African Journal of Agricultural Research

Volume 11 Number 23 9 June 2016
ISSN 1991-637X

Academic Journals

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Vol. 11(23), pp. 1990-1998, 9 June, 2016
DOI: 10.5897/AJAR2016.10863
Article Number: D6481C458883
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

The analysis of production function and farm marketing efficiency of pineapple (*Ananas comosus* L Merr) in South Sumatera Province, Indonesia

M. Syahirman Yusi

Department of Business Administration, State Polytechnic of Sriwijaya, South Sumatera, Palembang, Indonesia.

Received 2 February, 2016; Accepted 17 March, 2016

This study was based on input and output obtained from 200 random samples of pineapple farm in South Sumatera Province. The major objective of the study is to analyze the input allocation of pineapple farm from six angles: cultivated area, seed, man power, chemical fertilizer (Urea, TSP, and KCL), manure, and insecticide. The result of Cobb Douglas production function approach indicated that, inputs had not been used optimally. Cultivated area, seed, man power, chemical fertilizer, manure, and insecticide should be increased. In the 8 input factors, cultivated area has the biggest influence on pineapple output. In aggregate, the condition of pineapple farm in this area has increasing returns to scale. From the marketing analysis, the farmers who sold their pineapple by using the shortest channel of distribution received more contribution than the other two kinds. Thus, by using the marketing efficiency analysis, the shortest channel of distribution is more efficient.

Key words: Pineapple farm, production function, marketing efficiency.

INTRODUCTION

In the face of global economic era and free trade, horticulture products especially fruits will face intense competition. High quality fruits with sufficient volume and continuous availability are the key in this competition. Pineapple (*Ananas comosus* L Merr) contributes 8% of the world fresh fruit production; Indonesia is the third fresh pineapple producing and processing country after Thailand and Philippines (Hadiati and Indriyani, 2008).

Based on Directorate General of Horticulture-Ministry of Agriculture data, the development of pineapple harvest in Indonesia in 2000 to 2011 increased with an average growth of 10.77% per year. Significant improvement occurred in 2006, that is, 114.50%. Based on the cropping region, 2000 to 2011 harvest area outside Java is higher than outside Java with each of the growth being 18.81 and 9.17% per year. In 2011, pineapple's area harvest in Java is 2,289 ha, while outside Java is 9,506 ha.

The development of pineapple production in Indonesia from 2000 to 2011 showed a fluctuating pattern. The highest pineapple production occurred in 2011, that is, 124.90 ton/ha. Based on the region, in 2011, Java's pineapple has the highest productivity level compared to outside Java; the production of Java reached 158.55

E-mail: msyusi@gmail.com.

ton/ha, while that outside Java was only 114.86 tons/ha. Based on the data of the production of pineapple in 2011, pineapple production center in Indonesia was found in 5 provinces: North Sumatera (183,186 tons), South Sumatera (112,763 tons), Central Java (92,953 tons), Lampung (50,534 tons), and West Java (49,989 tons).

The development of pineapple as agricultural product in Indonesia has not received serious attention as reflected in the harvested area and fluctuating production. This is caused by a variety of reasons; some of them are undevelopment of the use of superior varieties, lack of optimal cultivation techniques, and lack of post-harvest handling by farmers. The available land in South Sumatera has also not been used optimally. The land which is available is 12,332 ha, but it is only 842 ha that is used. The rest of the available land is 11,490 ha. It has direct impact on pineapple production. The production in 2012 was 47,342 tons. In 2013, it was 57,887 tons, a decrease from the production in 2011, that is 112,763 tons (South Sumatera, 2014).

In reality, the purpose of agriculture development in Indonesia is to meet the food needs of the society, the needs of raw material in the country, to prevent migration of villagers to cities, reduce poverty of villagers, increase foreign exchange through export, expand employment opportunities, increase the income of the society, and support the distribution of development results.

Based on the purpose, pineapple as one of agricultural products needs more attention, either in terms of its quantity or marketing system. An increased product without a good support from the marketing system cannot last for long, and can reduce the motivation of farmers to increase their production. Therefore, efficient marketing activities will really affect farmers' passion to be more active in production in order to increase their income and well-being. This has an impact on the overall productivity results.

In narrowedly cultivated areas, most of the farmers in Indonesia and pineapple farmers in South Sumatera, especially farmers weak in capital, when faced with urgent needs and having less information about the market often receive the price. The middlemen, who have the capital, provide temporary loans to the farmers, either in cash or kind. This situation indirectly makes the farmers bound to the traders concerned, that in the time of marketing, the farmers cannot determine their products' price freely. In this situation, the farmers will always get the smallest part of the price paid by the consumers, compared to the price that the brokers get as the marketing agent.

The involvement of the middlemen, if associated with the farmers' skill, actually has a big enough meaning. Moreover, when reviewed in terms of economics which is shown by the function of the traders concerned, collecting pineapple from small quantity to bigger quantity becomes more efficient in its marketing. But the number of the middlemen level involved makes a product distribution channel become longer and it affects the marketing cost of that product (Kotler, 2005). It is known that high or low marketing cost of a product will affect the price of the product that is marketed either at producers' or consumers' level.

Therefore, in an effort to increase the farmers' income, at least, the development of this pineapple's farm must follow not only on how to improve the production, but also on how to market to obtain reasonable profit. So, a research is needed to review the average result from the development of pineapple program in South Sumatera which is closely related with the effort to increase the net income of the farmers as the center point of agriculture development. Research on production and marketing should create the opportunity to increase the farmers' income, so they can be motivated to increase the yield and level of farm production.

MATERIALS AND METHODS

This research was conducted in March 2015 to December 2015 in South Sumatera Province, which is one of the biggest pineapple producers in Indonesia. South Sumatera has an area of 8,701,742 km^2. Agricultural sector has an important role in South Sumatera's economy. This sector was in third position as the sector that gave the highest contribution for the economy besides mining and manufacturing. The contribution from agricultural sector for Gross Regional Domestic Product was 17.28%. The scope of agriculture activities in this province covers several kinds of activities. In order to have detail data, agriculture is classified into some sub sectors which are food crops, estates, forestry, animal husbandry, and fishery. South Sumatera has a variety of horticulture crops such as vegetables and fruits. In 2013, there were 23 commodities of vegetables grown in different areas of regencies. The production of fruits in South Sumatera in 2013 was about 109,131 tons for banana, 57,887 tons for pineapple, and 17,934 tons for siam orange.

Pineapple farm prospect

One of the identified commodities with a great business potential to achieve the goals of the development of Indonesia agribusiness is pineapple (Astoko, 2014). Harvested area of pineapple in Indonesia is 165,690 ha or 25.24% of the national fruit harvest target (657,000 ha). In the last few years, harvested area of pineapple plants was ranked first of 13 types of commercial fruits which are cultivated in Indonesia (TFSC, 2007). In line with this, the market demand of pineapple in the country tends to increase more. In line with the population growth, the society's income is good, due to the increasing awareness of the population about the nutritional value of fruits. Pineapple, besides being consumed fresh, it can also be processed into variety of food products and drinks. Also the waste or by product of the fruits peel and pineapple leaves can be used for food production, paper, and textiles (The Ministry of Agriculture, 2007).

Pineapple is a proven commodity which has good enough market opportunity domestically and abroad. This time, Indonesia has been able to shift the competitors, especially Asian countries; the countries that have become Indonesia export destinations are USA, the European Union, the Middle East, and Latin America. Based on Statistics Center Agency (SCA) data, the volume of pineapple export, either fresh or canned fruit from January to October 2011 reached 161,386 tons or 173.89 million US$. Total production of pineapple in Indonesia reached 1.5 million tons in 2011; 90% is a kind of queen pineapple and the rest 10% is smooth cayenne. In a total of pineapple export (divided into fresh pineapple and processed pineapple), the biggest export in fresh pineapple was addressed to Malaysia with a share of 74%, while Japan had 24.54%. Meanwhile, the countries with the biggest share of processed pineapple are United States of America (22.62%), Netherlands (15.19%), Singapore (13.94%), German (13.86%) and Spain (10.58%). The average volume export to United States of America since 1999 to 2005 is 562,054 tons and is relatively stable every year. But, export to Netherlands, Singapore, German and Spain continues to increase.

