**	
	2014	**2015**	**OFT**	**2014**	**2015**	**OFT**	**Boni**	**Dori**
0	0.39	0.56	0.51	0.87	0.84	0.71	1.53	0.60
7.5	0.48	0.63	0.63	1.02	0.94	0.87	1.69	0.65
15	0.51	0.78	0.67	1.03	1.15	0.94	1.87	0.74
22.5	0.59	0.99	0.83	1.15	1.40	1.11	1.95	0.82
P effect	***	***	***	***	***	***	*	**
P×K effect	ns	ns	ns	ns	ns	ns	ns	ns
P response coefficients	**A**‡	**L**	**L**	**A**	**L**	**L**	**A**	**L**
a (Mg ha^{-1})	0.68	0.524	0.510	1.16	0.799	0.717	2.25	0.590
b (Mg ha^{-1})	0.29	0.019	0.013	0.31	0.025	0.017	0.72	0.010
c	0.95			0.91			0.96	
K rate								
0	0.56	0.74	0.68	0.96	1.12	0.94		
10	0.50	0.81	0.71	0.93	1.20	1.11		
20	0.47	0.81	0.67	0.84	1.15	0.93		
30	0.45	0.89	0.70	0.83	1.29	0.94		
K effect	*	*	ns	*	ns	ns		
K response coefficients								
A	0.549	0.745		0.962				
B	-0.004	0.005		-0.005				
Orthogonal contrasts								
Mg-S-Zn-B†	ns	0.077*	0.170*	ns	0.019*	ns	ns	ns
N	ns	0.145*	ns	ns	0.026**	ns		
Manure with 0P	ns	ns	ns	ns	ns	ns	ns	ns
Manure with P	ns	ns	ns	ns	0.298*	ns	ns	ns

ns: not significant; * and ** indicate significant effects at P ≤ 0.05 and 0.01, respectively. †Mg-S-Zn-B, a diagnostic treatment combined with N, P, and K at rates comparable with another treatment. ‡A, asymptotic function of $Y = a - bc^r$; L, linear function of $Y = a + br$, where r is the rate of nutrient application (kg ha^{-1}).

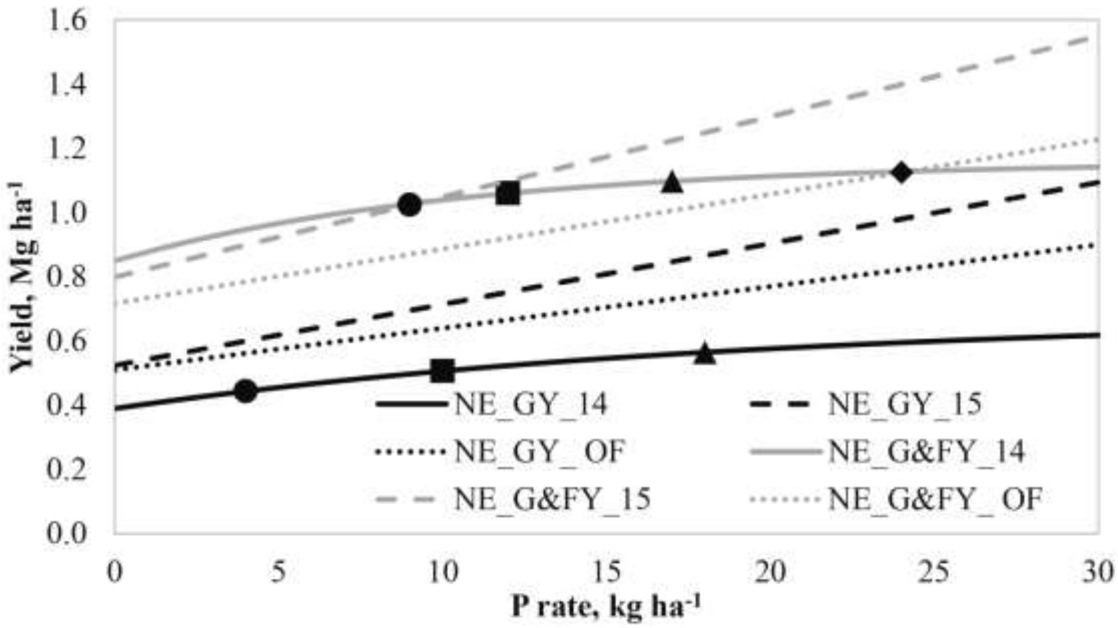

Figure 2. Response of cowpea grain yield (GY) and grain plus fodder yield (G&FY) to applied P in Niger in 2014 (_14) and 2015 (_15) with trials conducted on-station. Other trials were conducted on-farm (_OF). The economically optimal rates (EOR) of P for four fertilizer P use costs where the cost of using one kilogram of P equal to the value of 3 (●), 6, (■), 9, (▲) and 12 (♦) kg of cowpea grain, respectively. Grain plus fodder yield was the sum of grain and 25% of fodder yields.

Table 5. Cowpea foliar nutrient concentrations with sampling of upper fully expanded leaves near flowering in Niger.

Variable	N	P	K	Ca	Mg	S	Zn	Cu	Fe	Mn	B	Mo
Mean	4.0	0.39	2.7	2.6	0.66	0.28	57	12.9	1628	333	39	0.96
Minimum	3.6	0.23	1.5	1.6	0.39	0.20	46	7.2	282	216	24	0.16
Maximum	4.3	0.49	3.4	3.1	0.90	0.33	73	16.2	3440	466	46	1.53
Critical value	3.4	0.25	1.6	0.7	0.22	0.20	21	4	25	12	15	0.05

Sampled plots had 10, 15, and 20 kg ha^{-1} N, P, and K, respectively, applied after crop emergence.

EOR ranged from < 17.5 to 19.9 kg kg^{-1}, while the corresponding ranges for Niger 2014 were <7.6 to 13.5 kg kg^{-1} for grain alone and 11.6 to 19.7 kg kg^{-1} for grain plus fodder. For Boni, PCR at EOR ranged from >4.0 to 0.66 \$ $\$^{-1}$, respectively, for CP = 3 to 12. The corresponding ranges of PCR at EOR for Niger 2014 were >1.5 to 0.1 \$ $\$^{-1}$ for grain alone and 2.9 to 0.6 \$ $\$^{-1}$ for grain plus fodder.

DISCUSSION

Cowpea yields were low for the Sahel trials but much higher for Boni in the Sudan Savanna. Soil organic C and nutrient availability were low for all sites with some exceptions for Mehlich-3 P (Table 1). Rainfall was relatively more at Boni and Bengou compared with the Sahel sites in both years. Boni also had a relatively great response to applied P, apparently due to less soil water deficits. The grain yield equivalent was much increased by consideration of fodder yield (Table 2).

The greatest fertilizer use opportunity for sole crop cowpea production was with P application and response occurred independent of Mehlich-3 P (Table 4). This confirms the first hypothesis. The grain yield equivalent response to P was greater for grain plus fodder compared with grain alone. The linear and near-linear responses to P were generally associated with low to modest AE, and probably low recovery efficiency. For example, if P uptake was 5 g kg^{-1} of grain, recovery efficiency would be just 7.5% when AE is 15 kg kg^{-1}. The available information was not sufficient to determine the cause of low recovery but does suggest low capacity for P uptake and the possibility of low vascular arbuscular mycorrhizal colonization of the roots. The low to modest AE for P also implied that fertilizer P use may not always be financially practical. With the more costly fertilizer P scenario considered, fertilizer P use at Dori had a negative PCR. A PCR > 1 will likely be needed to attract investment by financially constrained farmers (CIMMYT, 1988). This was always achieved with the lower cost of fertilizer P use scenarios but never achieved with the most costly scenario considered.

Cowpea yield response to 10 kg ha^{-1} N occurred only for on-station trials conducted in Niger in 2015 (Table 4) indicating less potential than previously reported (Abayomi et al., 2008; Dugje et al., 2009). Application of 10 kg ha^{-1} N, if CP < 4, may give a satisfactory PCR. The response in 2015 and with the on-farm trials in Niger to Mg-S-Zn-B is of interest and deserves more investigation. The foliar test results show that only S was occasionally of low availability (Table 5). Sulfur is abundant and of modest cost globally suggesting feasibility for fertilizer S use, but further research is needed to determine if mean cowpea response to S, can justify the cost of fertilizer S application.