Cobb Douglas production function

The effort of increasing the pineapple farm productivity in rural areas of South Sumatera is the utilization of cultivated area which is relatively still small. Chavas (2008) said that the small agriculture area is less economical in production process. In line with this, the labor in the village does not work optimally in managing their farming, because the land that is available for cultivation is too small compared to the labor. As a result, the productivity of labor on average is also low. In terms of the availability of land that is relatively small, the intensification result becomes less comparable with the cost paid and the number of laborers who participated in the production process. The next effect in growing larger number of labor can be classified as visible and invisible underemployment in agriculture sector which is often referred to as disguised unemployment.

In classical economic theory, land is one of the natural resources components which dominantly act as obstacle to output and lead to a marginal decline in labor and capital. It must be admitted that the problem of business capital is a dilemma for the farmers, especially in the research area. The effect of the limitation of capital, is that, many available land cannot be utilized optimally; also, the utilization of the means of production by the farmer is often not in accordance with the one recommended by agriculture extension. The capital function is not only a production factor, but also plays a role in improving the capacity to adopt the technology (Ashari, 2009).

There are many factors that cause low productivity in agriculture sector in developing countries. The most important of all is the lack of agriculture infrastructure. Also farming methods used are traditionally, modern facilities are rarely used, the level of education and farmers' knowledge are low, there are some socio-cultural factors which decrease the farmers' motivation to increase production, and the farmers do not have the means to buy their own necessary agriculture inputs (Todaro and Smith, 2003).

One of the technologies used for production in farming is production function. According to Rasmussen (2013), the production function showed the relation between physical production results and production factors. It is useful as the basic framework for understanding the problem of utilizing the production factors including production process.

In analyzing the production function, there are so many forms of mathematical equations which can be used, but basically there is no singular function of the production function which can reveal the characteristic of the agriculture production appropriately (Echevarria, 1998). The phenomenon is abstracted into the production function model with regard to the assumptions that restrict the model prediction. The production form and magnitude of the parameters coefficient vary by the production factor and specific environment from every production center. However, Cobb Douglas's production function is more specific to be used to predict the function of agriculture production. The advantage of this function is the ranking of the function as well as the elasticity of production from the production factors that are used (Biddle, 2010).

Yuan (2011) used the Cobb Douglas's function model in studying the level of rationality and the efficiency of the uses of agriculture resources in Hebei Province-North China. The independent variables included in this model are cultivated area, effective irrigation area, chemical fertilizer usage, agriculture machinery power, rural electricity consumption, and manpower. From this research, it was found out that the effective irrigation area had the highest influence on agriculture output. Before that, Ionita and Andrei (2010) also used Cobb Douglas's production function in analyzing input-output of agriculture in Rumania. The independent variables included in this model are the capital in agriculture and work force employed in agriculture. The research result showed that the capital in agriculture has dominant influence on agricultural out puts.

Marketing efficiency

In order to increase the farmers' income, besides the needs of increased production, the efficiency of the relevant commodity marketing should also receive an attention, because inefficient marketing system will cause them to receive small profits. This analysis is important, because generally, the agricultural product marketing in Indonesia is the weakest part in the chain of the economy or in the flow of goods. That statement means that the efficiency in this sector is still low, so the possibility to enhance it is still big (Mubyarto, 1995).

The efficiency concept can be divided into two: efficiency economic or the efficiency of the price and technical efficiency or operational efficiency (Norwood and Lusk, 2007). The economic efficiency concerns the operation of the aspects of the services and the marketing cost which consists of the purchase, sales, and price formation. The technical efficiency is related to the reduction of input expenses to produce a number of goods and services, and whether the physically distributed goods has been conducted well.

In agricultural product marketing, the formation price that occurs in each of the market agents is different, where the marketing margin in each of the agent is bigger with an increase in the marketing channel that has been through, besides the change in demand and supply in every level (Abassian et al., 2012). Many levels of marketing organization involved in the process of distribution of the goods from the producer to the consumer indicated the length of the marketing distribution. The length of marketing distribution of a product makes the marketing cost bigger and the profit accumulated value will become bigger. Situations like this will affect the price of a product either at producers' level or consumers' level; and at the end it will affect the amount of the marketing margin.

Rit (2014) said that, the marketing system is considered efficient if it can divide the margin of a whole price paid by the last consumer to all the agents involved in the production activity and commodity marketing. Tomek and Kaiser (2014) define marketing margin as: (1) the difference between the price paid by the consumer with the price received by the producer, and (2) the collection of the retribution received by the marketing agent, as a result of demand and supply. Moreover, they declare that the efficient marketing system is when the price paid by the consumer and the number of the products offered by the farmers will not affect the marketing margin. The margin percentage for each level of the institution is constant. If this situation occurs, it means that the farmers as the producers, the middlemen, and the consumers are in a perfectly competitive market structure.

Research sample

In this research, the sample unit, besides the pineapple farmers and middlemen involved in the marketing activity, consists of

Table 1. The definition of variable operationalization.

Variable	Definition	Unit of measurement
Production (Y)	Production per farm size in 2013-2014 growing season	Quintal
Cultivated area (X_1)	The land area planted with pineapple in monoculture	Hectar
Seed (X_2)	The number of seeds used in every hectar of planted area	The number of seeds
Man power (X_3)	The number of hours per day	Male = 8 h, Female = 0.75 h
Urea fertilizer (X_4)	The number of urea fertilizer	Kilogram
Tsp fertilizer (X_5)	The number of TSP fertilizer	Kilogram
Kcl fertilizer (X_6)	The number of KCL fertilizer	Kilogram
Manure (X_7)	The number of manure	Kilogram
Insectiside (X_8)	The number of insectiside	Litre
Price per unit of variable X (P_X)	Average price of inputs	IDR
Price per unit of variable Y (P_Y)	Average price of outputs	IDR

Table 2. The definition of marketing variable.

Variable	Definition	Unit of measurement
Marketing margin	The number of margin obtained in every traders, counted by the difference between selling price and purchase price after minusing the amount of marketing cost	IDR
Price	Traders buying price of pineapple from farmers	IDR
The volume of goods	The amount of pineapple sold by farmers or traders in one transaction	unit
Marketing cost	The cost paid by every traders in selling their products	IDR
Marketing profit	The retribution obtained by every traders	IDR
The farmers's share	The share received for farmers from the price paid by consumers	IDR
Channel of distribution	The number of distribution channel involved in the process of delivery of goods	The number of channel distributions

traders collectors in village, district, local big traders collector, and traders outside the region. The retailers are not used as the unit of analysis, because the retailer role is more assumed by the trader collector at village level.

To obtain the data that can represent the situation of pineapple agriculture in South Sumatera, the stratified cluster sampling is needed. First, South Sumatera Province is divided into district classes. Then, the district with pineapple planting area are more than 500 ha is defined as the unit of sampling primer, and from that sampling unit, primer is taken entirely. The number of sample farmers is determined randomly, that is, 200 farmers who are the number of samples in every district according to the proportional allocation.

In marketing, the first thing is to observe the existing marketing channels. Then, note every pineapple distribution from the farmers as producers to the last customers, either at the price level, marketing cost paid, and profit level received by each of the middlemen involved.