The results from Niger indicate that manure applied alone has little value but that synergistic effects of both P and manure applied occur. This supports the hypothesis of enhanced nutrient response with manure application, but only for fertilizer P. This agrees with other results from the Sahel indicating synergism of manure use with fertilizer P but not with other nutrients applied for cowpea (Garba et al., 2018).

Consideration of fodder together with grain added 60% to the value of the harvest and increased AE and PCR. Reducing CP adds greatly to profitability of fertilizer use as shown above for P. Reduced CP might also be achieved through fertilizer use subsidization, more efficient fertilizer supply, or more efficient cowpea marketing for increased farmgate value. Often PCR and AE can be increased by applying at less than EOR due to curvilinear responses such as with pearl millet, sorghum and their intercrops with cowpea and groundnut (Maman et al., 2017b, c). This has led to recommendation of microdose nutrient application for some semiarid production conditions (Bagayoko et al., 2011; Tabo et al., 2011). However, the potential for increased PCR with rates of less than EOR is much less for cowpea sole crop response to P due to the linear and near linear responses found in this study. The results support the use of cost-effective P fertilizer, such as triple super phosphate, but do not support the recommended rates and use of N-P-K fertilizer blends (Dugje et al., 2009). This consideration is especially important to financially constrained farmers as any of use of their limited resources for unprofitable fertilizer use means less available for more profitable fertilizer use.

The results of this research need to be considered together with other field research based information as was done for the development of fertilizer use decision tools (Dicko et al., 2017; Maman et al., 2017c; Ouattara et al., 2017). The decision tools are commonly used on behalf of financially constrained farmers and need to optimize fertilizer use choices for profit maximization. For example, Maman et al. (2017a) found that fertilizer use for pearl millet-cowpea intercropping had much more profit potential than for pearl millet sole crop. The computer decision tools and their paper formats are available at http://agronomy.unl.edu/ofra.

Conclusion

The yield potential of cowpea in the Sahel is low but it is much higher in Sudan Savanna where soil water deficits are generally less severe. The hypothesis that cowpea is most responsive to applied P with occasional responses to other nutrients was supported by the results of this study. The second hypothesis that nutrient response would be enhanced with manure application was found to be true only for fertilizer P. Cowpea response to P has good profit potential in both the Sahel and the Sudan Savanna if the cost of fertilizer P use relative to grain value is not too great. Phosphorus use efficiency is likely to be low which may imply P residual effects for the following crop. Cowpea fodder adds to the value of the harvest and to the response to P. Current fertilizer use recommendations for sole crop cowpea in these semi-arid areas of West Africa was found to be excessive. Fertilizer use should be limited to P, and maybe low-cost N, application until additional information justifies application of other nutrients. A research priority should be to determine the effect of S application for cowpea production. Combining fertilizer P with manure application should be practiced where feasible.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ABBREVIATIONS

AE, Agronomic efficiency of applied nutrient use; **CP**, cost of nutrient use relative to cowpea grain value or kilogram of cowpea grain required to equal the cost of one kilogram of nutrient applied; **EOR**, the economically optimal rate of nutrient application or the rate expected to maximize net return to nutrient application; **PCR**, profit cost ratio or the net returns divided by the costs of a nutrient application.

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Cultivation of watermelons submitted to water deficit in the experimental area of Brazilian semiarid

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The culture of watermelon is mostly responds to technological advancement. The fruit is analyzed in terms of its production and quality. Among the factors involved in its production, irrigation is very important; however it must be well managed. There are phases of the culture that requires greater or lesser amount of water for maximum productivity and high quality. In this context, the present research was conducted to evaluate the cultivation of watermelon under water deficit in the experimental area of semiarid Brazilian. The experiment was conducted at the National Department of Works Against Drought, Ayres of Souza, Ceará, Brazil. Growth variables were evaluated, such as the number of leaves, leaf area, length, and number of branches. The evaluations were conducted at 36, 42 and 49 days after transplanting (DAT). The data obtained showed that culture has water deficit tolerance, and if properly handled, using the right amount of irrigation water, there would be no damage to the culture.

Keywords: *Citrullus lanatus*, water stress, irrigation.

INTRODUCTION

Population growth has consistently impelled the use of irrigation technologies for production of foods, not only to complement water needs of crops in the humid regions but also to make agricultural semiarid areas productive (Lima et al., 2012).

According to Lima et al. (2010), in a bid to increase productivity, create new needs and prospect of development, some sectors seek more efficient means of production; these include in this context: expansion of irrigated areas and environmental sustainability (e.g. efficient management of water use, minimizing the impact on demand for water resources, leaching of nutrients and the risk of secondary salinization).

Water resource is increasingly becoming scarce, and for greater use of irrigation, the use of practices that can efficiently contribute to reducing waste of water, using appropriate water levels and time of application suitable for the crop is recommended (Valnir Júnior, 2007).

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Table 1. Treatments submitted to watermelon culture at different times.

Treatment	Phenology stage		
	36 DAT	42 DAT	49 DAT
T1	ET_0 = ETC	ET_0 = ETC	ET_0 = ETC
T2	ET_0 = ETC	ET_0 = ETC	ET_0 < ETC
T3	ET_0 = ETC	ET_0 < ETC	ET_0 = ETC
T4	ET_0 < ETC	ET_0 = ETC	ET_0 = ETC
T5	ET_0 = ETC	ET_0 < ETC	ET_0 < ETC
T6	ET_0 < ETC	ET_0 = ETC	ET_0 < ETC
T7	ET_0 = ETC	ET_0 < ETC	ET_0 = ETC

The application of a significant quantity of water in irrigation influences the development of the crop; water deficit can lead to low productivity. Some benefits of irrigation include: irrigation efficiency, reduction of costs, and environmental risks (Frizzone, 2010).

Due to water deficit, it is necessary to supply the water demand of the crop during evapotranspiration. This type of management can be practiced, with full and supplemental irrigation. Water can be applied during the crop cycle or during stages not critical to the water deficit. In the latter case, smaller reductions in crop productivity are possible, according to Frizzone (2010).

In the state of Ceará, the high temporary and space variability of precipitations make irrigation the only way to guarantee agricultural production. Irrigated Perimeter Ayres of Souza around river Jaibaras's alluvial plain, located in the municipal district of Sobral, stands out among the areas irrigated in the state. The irrigation of the perimeter is fed through the dam on Jaibaras River with a maximum accumulation of 104 × 106 m^3, at Federal Public Ayres of Souza (Carneiro Neto, 2005). Although in the perimeter, there is overhead irrigation on the surface, there are implanted overhead irrigations through leakage in small agricultural areas, where water is usually scarce. This is done with the purpose of optimizing the available water in the rural community (Moreira et al., 2015).

Watermelon (*Citrillus lanatus*) is one of the Cucurbitácea's family species and is produced more in Brazil. Northeast stands out as the largest producing area, because it has a favorable climate. In other areas where the climate is cold, watermelon does not develop satisfactorily. Small producers make more use of this culture, because it is easy to handle and has smaller production cost; however, the producer needs to adopt an appropriate handling, minimize production costs and optimize the use of irrigation water, according to Moreira et al. (2015).

The main watermelon producing regions in the country are the Northeast with 34.15 of the national total (Silva et al., 2015). According to Amaral et al. (2016), the productivity and final quality of the watermelon crop are related to several factors, which act during all phases of its growth and development.

In this context, the present research was conducted aiming to evaluate the cultivation of watermelon under water deficit in the experimental area of semiarid Brazilian.

MATERIALS AND METHODS

The experiment was conducted in an area of 2000 m^2 in the irrigated perimeter of National Department of Works Against Drought (NDWAD), Ayres of Souza, in the district of Jaíbaras, municipality of Sobral-Ceara, Brazil; it has geographical coordinates of 3°45"5" South and 40°27" West. Its temperatures vary based on the time of year and location. It has a minimum of 21°C and maximum of 36°C. During the trial period, the average rainfall is 12.06 mm.