Operationalization of variables

The concept of basic relation in production process is shown by algebraic relation, which is a production function. In certain cases, economic theory gives the indication of the dependent variable (Y) which is influenced by the independent variable (X_i). The operational definition from the independent variable and dependent variable is shown in Table 1. Meanwhile, the measurement of marketing variable is shown in Table 2.

Analysis method

Analysis method which is used to suspect production factor affects the development of pineapple production in South Sumatera using Cobb Douglas's production function. Systematically, that function is as follows:

$$Y = \alpha \sum_{i=1}^{8} X_i^{\beta i} e^u$$

The linear form from the equation is:

$$\ln Y = \ln \alpha + \sum_{i=1}^{8} \beta_i \ln X_i + \ln u$$

Based on the assumptions used to obtain a good prediction, the value of e^u is zero; so the marginal product (MP_{Xi}) and elasticity of production are considered as follows:

Table 3. The parameter estimation of the production function of pineapple farm in South Sumatera.

S/N	Variable	Parameter	Coefficient	t_{cal}
1	Intercept	lnα	4.2158	-
2	Cultivated area	β_1	0.3175***	2.6046
3	Seed	β_2	0.2094**	2.3205
4	Man power	β_3	0.0846	1.6583
5	Urea fertilizer	β_4	0.2163***	3.2162
6	Tsp fertilizer	β_5	0.2012***	2.4913
7	Kcl fertilizer	β_6	0.2145**	3.1202
8	Manure	β_7	0.0492^{ns}	-
9	Insectiside	β_8	0.1461**	2.2683
		n	150	-
		R^2	0.9274	-
		F	36.4235	-

***Significant at 0.01, **significant at 0.05, ns: no significant.

$$MP_{Xi} = \frac{dY}{dXi} = \alpha\beta_i X_i^{\beta i-1}$$

$$= \alpha\beta_i \frac{X_i^{\beta i}}{X_i}$$

$$= \beta_i \frac{Y}{X_i}$$

$$E_{Y.Xi} = \frac{dY}{dXi} \times \frac{Xi}{Y}$$

$$= \beta_i \frac{Y}{X_i} \times \frac{Xi}{Y}$$

$$= \beta_i$$

The sum of the regression coefficients (elasticity) in Cobb Douglas's production function can automatically indicate the returns to scale (Kilmer and Armnbruster, 1984). If $\sum \beta i > 1$ then production function is in incerasing returns to scale phase. Constant returns to scale phase is if $\sum \beta i = 1$, and decreasing returns to scale is if $\sum \beta_i < 1$. The criteria for the determination of efficiency level can be done by comparing the marginal product value from every production factor (MPV_{Xi}) with the price per unit of production factor (P_{Xi})

In marketing analysis, that is, by studying the relation between the price received by the farmers with the retail price, the cost and the profit of marketing institution, effort to increase market share received by the farmers as producers with the formula can be analyzed:

$$M = \sum P_{ci} - P_{fi}$$

M = marketing margin

P_{ci} = the price in the level of consumers per unit to i

P_{fi} = the price in the level of farmers per unit to i

$$\frac{MPV_{xi}}{P_{xi}} \begin{cases} =1: \text{the utilization of production function is efficient} \\ >1: \text{the utilization of production function is not efficient (should be increased)} \\ <1: \text{the utilization of production function is not efficient (should be reduced)} \end{cases}$$

The large number of the farmers' part can be known with the formula:

$$F_S = \frac{P_f}{P_c} \quad \text{or} \quad F_S = \frac{P_c - M}{P_c} = 1 - \frac{M}{P_c}$$

where F_S is the share received by farmers, P_f is the price at the level of farmers, and P_c is the price at the level of consumers.

Next, in order to measure the efficiency level of marketing, the concept of Shepherd (1982) is used, that is, the comparison between the amount of sales and marketing cost. If the result of the measurement is high, it means that the marketing activity is efficient. Otherwise, if the result of the measurement is low, it means that the marketing activity is not efficient.

RESULTS

Coefficient estimation of production function

The estimation of production function of pineapple farm in South Sumatera Province is shown in Table 3.

Table 4. Marginal product value and price per unit of production factors.

S/N	Variable	β_i	MPV_{xi}/P_{xi}
1	Cultivated area (ha)	0.3175	4.6982
2	Seed (number of units)	0.2094	3.8263
3	Man power (working hours)	0.0846	1.0531
4	Urea fertilizer (kg)	0.2163	6.9485
5	TSP fertilizer (kg)	0.2012	9.3876
6	KCL fertilizer (kg)	0.2145	8.5967
7	Manure (kg)	0.0492	3.8563
8	Insecticide (L)	0.1461	4.2918

Marginal product value and price per unit of production factors

The result of the analysis about the efficiency of the production factor in pineapple farm in South Sumatera is shown in Table 4.

Marketing margin analysis

The spread margin on the third marketing distribution channel found in pineapple marketing in South Sumatera showed variation among all the distribution channels.

DISCUSSION

In Table 3, we can see that, partially the production factor of cultivated area, seed, manpower, chemical fertilizers (Urea TSP and KCL) significantly influence the pineapple production at 95% level. Even though positive, the variable of the manure has no effect on the pineapple production. This is due to inappropriate use of manure by some farmers; so it is not suitable with the recommended size. Meanwhile the determination coefficient (R^2 = 0.9274) showed that the capability of independent variable to explain the diversity that occurs in the dependent variable is 93%.

As the value of the coefficient of Cobb Douglas's rank function is an elasticity value of the production factors involved partially, then the result estimation of the coefficients rank function can be used to explain the effect of each production factors on pineapple production result in South Sumatera. From the production elasticity of the cultivated area (0.3175), an additional cultivated area of 100% will increase the production by 31.75%. Production elasticity of seed and man power of 0.2094 and 0.0846 means that an additional seed and man power of 100% will increase the production by 20.94 and 8.46%. Urea fertilizer, TSP and KCl's production response of 0.2163, 0.2012, and 0.2145 mean that additional urea fertilizer, TSP and KCL of 100% will increase the production by 21.63, 20.12 and 21.45%. The manure did not affect the production, but the value is still positive. The elasticity of the insecticide production factor of 0.1461 shows that an additional 100% of that production factor will increase the production by 14.61%.

According to the Cobb Douglas production function, if the number of the regression coefficients result is summed may indicate the returns to scale conditions. The estimation result of the production function in Table 3 showed that the number of the function coefficients is bigger than 1.2076. Therefore, it can be concluded that the scale of pineapple farm condition in South Sumatera region has increasing returns to scale condition. It means that, the enhancement of the used production at 100% will increase the production by 120.76%.

The allocation levels of factors of production in pineapple farm in South Sumatera are close by the economic efficiency approach. The magnitude of the ratio between the marginal product value from each of the production factor (MPV_{Xi}) with price per unit of production factors (P_{Xi}) may give an indication of the needs for the addition or subtraction of the use of the production factors concerned (Darko and Gilbert, 2013). Efficiency analysis of the use of production factors is not only connected with the farming production activity in a certain period which is not affected by the previous period. Therefore, this approach shows that the production process is a closed system. That assumptions provide the limits that every variable which is used in the process of production purchased at the start of the production activity and sold at the time of production period ended; so the time element has no effect on the price variation.

In Table 4 the use of the cultivated area production factor, seeds, man power, chemical fertilizers (Urea, TSP and KCL), manure, and insecticide is at optimal level. It shows the ratio value is bigger than 1. The bigger profit can be achieved by increasing the use of production factors. The addition of land area for pineapples farm in South Sumatera will increase the number of production for the farmers, and until now, there are many land which has not been used economically (South Sumatera in Figure 2014). This result is in line with the research of Blank et al. (2009) who state that larger cultivated areas will have significant effect to the welfare of the farmers

Table 5. The spread of marketing margin on three distribution channels.

Description	Marketing channels (%)		
	First	Second	Third
The margin of traders in village			
Profit margin	2.46	-	3.54
Cost margin	2.62	-	3.26
The margin of traders in district			
Profit margin	3.25	2.84	-
Cost margin	3.06	3.62	-
The margin of local wholesalers			
Profit margin	1.86	2.52	4.61
Cost margin	5.16	4.95	5.73
The margin of traders in outside region			
Profit margin	2.89	2.96	-
Cost margin	2.76	2.81	-
Farmers share	75.94	80.30	82.86
Amount of profit margin	10.46	8.32	8.15
Amount of cost margin	13.60	11.38	8.99

compared to narrow cultivated areas.

Besides that, buying other production factors, for example the use of chemical fertilizers (Urea, TSP and KCL), manure and insecticide become very important. But the problem is lack of capital, making the farmers unable to fund that production factor. This would make the developments of pineapple farms in the areas of research become difficult.

The research is in line with the invention of Ionita and Andrei (2010) who said that the availability of capital in agriculture is the most important thing. Yao and Alles (2006) also said that the theoretical rules are to get enough resources to fund a business activity in order to increase operational performance and profit at a specific time. Capital is the main drive in developing an enterprise. Capital can: (1) assist farmers in overcoming the limitations of capital with relatively small interest, and (2) reduce the dependence of the farmers on middlemen and money lenders (Ashari, 2009).

From three existing marketing channels in Table 5, it is shown that the percentage of the farmers' share is smallest is in the marketing distribution in the first type. Next is in the marketing channels, which are the spread of margin, either profits or cost margin. The spread is uneven. The biggest profits margin is obtained by the traders collectors at district level, while the biggest margin cost is obtained at local wholesaler level. This situation is same with the type of the second marketing, but here the biggest profits margin is obtained by the traders outside the regions. If we compare it with the marketing distribution in the third type, the percentage of the farmers' part which is received on this second marketing channel is still small. The spread of this third type of marketing margins also varies. The biggest profits and costs margin in this channel are found in the local wholesalers.

According to Mubyarto (1995), the marketing system could be said to be efficient if in delivering the result of the production from the farmers to the customers (end users), it is implemented with the lowest cost and equitable share from the overall price paid by the consumer to all parties involved in the marketing activity. According to Penson et al. (2015), the magnitude of profits margin taken by the middlemen and the low parts taken by the farmers reflect the marketing system that is not efficient.

Regarding the magnitude part received by the farmer from the price paid by the last consumer, the situation of the spread margin and the large amount of profits margin in the third channel of pineapple distribution in South Sumatera, the third marketing channel is more efficient than the first and the second marketing channel. On the third marketing channel, the farmers obtained the biggest part from the price paid by the consumer, compared to the first and second channels. It is because the third marketing channel between the farmers as the producers with the last consumer is relatively closer. Therefore, the selling price which is the absolute acceptance of farmers becomes larger. This thing is appropriate with the invention of Setyowati (2008), who researched on the milk marketing in Boyolali, Central Java. She said that the shorter marketing channel will give the bigger profits for the farmers as the producers. Asogwa and Okwoche (2012) also emphasize that the need for the establishment of farmer's cooperative in marketing their product in order to reduce the role of the middlemen in deciding the price of agricultural products in market.

Conclusion

The variance analysis conducted on a model production function of Cobb Douglas provides the significant ratio value. It means that the pineapple farm in South Sumatera simultaneously is affected by the production factor: cultivated area, seed, man power, chemical fertilizers (Urea, TSP and KCL), manure and insecticide. The production factor which has the dominant influence is the cultivated area. Every increase and decrease from the production factors will cause a change in the number of overall pineapple production results. Pineapple farming scale conditions in South Sumatera are increasing returns to scale. It means that the number of the result can be improved if the production factors used are maximum.

The process of delivering the pineapples from farmers as producers to the hand of consumers consists of three types of marketing channels, each of which is different. A longer marketing channel will give the farmers relatively smaller value compared to the shorter marketing channel. The shorter marketing channel is more efficient and profitable because it provides bigger income for the farmers in marketing their products.

Bigger profits can be achieved with increased use of all the factors of production. To increase the use of other production facilities, government‘s aid in terms of capital assistance is really expected. Generally, the capital of the pineapple farmers in this region is still low; they need help.

In marketing, there is need to reduce magnitude of the marketing margins as one of the effort to create an efficient marketing system. This can be done by reducing the gains and marketing spending of every middlemen involved. It is necessary to establish a cooperative that can act an agency liaison between the farmers and the consumers.

Conflict of Interests

The author have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author is thankful to the Director of State Polytechnic of Sriwijaya for supporting this research. Sincere thanks are due to the farmers of pineapple in South Sumatera involved in this research for their cooperation.

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academicJournals

Vol. 11(23), pp. 1999-2006, 9 June, 2016
DOI: 10.5897/AJAR2016.10955
Article Number: 572DEB258888
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Productivity and economic analysis of sunflower/maize crop rotation under different levels of salinity and nitrogen

Hernandes de Oliveira Feitosa[1], Claudivan Feitosa de Lacerda[2]*, Albanise Barbosa Marinho[3], Raimundo Nonato Távora Costa[2], Clayton Moura de Carvalho[1] and Hans Raj Gheyi[4]

[1]Technological Education Center Institute, Juazeiro do Norte, Fortaleza, Ceará, Brazil.
[2]Agricultural Engineering Department / Federal University of Ceará, Campus do Pici, CEP: 60.055-460, Fortaleza, Ceará, Brazil.
[3]Rural Development Institute / University of International Integration Lusophone African-Brazilian, Redenção, Ceará, Brazil.
[4]Nucleus of Soil and Water Engineering, Federal University of Recôncavo of Bahia, Cruz das Almas, Bahia, Brazil.

Received 2 March, 2016; Accepted 23 April, 2016

This study aimed to assess the effect of salinity of irrigation water and nitrogen fertilization on yield and oil production of sunflower, cv. BRS 324, and yield of maize hybrid AG1051, as well as economic analysis of crop rotation (sunflower/maize). The experimental was laid out in a randomized complete block design in split plot with five replications, the plots were formed by five levels of electrical conductivity of the irrigation water (0.8, 2.2, 3.6, 5.0 and 6.4 dS m^{-1}) and the subplots by four rates of nitrogen (0, 25, 50 and 75 kg ha^{-1}), in sunflower crop. For maize crop the same design was used, studying on the same plots the residual effects of salts and four nitrogen rates (0, 108.5, 217 and 325.5 kg ha^{-1}). Sunflower was grown in dry seasons (2011 and 2012), while maize was grown in the rainy seasons (2012 and 2013). For the sunflower crop, the productivity and potential oil production showed the highest values when the plants were not salt-stressed and subjected to the maximum dose of nitrogen. But yield was decreased with increasing salinity, even with increasing the rate of nitrogen. For the maize crop, productivity was strongly influenced by increasing the rate of nitrogen; the leaching of salts, caused by rainfall, virtually eliminated any residual effect of the salts on this crop. A soil management system employing sunflower-maize crop rotation was found to be economically viable, being more attractive when using low saline water and the highest application rate of nitrogen.

Key words: *Helianthus annuus* L., *Zea mays* L., salt stress, nitrogen fertilization, economic viability.

INTRODUCTION

In Brazil and around the world, the demand for energy sources that meet the goals set by the Kyoto Protocol has generated the need to replace fossil fuels (the greatest generator of greenhouse gasses), by energy

*Corresponding author. E-mail: cfeitosa@ufc.br.

Table 1. Physical and chemical attributes of the soil in the experimental area.