The experimental design was randomized blocks, with seven treatments and four replications; totaling 28 parcels in an area of 2000 m^2. Each parcel had 12.5 × 2.5 m, containing five rows of watermelon with a plant per plot, and the plants are located on the sides considered as surrounding.

The treatments were defined based on the time of application of water at different phenological stages of the culture: vegetative stadium, Estadio deflowering, and development of the fruit, as noted in Table 1. 50% level of water was used in treatment 1 (without limited water). The crop evapotranspiration (ETc) = crop coefficient (CC) × reference evapotranspiration (ET0); it was estimated using the method of Pennam Monteith, which was recommended by Allen et al. (1998).

The seeds of the variety of watermelon Top seed Premium Explorer 1 (one), used in the experiment, were acquired in AGRIMASTER, Marco-CE, Brazil. Watermelon seeds were sown in styrofoam trays with 128 cells. Eight trays were used in total, they were filled with sand and livestock manure in a simple sieve. The transplanting and irrigation were done 13 days after sowing (DAS).

For irrigation, the transmitter (Dripper tube Taldrip), has five plot lines with 12.5 cm space; it is controlled by a 32 mm socket ball on each parcel. Characteristics of water used for irrigation are: 0.38 $mmcL^{-1}$ (Ca^{2+}), 0.38 $mmcL^{-1}$ (Mg^{2+}), 1.22 $mmcL^{-1}$ (Na^+), 0.08 $mmcL^{-1}$ (K^+), 7.7 (pH), 0.11 dSm^{-1} (CE), and classification in C1S1. Soil chemical characteristics used in the experiment are: Calcium (4.35 meq/100 g of soil), Magnesium (2.73 meq/100 g soil), Sodium (0.20 meq/100 g of soil), Potassium (0.07 meq/100 g soil), Hydrogen (5.73 meq/100 g of soil), Aluminium (0.60 meq/100 g of soil), Calcium Carbonate Qualitative is Absent, Organic Carbon (0.72%), Organic matter (1.24%), Nitrogen (0.07%), Phosphorus Assimilable (4.52 mg/100 g), 6.75 of pH H_2O (1:2.5), Electrical Conductivity (Soil-Water Suspension) of 0.08 mmhos/cm, and Normal Ground

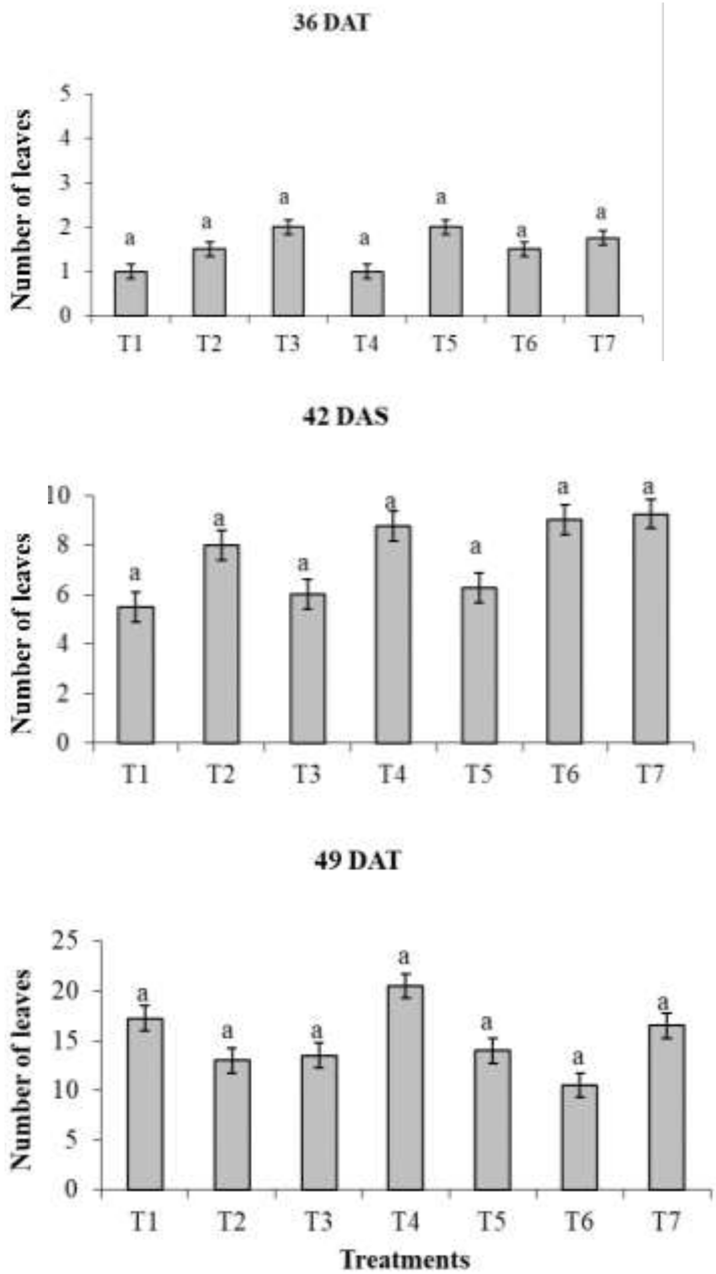

Figure 1. Number of sheets of water deficits with watermelon. Columns with the same letters indicate that there is no statistical difference between the treatments.

Class.

The variables analyzed were number of leaves, leaf area, number of secondary branches and length of the main branch. All variables were valued at 36, 42 and 49 days after transplanting (DAT). The number of leaves and number of branches were obtained through the count. For the determination of leaf area per plant-LA (cm^2), measurements of length and width of each sheet were carried out, using a ruler graduated in centimeters and then calculated using the formula: LA = length × breadth × 0.70 watermelon. The length of the branch was taken.

The data obtained in the experiment for dealing with qualitative variables were subjected to analysis of variance (Tukey test) and there was significant deployment of the degree of freedom, by means of polynomial regression analysis, using statistical software SISVAR, according to Ferreira (2014).

RESULTS AND DISCUSSION

Figure 1 shows that the variable, number of leaves per plant (unit) was analyzed in 36, 42 and 49 days after

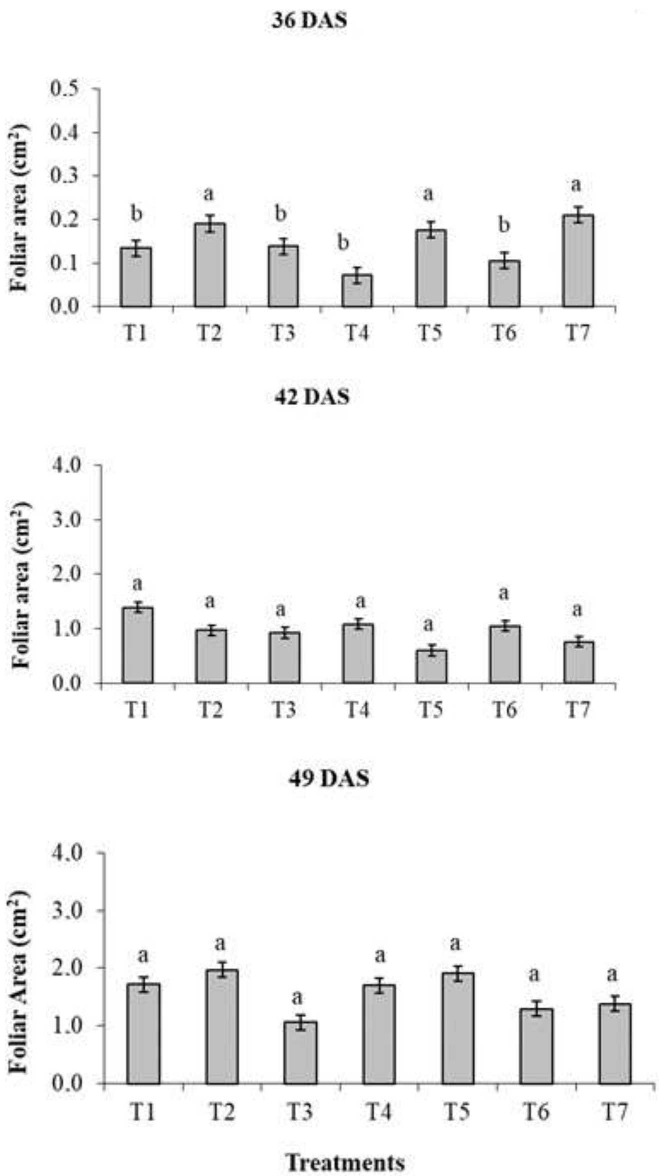

Figure 2. Watermelons foliar area exposed to water deficits. Columns with the same letters indicate that there is no statistical difference.

transplanting (DAT). No statistically significant difference was observed between treatments in any of the seasons. In Figure 1, although there is no significant difference in treatment T4, there was decrease in the number of leaves in 36 DAT, possibly due to water deficit. According to Doorenbos and Kassam (1994), limited provision of water causes a decrease in the production and development of the culture.