Attribute	Depth (m)	
	0 - 0.30	**0.30 - 0.60**
Textural class	**Sandy loam**	**Sandy clay loam**
Soil Density (kg dm^{-3})	1.39	1.42
Ca^{2+} ($cmol_c$ dm^{-3})	0.83	1.2
Mg^{2+}($cmol_c$ dm^{-3})	0.82	0.80
Na^{+} ($cmol_c$ dm^{-3})	0.25	0.33
K^{+} ($cmol_c$ dm^{-3})	0.12	0.12
ESP (%)	5	7
H^{+}+Al^{3+} ($cmol_c$ dm^{-3})	2.0	2.4
Al^{3+} ($cmol_c$ dm^{-3})	0.30	0.45
pH in water (1:2.5)	5.6	5.4
ECse (dS m^{-1})	0.20	0.28

EC_{se}, Electrical conductivity of saturation extract; ESP, exchangeable sodium percentage.

derived from biomass or other alternative sources (wind, solar radiation, etc.). As it is a renewable resource, and due to its sustainable use considerably reducing damage to the environment, there has been much research into the use of biodiesel in programs of renewable energy. Projections indicate Brazil as the country with the greatest potential in the production of vegetable oil for biodiesel, especially of oilseed crops such as castor bean, palm oil, sunflower and cotton, among others.

The European Union currently leads the market in the global production of biodiesel, despite the relative scarcity of arable land, designing its market for the addition of 8% biofuel by 2020, and with a focus on the environment. In Brazil, and around the world, competitiveness of the biodiesel market depends closely on tax exemption of the product, since production costs are higher compared to diesel.

In addition to economic problems, the productivity of oilseed crops is also hampered by such abiotic factors as water deficit, salinity and nutrient deficiency. Salinity is a common problem mainly in the soils and sources of water of semi-arid regions, and leads to reduced productivity in most crops due to the direct and indirect effects of a high concentration of salts on plant physiology and metabolism. This limits the expansion of irrigated agriculture, an extremely important factor in the development of these regions (Chen et al., 2009).

Mineral nutrition is another aspect that is directly linked to agricultural production, due to the high demand for nutrients such as nitrogen for obtaining high productivity. This problem becomes more complex under saline conditions, considering that the excess of certain ions in the root zone can affect the absorption of some essential elements; for example, an excess of Cl can limit the uptake of nitrates. On the other hand, the restrictions on growth caused by salinity also reduce the need for nutrients (Neves et al., 2009; Lacerda et al., 2016). This may increase nutrient loss, which is already quite high in non-saline environments.

Medium and long-term studies have demonstrated the need to work simultaneously with different management strategies under saline conditions, and particularly when using brackish water for irrigation. Among these strategies can be highlighted the use of tolerant or moderately tolerant crops, the use of crop rotation and the use of organic and mineral inputs. For example, the use of high doses of nitrogen has been suggested as a way to minimise the harmful effects of salinity (Gengmao et al., 2014), although this has not often been demonstrated by results (Lacerda et al., 2016).

The aim of present study was to evaluate the productivity and economic viability of sunflower/maize crop rotation, as a function of the salinity of the irrigation water used during the dry season. The survey also sought to evaluate whether an increase in the rate of nitrogen overcomes the effects of salinity under this cropping system.

MATERIALS AND METHODS

The study was carried out in the Experimental area of the Department of Agricultural Engineering at the Federal University of Ceará, in Fortaleza (3°45' S, 38°33' W, at an altitude of 20 m), Ceará, Brazil, during a period of 24 months (06/2011 to 06/2013). A sunflower and maize crop rotation system was used, with cultivation of sunflower during the dry seasons of 2011 and 2012, and maize in the rainy seasons of 2012 and 2013.

According to the Köppen classification (Köppen, 1948), the local climate is of type Aw', that is, rainy tropical, very hot, with a predominance of rainfall in the summer and autumn, and an average annual temperature of 27.1°C. Table 1 shows the physical and chemical properties of the soil, obtained before starting the experiment.

Information on the weather variables obtained during the dry season (sunflower crop) and the rainy season (maize) are shown in Table 2. The sunflower (*Helianthus annuus* L.) cultivar BRS 324 was used. This is an open-pollinated, early variety, displaying, on average, high oil content, around 47%, with a productivity

Table 2. Climatic parameters of the experimental area and irrigation depths (ID) applied during each cultivation.

Month	Maximum temp. (°C)	Minimum temp. (°C)	RH (%)	Insolation (h month^{-1})	ETo (mm)	Rainfall (mm)	ID (mm)
First cycle (sunflower) – 2011							
Sept	31.6	22.8	59	168.4	159.4	0.0	76.87
Oct	31.0	23.6	66	288.5	258.6	23.8	166.24
Nov	31.1	24.0	68	296.5	242.4	7.3	133.53
Dec	31.2	24.3	65	202.9	163.7	2.8	45.61
Mean/sum	**31.2**	**23.7**	**64.5**	**956.3**	**824.1**	**33.9**	**422.25**
Second cycle (maize) – 2012							
Feb	30.2	22.8	81	95.8	108.8	237.6	9.2
Mar	30.5	23.1	78	216.3	194.4	488.6	75.1
Apr	30.6	23.7	77	240.6	201.4	170.3	75.4
May	30.7	23.6	72	284.2	208.7	101.3	94.5
Mean/sum	**30.5**	**23.3**	**77**	**836.9**	**713.3**	**997.8**	**254.2**
Third cycle (sunflower) – 2012							
Sept	31.0	23.4	61	203.2	194.3	0.0	75.53
Oct	30.9	23.4	63	294.9	276.4	10.0	198.78
Nov	31.3	24.0	63	308.9	263.7	0.9	155.58
Dec	31.4	24.5	67	187.9	163.0	2.0	46.58
Mean/sum	**31.1**	**23.8**	**63.5**	**994.9**	**897.4**	**12.9**	**475.94**
Fourth cycle (maize)- 2013							
Apr	31.1	23.7	78	143.6	116.4	111.1	68.4
May	31.2	23.4	73	234.6	173.7	155.6	129.5
Jun	30.7	22.6	77	216.2	164.1	168	125.2
Jul	30.3	22.5	69	266.1	190.1	91.0	53.2
Mean/sum	**30.8**	**23.0**	**74.2**	**860.5**	**488.3**	**525.7**	**373.3**

RH, Relative humidity; ETo, Evapotranspiration of reference.

of 1,600 kg ha^{-1} as a rainfed crop. The other crop used was maize (*Zea mays* L), double cross hybrid AG1051. This has a semi-early cycle, high ear insertion, a cycle from 90 to 120 days, with a productivity of 4,500 kg ha^{-1} under rainfed conditions.

The experimental design was a randomized block in a split plot. The plots (40 m^2) consisted of five levels of irrigation water salinity , and the subplots (10 m^2) of four doses of nitrogen fertilizer, with five repetitions. Irrigation with different salinity water was only used in sunflower cultivation during the dry season.

For sunflower crop, the treatments with saline water were S1 = 0.8 (well water), S2 = 2.2, S3 = 3.6, S4 = 5.0 and S5 = 6.4 dS m^{-1}; nitrogen doses were N1 = no fertilizer, N2 = 25, N3 =50 and N4 = 75 kg N ha^{-1}, with the dose of 50 kg N ha^{-1} being recommended. Urea was used as the source of N. To prepare the saline water treatments, the salts NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ were added to the well water, using an equivalent ratio of 7:2:1, complying with a relationship between EC and salt concentration (mmol$_c$ L^{-1} = EC x 10), as per Rhoades et al. (1992). The characteristics of the well water were: EC= 0.8 dS m^{-1}, pH = 6.9, and Ca, Mg, Na, HCO_3^- and Cl^{-1} equal to 1.1, 1.2, 4.0, 2.7, and 3.7 mmol$_c$ L^{-1}, respectively.

The same plots with sizes of 50 m^2 and subplots with an area of 12.5 m^2 previously cultivated with sunflower were used for the purpose of evaluating the residual effect of saline water on maize plants. However, supplementary irrigation with well water (EC = 0.8 dS m^{-1}) was used for this crop, with the following levels of nitrogen: N1 = no fertilizer, N2 = 108.5, N3 = 217 and N4 = 325.5 kg N ha^{-1}; with the rate N3 (217 kg N ha^{-1}) being the dose recommended for maize according to technical recommendation. Urea was used as a source of nitrogen.

Sixty-five days before sowing the sunflower, liming based on soil analysis was carried out using dolomitic limestone (1.8 Mg ha^{-1}), and incorporated by ploughing followed by a single harrowing. Five days before sowing, the soil was ploughed and then cross-harrowed twice.

Water depths applied in irrigation were calculated using data for reference evapotranspiration, as determined by the Class A tank method, and the crop coefficients of the sunflower and maize (Doorenbos and Kassam, 1994), in the latter case, only for supplementary irrigation. A pressurised drip-irrigation system was used for both crops.

The sunflower was sown with four seeds per hole, at a spacing of 0.8 × 0.3 m, and a density of 41,666 plants ha^{-1}. Ten days after sowing (DAS), thinning was carried out leaving only one plant per hole. Fertilization was performed in the furrows, and was based on soil analysis and the recommendations: 80 kg ha^{-1} of P_2O_5, 70 kg ha^{-1}of K_2O, and 10 kg ha^{-1} of FTE BR12. The single superphosphate and the FTE were applied as basal dose of sowing, while the urea and potassium chloride were divided up, with 1/3 being applied as basal dressing and 2/3 as top dressing at 31 DAS. To meet boron

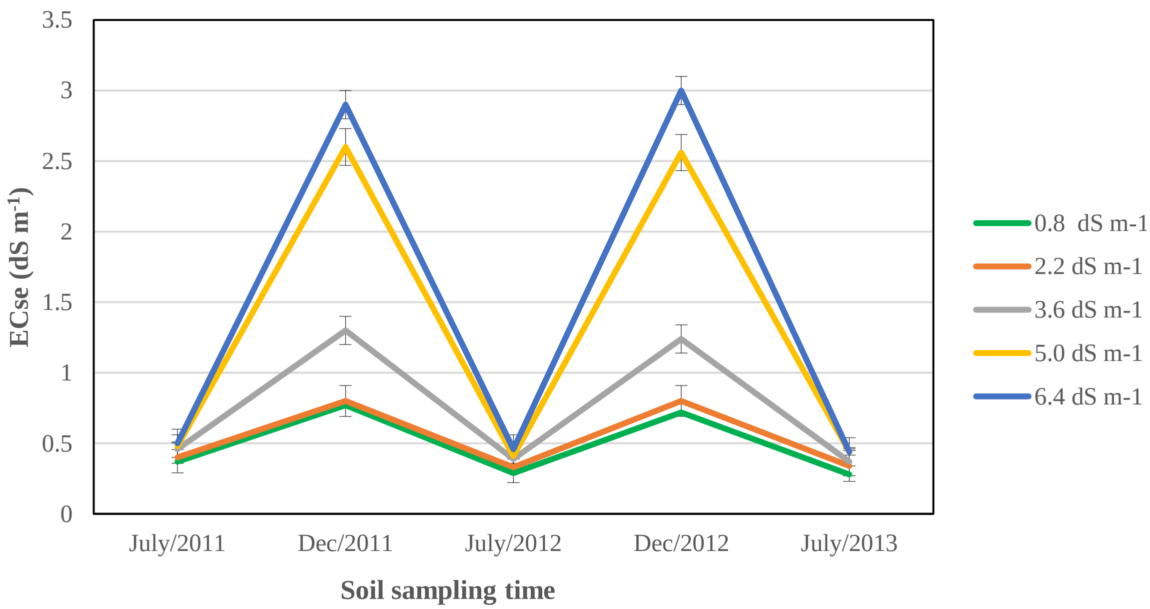

Figure 1. Variations in electrical conductivity of the saturation extract of the soil before and after each crop of sunflower and maize.

needs 4.0 kg ha^{-1} of boric acid were applied, as recommended by Castro et al. (2006).

After each crop, the plant residue was mown once, and all remaining biomass was maintained in the respective plots. At the beginning of the rainy seasons of 2012 and 2013, after sunflower crop, the maize was sown, using three seeds of the cultivar AG1051 per hole, following the same order of treatment, and following the same spacing, as the previous crop. On the tenth day after germination, the plants were thinned, leaving only one plant per hole.

Fertilization of the maize crop was also carried out in the furrows, using 42 kg ha^{-1} of P_2O_5, 157 kg ha^{-1} of K_2O and 40 kg ha^{-1} of FTE-BR12. The superphosphate and the FTE were applied as basal dressing in a single application, while the potassium chloride and the rates of nitrogen were divided, with 1/3 being applied as basal dose and 2/3 as top dressing at 31 DAS.

At the end of each sunflower cycle (at 78 and 80 DAS) and maize cycle (at 95 and 90 DAS), ten plants from each sub-plot were collected to evaluate productivity of the crops under study. The productivity of both crops in kg ha^{-1} was taken as the product of the average mass of achenes per plant (sunflower) and grains (maize) of the two crop cycles and plant density of 41,666 plants ha^{-1}. The potential for oil production in kg ha^{-1} was estimated for each treatment according to its achene productivity and the respective percentages of oil.

The data were submitted to analysis of variance by F-test at 0.01 and 0.05 probability, using the statistical software ASSISTAT 7.6 (Silva and Azevedo, 2009). When significant effects of the interaction were observed, the response surfaces were plotted, using the Table Curve3D v.4.0 software.

Based on the productivity of the crops (sunflower and maize), the effects of the treatments were analysed by investment analysis, using profitability indicators (benefit to cost ratio, net present value and internal rate of return) for a planning horizon of eight years, as per Costa et al. (2005). All prices used in the economic analysis were obtained at the local market, taking the average prices over recent years, so as to represent the real economic potential of the alternatives tested in this study.

RESULTS AND DISCUSSION

The variations in electrical conductivity of the soil saturation extract (ECse) before and at the end of each crop cycle are shown in Figure 1. It can be seen that during the dry season, that is, the period in which the sunflower was grown using water of different salt concentrations, increases in soil salinity were observed, especially at the highest salinity levels. Whereas, in the rainy season, the period when maize was cultivated using well water for supplementary irrigation, ECse decreased, showing that the rainfall helped to reduce the soil salinity regardless of the level of water salinity used previously. It is noteworthy that the sunflower crop was always cultivated in periods (July/2011 and July/2012) and the corn crop in periods (Feb/2012 and Feb / 2013).