At 42 DAT, better treatments included T4, T6, and T7; they provided the area to accomplish photosynthesis. At 49 DAT only T4 was superior to other treatment measures.

There is reduction in the number of leaves due to the small amount of available water. Water stress causes decrease of production in foliar area, the closing of the stomata, the acceleration of senescence and abscission of the leaves; it does not just limit the size of the individual leaves, but also the number of leaves. It decreases the number and rate of growth of the branches (Taiz and Zeiger, 2009).

Aumonde et al. (2011) studying the mini hybrid watermelon, smile at 63 DAT observed increased number of leaves with mean of 109 m^2 leaves in grafted plants. These authors affirm that there was increased number of leaves and large area of photo-assimilation. Lower scores were obtained by Dalastra et al. (2016) with a number of leaves of watermelon with an average of 4.87 leaves.

Similar results were observed by Sousa et al. (2012) who evaluated watermelon plant cultivation under water deficit in the semiarid region of Brazil. Such results can be related to the maintenance of the balance between absorption and respiration. This can increase efficiency in the use of water. Salamoni (2008) enumerates the effects of water deficit on plants, leaf senescence, and reduction in the number of leaves.

There was a significant statistical effect in 36 DAT (5%). In Figure 2, the largest value of the foliar area (0.2103 cm^2) was found in T7 and there was reduction in other middle of that variable at 36 DAT.

Melo et al. (2011) studied water deficit in the culture of melon and found that starting from its cultivation at 18 DAT, foliar area had constant growth for approximately 53 DAT.

In Figure 2, the smallest average leaf area was found in T5 and T7 (0.5997 and 0.7596 cm^2, respectively), showing consistence with the results for the number of sheets in T5. It is observed in Figure 2 that, in general, there was almost a continuous growth of leaf area at 49 DAT, indicating that the leaves have expanded leaf area, thus ensuring the production of photoassimilate for the fruit and plant.

For Braga et al. (2011), reduction in leaf area is caused by the shading of leaves and also by intense photoassimilate allocation for the fruits and maintenance of existing organs. This causes acceleration in senescence and leaf abscission, reduces over cycle and plant efficiency in daily production of dry matter.

In evaluating the growth of watermelon subjected to different water qualities, Costa et al. (2012) discovered that with increased salinity of the water, there is less leaf area, while with reduced salts, the leaf area expanded; however, it was found that same irrigation was accomplished with salt water; there was growth in leaf area. This was also observed by Barros (2008). Superior results for leaf area were obtained by Dalastra et al. (2016), with a mean of 24.88 cm^2.

In Figure 3, at 36 DAT, the highest growth of secondary branches was found in T2 and T7 with values of 7, 5, and 4.75, although there was no statistically significant

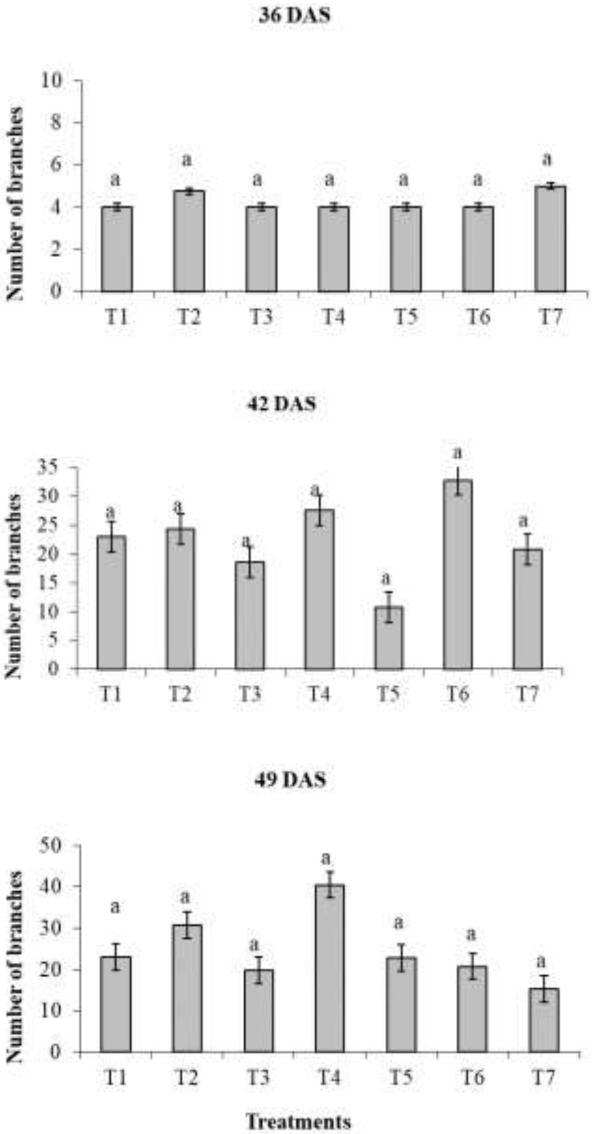

Figure 3. Number of secondary branches watermelon subjected to water deficits. Columns with the same letters indicate that there is no statistical difference.

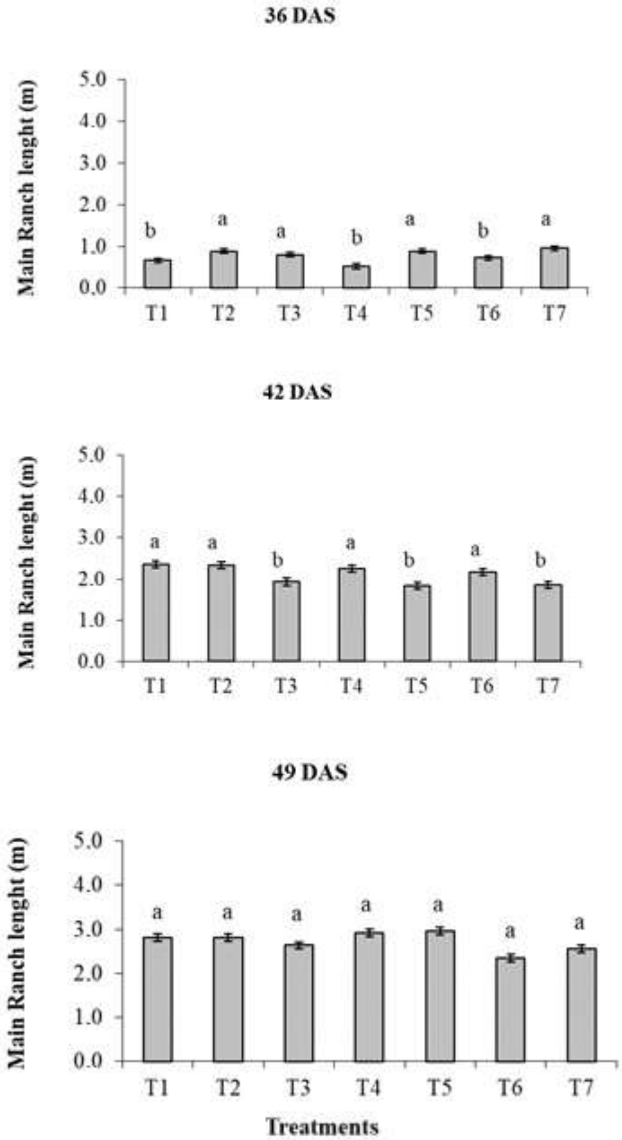

Figure 4. Length of the main branch of watermelon subjected to water deficits. Columns with the same letters indicate that there is no statistical difference.

differences in this variable analyzed during the entire experiment in all treatments. Applying the treatment will not affect the development of watermelon significantly. At 42 DAT, there are evidences of T6 as compared to other treatments. At 49 DAT, the mean was important in T4.