A quadratic mathematical model was the best fit for the average productivity values for the sunflower cultivar BRS 324, as a function of water salinity and rates of nitrogen, with a coefficient of determination (R^2) of 0.92. The maximum yield was 4,050 kg ha^{-1} was obtained with the treatments S1N4 (0.8 dS m^{-1} and 75 kg N ha^{-1}) (Figure 2). The lowest estimated yield (1,480 kg ha^{-1}) was obtained under the highest concentration of saline water (EC = 6.4 dS m^{-1}) and no nitrogen fertilization. Furthermore, it can be seen that response to the nitrogen was more significant in the non-stressed plants, indicating that salt stress reduces the efficiency of nitrogen fertilization (Semiz et al., 2014; Lacerda et al., 2016). Similar results were observed for oil yield (Figure 3). The maximal potential for oil production (2000 kg ha^{-1}) was obtained with the treatments S1N4 (0.8 dS m^{-1} and

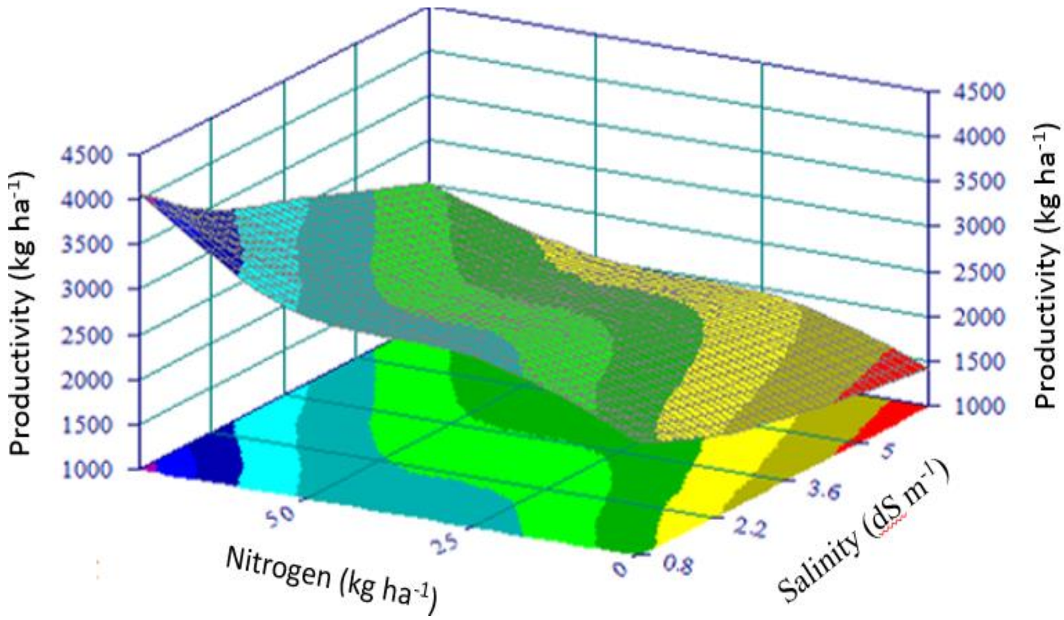

Figure 2. Response surface for the mean yield of two cycles of the sunflower cultivar BRS 324, as a function of salinity of irrigation water and levels of nitrogen fertilizer. Z= 2589.57 - 259.84 × salinity + 9.73 × $(\text{salinity})^2$ + 17.64 × nitrogen; $R^2 = 0.92$

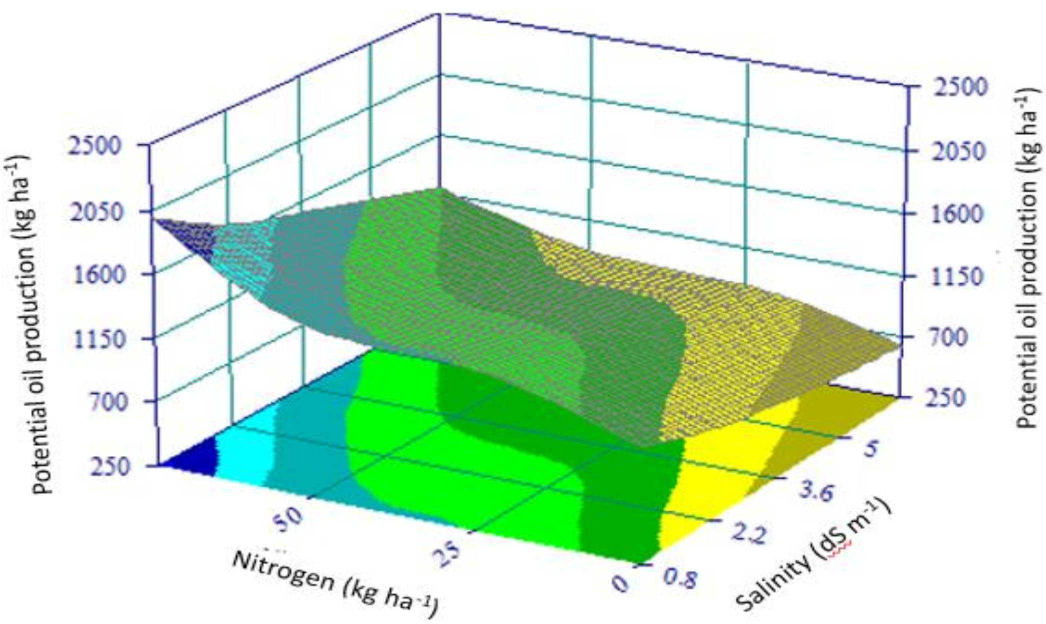

Figure 3. Response surface for potential oil production from two cycles of the sunflower cultivar BRS 324, as a function of salinity irrigation water and rates of nitrogen fertilizer. Z = 122.13 - 139.32 × salinity + 5.57 × $(\text{salinity})^2$ + 9.17 × nitrogen; $R^2 = 0.94$.

75 kg N ha^{-1}), while the lowest value (590 kg ha^{-1}) was observed in plants under S5N1 treatment (6.4 dS m^{-1} and without nitrogen fertilization).

Reductions in plant yield under saline stress conditions (Figures 2 and 3) are associated with the osmotic, toxic and nutritional effects resulting from the accumulation of salts in the root zone (Figure 1), which affects CO_2 assimilation, inhibits leaf expansion and accelerates the senescence of mature leaves. These effects reduce the area reserved for the photosynthetic process and the total production of photoassimilates, causing a reduction in crop yields (Munns, 2002; Wilson et al., 2006). Salinity

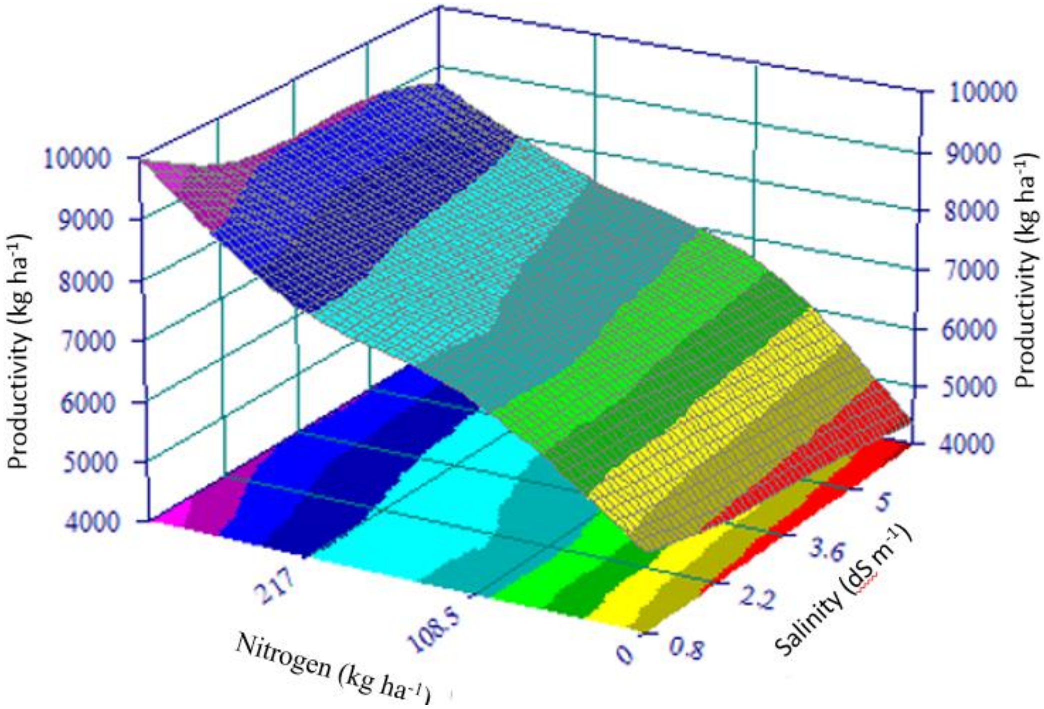

Figure 4. Response surface for the mean yield of maize cultivar AG 1051 from two cycles as a function of salinity of irrigation water and levels of nitrogen fertilizer. Z = 5394.28 - 137.40 × salinity + 17.93 x $(\text{salinity})^2$ - 0.0153 x nitrogen; R^2 = 0.97.

affect both quantity and quality of the seeds of many different oilseed crops, with negative impacts on oil production (Travassos et al., 2011; Sezen et al., 2011; Nobre et al., 2013).