High levels of T6, T4, T2, and T1 in 32.75, 27.5, 25 and 23, respectively and the lowest values were found in T3 and T5 to 18.5 and 10.75, respectively at 42 DAT. At 49 DAT, T4, T2 and T1 remained 40.75, 30.75, and 23, with increasing positive and T5, T6 and T7 with 22.75, 20.75 and 15.25 values were considered low. According to Taiz and Zieger (2009), these changes happen in cultures that insist on growth and reproduction in the place where there are limits on the number of branches and soil water regime in the vegetative stage.

Evaluating different fertilizers, Oliveira et al. (2013), obtained a mean of 3.91 numbers of secondary branches, lower value than those obtained in this experiment in all the evaluation periods.

In Figure 4, the growth variable length of the main branch had a significant effect on statistical treatments until 36 and 42 DAT. Therefore, in this trial period, the amount of water available in plants influences the development of length of the main branch of the culture.

For Melo et al. (2010), there is a strong correlation between leaf area and the length of the branches in watermelon, indicating that the prolongation of the same provides an increase in the number of leaves and photosynthetic capacity improvement. They add that this correlation is influenced significantly by the amount of water available in the soil.

In Figure 4, this variable had the best development at 49 DAT. At 49 DAT, the largest main branch lengths were found in T4 (2.9 cm) and T5 (2.96 cm) treatments. Conflicting results were found by Freitas (1999); there was a high moisture deficit during the phenological and flowering stage. For research on density in the cultivation of melons, Ramos et al. (2009) discovered that there was not a significant effect on the length of the main branch. The results obtained in this experiment and by Ferraz et al. (2011) were higher than those obtained by Alencar et al. (2003) on melon under irrigation. In agreement with Melo et al. (2010), there is an interaction of leaf area with the length of the branches in watermelon; therefore, the higher the length, the higher the number of leaves. It leads to a satisfactory photosynthetic capacity of the plant, being instigated by the amount of water available to the plant.

Oliveira et al. (2013) while studying the growth and production of watermelon with mineral and organic fertilization had the main branch length of 1.17 m, with 1.05 m for treatment with bovine manure, 1.84 m for mineral fertilization and lower average with 0.83 m without fertilization. These results are lower than those obtained in this research.

Researching the efficiency of water application and use in watermelon irrigation, Cavalcante et al. (2015) found that the different irrigation slides significantly influenced watermelon production; high amount of water resulted in greater water use efficiency.

There is a balance of water that favors better development of culture, proper treatment for the different phenological stages, greater efficiency of water use and better results.

Conclusion

Watermelon cultures submitted to two periods under water deficit had similar results with plants that did not suffer water stress. For these three appraisal times, the culture has a significant tolerance for water stress. When applying the correct amount of water to the plant, there is decrease in the amount of water used for irrigation and costs leading to satisfactory results.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Humic acids and brassinosteroid application effects on pineapple plantlet growth and nutrition during the aclimatization phase

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Humic acid and brassinosteroid applications may be an alternative to decrease the pineapple plantlet acclimatization in *in vitro* cultivation, since promising results have been observed when these substances were independently applied in other propagation methods. In this sense, the aim of the present study was to evaluate the effects of humic acids and brassinosteroid application on 'BRS Vitória' pineapple plantlets grown from *in vitro* cultivation during acclimatization. A randomized block design was used in a 5x2x4 factorial scheme, at five brassinosteroid doses (0, 0.25, 0.50, 0.75, 1.0 mg L^{-1}) in the presence and absence of humic acids during four sampling periods (60, 90, 120 and 150 days after transplanting), with five replicates for each treatment. BIOBRÁS-16 was used as the brassinosteroid source, and the organic soil conditioner Agrolmin HF® was used as the humic acid source. Plantlets were collected for evaluation every 30 days from 60 days after transplanting. The number of plantlet leaves, length and root mass were higher in the humic acid treatment without brassinosteroid application. Leaf, nitrogen, phosphorus, potassium, calcium and magnesium contents were of 13.04, 1.77, 40.2, 8.79 and 3.17 mg kg^{-1}, respectively. Nitrogen and potassium contents in the plantlets decreased, while phosphorus contents increased as a function of acclimatization time, regardless of treatment.

Key words: *Ananas comosus* var. comosus, propagation, *in vitro*.

INTRODUCTION

The most planted pineapple cultivars in Brazil are the Pérola and Smooth Cayenne cultivars (AGRIANUAL, 2015), both susceptible to fusariosis, a disease caused by the *Fusarium guttiforme* fungus and the main

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pineapple cultivar disease in the country, responsible for the loss of up to 40% of total pineapple production. In recent years, several research groups have been developing resistant cultivars. In this context, the 'BRS Vitória' pineapple is a promising alternative, since it displays several favorable agronomic traits (Ventura et al., 2007; Ventura et al., 2009).

Specialized biofactories already commercialize 'BRS Vitória' pineapple plantlets that originated from the tissue culture technique. This is an indispensable tool for the availability of matrices throughout the national territory, since it is possible to produce large amounts of excellent quality homogeneous plantlets in a short time and in small space (Berilli et al., 2011). However, it is worth mentioning that this type of plantlet is still not very accessible to Brazilian farmers, due to its high cost (Moraes et al., 2010).

The final cost of a propagule obtained by this technique can reach a cost of up to 10 times more than conventional plantlets. In this context, the development of new technologies that improve this technique is fundamental, in order to offer better standards and cheaper plantlets. The application of certain substances, such as humic acids and brassinosteroids, can serve this purpose, since some studies using these substances, applied in isolated form, in pineapples have obtained promising results (Catunda et al., 2008; Baldotto et al., 2009; Freitas et al., 2012; Santos et al., 2014).

The main brassinosteroid effects are cell stretching and expansion, resistance to stress and delay of leaf abscission (Fujioka and Saakurai, 1997). Deficiencies in the biosynthesis or perception of these hormones generates dwarf plants with a dark green color, presenting leaf epithelia with reduced or no fertility and developmental delays (Bishop and Koncz, 2002).

Humic acid applications can provide higher plant growth and productivity by positively influencing ion transport and facilitating nutrient uptake (Nardi et al., 2002). In addition, these compounds increase respiration and the rate of Krebs cycle reactions, resulting in higher ATP production (Canellas et al., 2002), while also supplying nutrients to plants through their mineralization (Cordeiro et al., 2010).

These technological approaches may lead to increased pineapple plantlet growth in nurseries and may impact the biofactory production system, therefore, making these plantlets more affordable to farmers. In this context, the aim of the present study was to evaluate the effect of humic acid and brassinosteroid application on 'BRS Vitória' pineapple plantlet growth and leaf nutrient content during the acclimatization period.

MATERIALS AND METHODS

The experiment was conducted under greenhouse conditions. During the experimental period, temperature and relative humidity data were collected by the WATCH DOG Weather Station (Weather Station, Spectrum Technologies, Inc), programmed to perform readings at one- hour intervals (Figure 1).

The experimental design comprised a randomized block design in a 5×2×4 factorial scheme, comprising five brassinosteroid doses (0, 0.25, 0.50, 0.75, 1.0 mg L^{-1}) in the presence and absence of humic acids during four sampling periods (60, 90, 120 and 150 days after transplanting), with five replicates for each treatment. The experimental unit was composed of 4 individually grown plantlets.

The BRS Vitória pineapple cultivar plantlets, propagated *in vitro*, were supplied by the BIOMUDAS biofactory, located at Venda Nova dos Imigrantes – ES, Brazil. The plantlets were transplanted into 200-cell polystyrene trays, pre-filled with the commercial Basaplant® Hortaliças substrate mixture, sieved through a 2-mm mesh and 20% of expanded vermiculite was added. The trays were then placed in a greenhouse, characterizing the beginning of the acclimatization process.

BIOBRAS-16® (spiro-static analog of castasterone - (25R) -2α, 3α-dihydroxy-5α-spirostan-6-one) was used as a brassinosteroid source, applied two days after plantlet transplanting and at 30 day intervals throughout the experimental period. The foliar route was used for the growth regulator applications, by means of solution sprays of each respective treatment (0.1% of Tween 20 was added as a surfactant).

The organic soil conditioner Agrolmin HF® was used as a humic acid source. Table 1 displays the chemical characteristics of this product. Humic acid applications were carried out at 15 days after plantlet transplanting and at 15-day intervals during the experiment, at 15 mmol L^{-1} of C, applied directly to the plantlet substrate, with the aid of an automatic pipette (totaling nine applications). Deionized water was applied in the control treatment.

Each week, 2 mL of the nutrient solution, with the following composition, in mg L^{-1}, were applied, per plantlet: $N(NO_3^-)$ = 112; $N(NH_4^+)$ = 3.5; P = 7.74; K = 156.4; Ca = 80; Mg = 24.3; S = 32.00; Cl =1.77; Mn = 0.55; Zn = 0.13; Cu = 0.03; Mo = 0.05; B = 0.27; Fe = 2.23, at pH = 5.5. Every 15 days, 2 mL of a urea solution at 1 g L^{-1} were also applied per plantlet.

The following characteristics were evaluated in the pineapple plantlets in each sampling period; foliar area was carried out on a bench leaf area measurement equipment (model LI-3100 LICOR, Lincoln, NE, USA), using a graduated ruler, with plantlet leaves grouped upwards, measuring from the base to the end of the larger leaf; root and aerial dry mass, after drying in a greenhouse under forced ventilation at 70 °C for 72 hours and finally, nitrogen, phosphorus, potassium, calcium and magnesium contents.

To determine the nutrient content of the aerial portions, the samples were ground and an aliquot from each treatment was weighed and sulfur digested, for nitrogen content determinations, while another was nitro-perchloric digested, for phosphorus, potassium, calcium and magnesium content determinations. Organic nitrogen was determined by the Nessler method (Jackson, 1965). Phosphorus was determined colorimetrically by the molybdate method, calcium and magnesium by atomic absorption spectrometry and potassium by atomic emission spectrophotometry (Malavolta et al., 1997).

The data were submitted to an analysis of variance by the F test, and the means obtained for the humic acid factor were compared by Tukey's test ($p = 0.05$), while the means for the brassinosteroid doses and sampling times were submitted to a regression analysis ($p < 0.05$).

RESULTS AND DISCUSSION

Table 2 displays the data obtained by the analysis of variance with the F values of each evaluated variable. The humic acid applications in pineapple plantlets led to a 6.2% increase in plantlet length at brassinosteroid dose

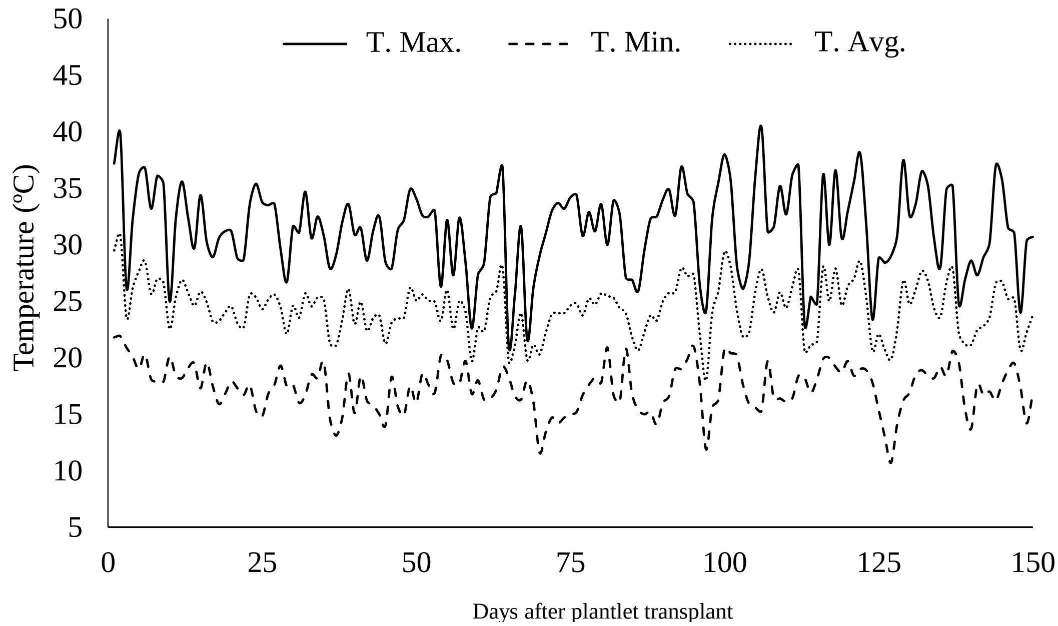

Figure 1. Minimum (T. Min.), average (T. Avg.) and maximum (T. Max.) temperatures recorded in the greenhouse during the experiment, at Campos dos Goytacazes - RJ.

Table 1. Chemical characteristics of Agrolmin® humic acid-based soil conditioner.

C_{org} total	Soluble N	K_2O	Zn	B	Density
		(g L^{-1})			
108	16.2	16.2	3.78	2.16	1.08

Lot: 400.014, Production: 02/25/11.
Source of raw material: Potassium Hydroxide, Urea, Peat, Zinc Sulphate, Boric Acid and Water.

0 (Table 3). Plantlet length is an important biometric characteristic in the acclimatization process, indicated by several biofactories for the determination of the end point of this phase. Normally, plantlets are commercialized for acclimatization when they are 6 to 7 cm long (Berilli et al., 2011).

During the acclimatization phase, pineapple plantlets undergo structural and physiological adjustments, altering their metabolism from mixotrophic to autotrophic. This stage is very time-consuming regarding productive crop cycles, especially due to the growth slowness of both the aerial portion and the root system. Several factors can influence this process and impact plantlet production and adaptation (Barboza et al., 2006).

Both the number of leaves and plantlet length are used to evaluate vegetative growth during the acclimatization phase, mainly because they are correlated to foliar area and the dry mass of the aerial portion. When pineapples grow in favorable climatic conditions and in the field, they emit on average one leaf per week until inflorescence production (Giacomelli, 1982).

The application of humic acids independent of brassinosteroid dose resulted in a 2.6% increase in the number of plantlet leaves (Table 3), in agreement with the results reported by Baldotto et al. (2009), who also evaluated 'BRS Vitória' pineapple plantlets during the acclimatization phase and verified increases in growth rates and in the aerial parts after treatment with humic acids at 15 mmol L^{-1} of carbon. Application of Humic acid applications may have promoted higher plant growth by positively influencing ion transport, facilitating nutrient uptake (Nardi et al., 2002).

As mentioned previously, although plantlet length is one of the most applied characteristics to estimate the end of the acclimatization period, foliar area could also be used for this purpose according to Moreira et al. (2006), since higher foliar area values can provide greater photosynthetic area for the production of organic matter.

Regarding plantlet leaf area in response to brassinosteroid doses as a function of acclimatization periods, the increase rates (regression equations) of the plantlets that received growth regulator applications were higher compared to the treatment that received the lowest dose (Table 4).

The aerial dry mass production of pineapple plantlets was not influenced by humic acid and brassinosteroid applications.

Table 2. Summary of the variance analysis of the variables: number of leaves, length, leaf area, aerial dry mass, root dry mass, nitrogen, phosphorus, potassium, calcium and magnesium contents of 'BRS Vitória' pineapple plantlets with the application of five brassinosteroid doses (BRs) with or without the application of humic acids (HA) evaluated at four sampling times.

Variation causes	F values				
	Number of leaves	Plantlet length (cm)	Aerial dry mass (mg)	Root dry mass (mg)	Foliar area (cm^2)
BRs	1.09^{ns}	2.52**	1.63^{ns}	5.86**	3.00**
AH	6.20**	0.87^{ns}	0.01^{ns}	32.00**	2.45^{ns}
Period	719.97**	856.01**	581.23**	360.42**	919.79**
BRs*AH	0.71^{ns}	3.08**	0.86^{ns}	6.69**	2.17^{ns}
BRs*Period	0.76^{ns}	1.94**	0.40^{ns}	3.42**	6.70**
AH* Period	1.68^{ns}	0.41^{ns}	1.18^{ns}	9.32**	1.35^{ns}
BRs*AH* Period	1.39^{ns}	1.06^{ns}	0.96^{ns}	3.16**	1.72^{ns}
General means	12.67	7.26	286.05	51.8	28.10
CV (%)	7.11	7.22	19.41	23.66	11.88

Variation causes	F values				
	Nitrogen	Phosphorous	Potassium	Calcium	Magnesium
			($g\ kg^{-1}$)		
BRs	2.98**	5.05**	1.24^{ns}	0.43^{ns}	7.68**
AH	0.67^{ns}	10.96**	2.16^{ns}	0.14^{ns}	0.52^{ns}
Period	424.89**	66.34**	84.55**	31.00**	16.18**
BRs* AH	1.53^{ns}	2.65**	4.44**	0.89^{ns}	1.25^{ns}
BRs* Period	2.10**	3.00**	1.55^{ns}	2.56**	1.59^{ns}
AH* Period	0.32^{ns}	1.93^{ns}	0.26^{ns}	9.88**	3.08**
BRs*AH* Period	1.38^{ns}	2.13**	3.24**	0.94^{ns}	0.93^{ns}
General means	13.04	1.77	40.2	8.79	3.17
CV (%)	13.16	15.19	8.78	7.89	13.05

^{ns} non-significant,* significant at $p \leq 0.05$ by the F test and ** significant at $p \leq 0.01$ by the F test.

Table 3. Length and number of leaves of 'BRS Vitória' pineapple plantlets as a function of the application of five brassinosteroid doses acclimatized in a greenhouse.

Application of humic acids	Plantlet length (cm)					Number of leaves
	Brassinosteroid doses (mg L^{-1})					Mean of all brassinosteroid doses
	0	0.25	0.50	0.75	1.00	
With	7.49^a	7.00^a	7.38^a	7.04^a	7.20^a	12.83^a
Without	7.05^b	7.19^a	7.50^a	7.31^a	7.40^a	12.51^b

Means followed by the same letter in the columns do not differ by the Tukey test ($p \leq 0.05$).

Their growth was adjusted with a quadratic equation ($\hat{y} = 0.0004x^2 + 4.7803x - 220.88$ $R^2 = 0.99$ **) as a function of the acclimatization period. However, Catunda et al. (2008) reported an 2.8-fold higher increase in dry mass accumulation in 'BRS Imperial' pineapples treated with 0.1 mg L^{-1} brassinosteroids compared to controls, while Freitas et al. (2012), when evaluating the effect of brassinosteroid doses ranging from 0 to 1 mg L^{-1} on the growth of 'Smooth Cayenne' pineapple plantlets through stem sectioning, found that the estimated dose of 0.68 mg L^{-1} provided higher aerial dry mass.

Plantlet root systems were significantly altered by humic acid applications, with a 63% increase in root dry matter at 150 days after transplanting (Table 5).

Micropropagated plantlets with greater root mass may be more vigorous and can present better adaptations to the acclimation and field planting phases, since the more developed the root system, the greater the nutrient absorption and, consequently, the greater the plant growth. The results of this study corroborate those reported by Baldotto et al. (2010) that observed positive effects on micropropagated pineapple plantlets when humic acids were applied.

The main effects of humic acids on growth are responses to the stimuli this substance causes, similar to the effects of the

Table 4. Increase of leaf area, nitrogen and calcium content in 'BRS Vitória' pineapple plantlets as a function of brassinosteroid (BRs) application at 150 days after the beginning of acclimatization.

BRs (mg L^{-1})	Equation	R^2	Foliar area (cm^2)	Increment in relation to BRS dose 0 (%)
0	$\hat{y} = 0.312x - 5.781$	0.99**	41.02	-
0.25	$\hat{y} = 0.386x - 11.67$	0.84**	46.23	12.7
0.50	$\hat{y} = 0.363x - 8.96$	0.95**	45.49	10.9
0.75	$\hat{y} = 0.344x - 8.134$	0.93**	43.46	5.9
1.00	$\hat{y} = 0.354x - 8.827$	0.97**	44.27	7.9

BRs (mg L^{-1})	Equation	R^2	Nitrogen content (g kg^{-1})	Increment in relation to BRS dose 0 (%)
0	$\hat{y} = -0.1351x + 27.50$	0.84**	7.24	-
0.25	$\hat{y} = -0.1306x + 27.25$	0.85**	7.66	5.8
0.50	$\hat{y} = -0.1033x + 23.13$	0.89**	7.63	5.4
0.75	$\hat{y} = -0.1249x + 26.10$	0.86**	7.36	1.7
1.00	$\hat{y} = -0.1085x + 24.50$	0.88**	8.23	13.7

BRs (mg L^{-1})	Equation	R^2	Calcium content (g kg^{-1})	Increment in relation to BRS dose 0 (%)
0	$\hat{y} = -0.0003x^2 + 0.07x + 5.11$	0.60**	8.41	-
0.25	$\hat{y} = -0.0003x^2 + 0.07x + 5.07$	0.93**	8.36	-0.6
0.50	$\bar{y} = 8.80$	-	8.80	4.6
0.75	$\bar{y} = 8.78$	-	8.78	4.4
1.00	$\hat{y} = 0.0131x + 7.33$	0.59**	9.30	10.6

* Significant at $p \leq 0.05$ by the F test and ** significant at $p \leq 0.01$ by the F test.

phytohormone auxin, which stimulates plasma membrane H-ATPase electrogenic pump activity (Hager et al., 1991), causing cell expansion and activation of secondary transporters in cell membranes (Sondergaard et al., 2004).

When humic acids were applied with 0.25, 0.50, 0.75 and 1.00 mg L^{-1} brassinosteroids, 16, 19, 23 and 39% reduction in root dry mass were observed, respectively, when compared to treatments with humic acid and 0 mg L^{-1} of brassinosteroids (Table 5). These results suggest that the biostimulating effect of humic acids on the root system can be reduced with brassinosteroid applications. Roddick et al. (1993) reported that continued brassinosteroid applications promote root growth inhibition.

Pineapple development is dependent on nutritional status, and when plantlets are cultivated under elemental deficiencies, their growth rate and development can be reduced and may even interfere in later developmental stages (Silva et al., 2016). However, little is known about adequate nutrient content during the pineapple acclimatization phase.

Increasing brassinosteroids doses caused a 13.7% increase in foliar nitrogen levels, when comparing the plantlets that received the lowest dose of this growth regulator to those that received the highest dose (Table 4). It is noteworthy that, regardless of growth regulator dose, nitrogen contents were reduced in 63, 61, 55, 69 and 54% for the 0, 0.25, 0.50, 0.75 and 1.0 mg L^{-1} brassinosteroid doses, respectively, from 60 to 150 acclimatization days (Table 4). However, this effect was lower for the 0.50 and 1.00 mg L^{-1} doses.

These results are in agreement with those reported by Freitas et al. (2012), who verified that brassinosteroid doses exhibited a significant effect on nitrogen content, with an increasing linear behavior, with 1.0 mg L^{-1} providing an 11.1% increase in the nutritional content of pineapple plantlets when compared to the controls. Freitas et al. (2015), when working with 'Cleopatra' mandarin plantlets, reported that 1.0 mg L^{-1} brassinosteroids associated with arbuscular mycorrhizal fungi led to a 15.4% increase in the aerial dry mass nitrogen content.

Diniz et al. (1999) evaluated *in vitro* macronutrient absorption by banana explants, and reported that nitrogen content was higher during the first 10 days, decreasing as a function of culture time. According to the authors, this decrease in nitrogen content in tissues may occur as a function of the dilution effect, due to higher dry mass production. This observation corroborates the results obtained herein, where higher nitrogen content was also observed in aerial portions at 60 days after transplanting, followed by a decrease as a function of time (Table 4).

The highest phosphorus levels were recorded in plantlets that received humic acids alongside brassinosteroids, while the opposite was observed for potassium, where the joint application of humic acids and brassinosteroids led to lower potassium contents when compared to plantlets treated with humic acids only (Table 5).

The 1.00 mg L^{-1} brassinosteroids dose led to a 10.6% increase in leaf calcium content in pineapple plantlets at 150 days of acclimatization (Table 4), while magnesium

Table 5. Increment of root dry mass and phosphorus and potassium contents in 'BRS Vitória' pineapple plantlets as a function of brassinosteroid (BRs) and humic acid (HA) applications at 150 days after acclimatization.

HA	BRs (mg L^{-1})	Equation	R^2	Dry root mass (mg)	Increment in relation to BRS dose 0 (%)
Without	0	$\hat{y}$= 0.7048x - 31.11	0.94**	74.6	-
	0.25	$\hat{y}$ = 0.663x - 25.34	0.88**	74.1	-1
	0.50	$\hat{y}$ = 0.5551x – 13.04	0.87**	70.2	-6
	0.75	$\hat{y}$ = 0.6534x - 21.27	0.91**	76.7	3
	1.00	$\hat{y}$ = 0.4838x – 4.965	0.78*	67.6	-9
With	0	$\hat{y}$= 1.1913x - 56.71	0.97**	121.9	63
	0.25	$\hat{y}$ = 1.0161x - 49.99	0.85**	102.0	37
	0.50	$\hat{y}$= 0.8735x - 31.93	0.83**	99.01	33
	0.75	$\hat{y}$ = 0.8815x - 38.43	0.81**	93.8	26
	1.00	$\hat{y}$ = 0.591x – 14.03	0.86**	74.6	0

HA	BRs (mg L^{-1})	Equation	R^2	Phosphorous content (g kg^{-1})	Increment in relation to BRS dose 0 (%)
Without	0	$\hat{y}$= 0.0002x² - 0.05x + 3.97	0.55**	1.03	-
	0.25	$\hat{y}$=0.0001x² - 0.019x + 2.32	0.68**	1.72	67
	0.50	$\hat{y}$= 0.0002x² - 0.048x + 4.03	0.67**	1.30	26
	0.75	$\hat{y}$= 0.0001x² - 0.02x + 2.44	0.69**	1.69	64
	1.00	$\hat{y}$=0.0002x² - 0.043x + 3.62	0.83**	1.74	69
With	0	$\hat{y}$ = 0.0001x² - 0.019x + 2.48	0.73**	1.86	81
	0.25	$\hat{y}$= 0.0004x² - 0.070x + 4.51	0.99**	3.00	191
	0.50	$\hat{y}$ = 0.0003x² - 0.055x + 4.3	0.73**	2.85	177
	0.75	$\hat{y}$= 0.0002x² - 0.05x + 3.78	0.74**	2.62	154
	1.00	$\hat{y}$= 0.0002x² - 0.035x + 3.20	0.73**	2.30	123

HA	BRs (mg L^{-1})	Equation	R^2	Potassium content (g kg^{-1})	Increment in relation to BRS dose 0 (%)
Without	0	$\hat{y}$= -0.1831x + 57.49	0.95**	30.0	-
	0.25	$\hat{y}$= -0.1345x + 55.34	0.97**	35.3	18
	0.50	$\hat{y}$= -0.0752x + 47.99	0.47**	36.7	22
	0.75	$\hat{y}$= -0.0821x + 48.81	0.77**	36.5	22
	1.00	$\hat{y}$= -0.0791x + 48.88	0.52**	37.0	23
With	0	$\hat{y}$= -0.0579x + 48.49	0.96**	39.8	33
	0.25	$\hat{y}$= -0.0794x + 46.25	0.61**	34.3	14
	0.50	$\hat{y}$= -0.1506x + 55.92	0.87**	33.3	11
	0.75	$\hat{y}$= -0.1651x + 55.75	0.78**	30.9	3
	1.00	$\hat{y}$= -0.1164x + 53.71	0.79**	36.3	21

* Significant at $p \leq 0.05$ by the F test.

content was observed as a function of brassinosteroid application, by means of the following equation: $\hat{y} = -0.368x + 3.35$ $R^2 = 0.65$ **, where contents decreased linearly, with a 11% reduction when a higher dose of the growth regulator was applied compared to the 0 mg L^{-1} brassinosteroid dose.

The highest calcium and magnesium contents were observed when humic acids were applied (Figure 2). Cordeiro et al. (2010) cite that, in addition to the biostimulating effect on plant growth, humic acids can provide nutrients to plants through their mineralization.

The results of this study point out the potential of the use of products based on humic acids and brassinosteroids in decreasing pineapple propagation periods. However, further studies on these treatments regarding acclimatization and on the effective behavior of the plantlets after transplanting them to beds during the acclimatization phase should be carried out.

Conclusions

The application of humic acids via substrate in 'BRS Vitória' pineapple plantlets favors the growth of both the aerial portion and root system, while also increasing phosphorus, potassium, calcium and magnesium

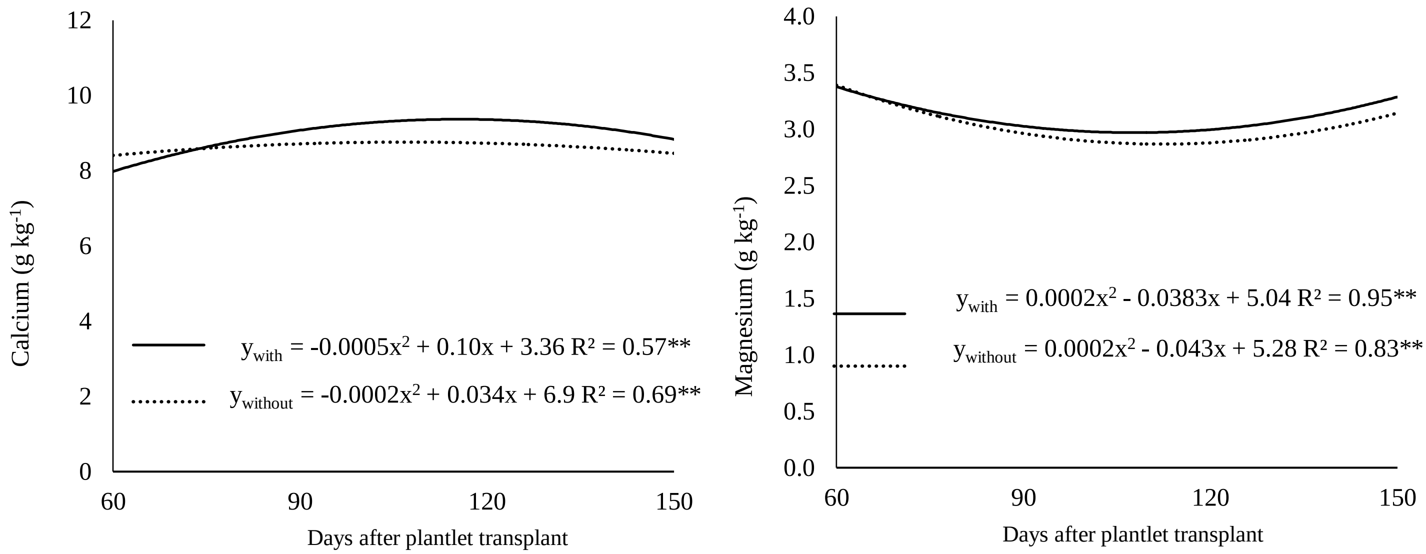

Figure 2. Calcium and magnesium contents of 'BRS Vitória' pineapple plantlet grown with or without humic acids as a function of four periods (60, 90, 120 and 150) after transplanting. * Significant at $p \leq 0.05$ for the F test.

contents, optimizing the acclimatization period. Therefore, this substance can be considered an ally in reducing the acclimatization period.

The application of brassinosteroids at 0.25 to 1.00 mg L^{-1} doses, allied or not to the application of humic acids, promotes a depression effect on the root systems of BRS Vitória pineapple plantlets. On the other hand, the application of this substance increases leaf area and nitrogen, phosphorus, potassium and calcium contents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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