Increasing rates of nitrogen caused considerable effect on maize productivity (Figure 4). When maize was grown without nitrogen fertilization, the yields were very low at different salinity levels previously used during the dry-season crop (sunflower). Increasing the rate of N resulted in significant increases in maize yield, and these effects were not influenced by residual soil salinity. The absence of a residual effect of salinity on maize plants can be related to leaching of salts (Figure 1) caused by rainfall and by the supplementary irrigation (Table 2) performed during the cultivation in the rainy seasons. The surplus irrigation and / or rainwater leaches the excess salts from the soil profile, resulting in a less detrimental effect from salinity on the rooting medium, which favours the growth and development of the crop (Murtaza et al., 2006; Assis Junior et al., 2007; Bezerra et al., 2010; Lacerda et al., 2011; Neves et al., 2015).

The benefit to cost ratio (BCR) shows values greater than one for the majority of treatments or scenarios (Table 3), that is, the expected benefits are greater than the costs, indicating the economic feasibility of such arrangements. However, for some of these arrangements the BCR was less than unity, such as S2N1 (0.91), S3N1 (0.87), S4N1 (0.83) and S5N1 (0.78), that is, all the treatments with saline water greater than 2.2 dS m^{-1} and with no nitrogen fertilization have a BCR of less than one.

The arrangements S1N4, S2N4, S3N4, S4N4 and S5N4 showed the highest BCR values, with 1.4, 1.29, 1.25, 1.24 and 1.19, respectively (Table 3). All of these treatments represent the lowest to the highest levels of salinity at the maximum rates of nitrogen, and it is clear that BCR was decreased with increasing water salinity. Despite these reductions in BCR, it can be seen that the results are always greater than 1.0, which is due in large part to the greater influence of the maize crop, whose productivity was not affected by the residual effect of the salts (Figure 2).

Only the arrangements S2N1, S3N1, S4N1 and S5N1 show a negative net present value (NPV), thereby indicating economic unviability (Table 3). Conversely, the other treatments had positive NPVs, particularly S1N4, S2N4, S3N4, S4N4 and S5N4, indicating that investment in these systems would be possible without loss to the investor. It is important to emphasize that saline water was used in these treatments only for irrigation of the sunflower, and practically no residual effect of salinity was observed on the maize crop. On the other hand, increasing rates of N caused significant yield responses for both crops, which contributed to the positive impact on the economic indicators. This is confirmed by the negative NPV values for those treatments where there was no nitrogen fertilization, even under low salinity water for irrigating the crops during the dry season.

The internal rate of return (IRR) of the present study

Table 3. Benefit to cost ratio (BCR), net present value (NPV) and internal rate of return (IRR) for the treatments under analysis, submitted to sunflower-maize crop rotation using saline water and rates of nitrogen.

Salinity (dS m^{-1})	Nitrogen (%)*	BCR	NPV (R$)	IRR (%)
S1 - 0.8	N1 - 0	1.03	1667.88	3.05
	N2 - 50	1.17	10516.13	17.44
	N3 - 100	1.22	14110.48	21.75
	N4 - 150	1.40	28267.90	40.14
S2 - 2.2	N1 - 0	0.91	-5020.21	-9.17
	N2 - 50	1.16	9505.06	15.76
	N3 - 100	1.20	12789.12	19.72
	N4 - 150	1.29	20363.17	28.91
S3 - 3.6	N1 - 0	0.87	-6863.74	-12.54
	N2 - 50	1.12	7424.70	12.31
	N3 - 100	1.17	10732.70	16.55
	N4 - 150	1.25	17921.03	25.45
S4 - 5.0	N1 - 0	0.83	-9139.74	-16.70
	N2 - 50	1.09	5656.87	9.38
	N3 - 100	1.14	8938.68	13.78
	N4 - 150	1.24	17128.20	24.32
S5 - 6.4	N1 - 0	0.78	-12009.77	-21.94
	N2 - 50	1.06	3746.43	6.21
	N3 - 100	1.10	6697.98	10.33
	N4 - 150	1.19	13126.57	18.64
	Mean	**1.112**	**7777.971**	**11.170**

-12.54, -16.70 and -21.94% for the treatments S2N1, S3N1, S4N1 and S5N1, respectively. When the IRR is equal to the minimum attractive rate (MAR), the NPV is equal to zero; but when the IRR is greater than the MAR, it means that the project yielded a higher return than the expected minimum rate, that is, the project should be accepted. The results obtained with the above treatments show the project can not be attractive. It can also be seen that the other scenarios showed a positive IRR, that is, greater than the MAR of 2% per annum, showing that these arrangements yielded a financial return to the investor. It was also found that scenarios that used the maximum levels of nitrogen, even with saline water, such as S1N4, S2N4, S3N4, S4N4 and S5N4, obtained a higher IRR respectively of 40.14, 28.91, 25.45, 24.32 and 18.64%, compared to the other scenarios, decreasing with the increase in salinity. For those scenarios in which nitrogen fertilizer was not used, it appears that increasing salinity also substantially reduced gains, making the BCR less than 1.0, and the values of NPV and IRR increasingly more negative.

Conclusion

The highest productivity and potential oil production of sunflower were observed in unstressed plants and subjected to the maximum rate of nitrogen. But yield decreased with increase in salinity, even with increase in the rate of nitrogen. The productivity of maize was strongly influenced by increases in the rate of nitrogen and the leaching of salts, caused by rainfall, virtually eliminating any residual effect of the salts on this crop. A soil management system employing sunflower/maize crop rotation is economically viable, being more attractive when using low salinity water and the highest application rate of nitrogen (75 kg ha^{-1} for the sunflower and 325 kg ha^{-1} for the maize).

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

Acknowledgments are due to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Instituto Nacional de Ciência e Tecnologia em Salinidade (INCTSal), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil, for financial support and scholarship provided to the first author.

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academicJournals

Vol. 11(23), pp. 2007-2012, 9 June, 2016
DOI: 10.5897/AJAR2016.11113
Article Number: 71F6C0958894
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

In silico identification of putative expressed sequence tag (EST)-simple sequence repeats (SSRs) markers of resistance to *Meloidogyne* spp. in common bean

Lucas Donizetti Vieira[1], Juliana Oliveira da Silva[2], Caio César de Oliveira Pereira[2], Solange Aline de Carvalho[3], Ricardo Diogenes Dias Silveira[3], Guilherme Malafaia[3] and Ivandilson Pessoa Pinto de Menezes[3]*

[1]Department of Genetics and Molecular Biology, Universidade Federal de Goiás, Goiânia, Brazil.
[2]Department of Agronomy, IF Goiano, Urutaí, Goiás, Brazil.
[3]Department of Biology, IF Goiano, Urutaí, Goiás, Brazil.

Received 10 April, 2016; Accepted 16 May, 2016

Expressed sequences are important sources in the development of functional heterologous microsatellite markers in phylogenetic related groups, that is, soybean and commom bean. The objective of this work was to identify and characterize expressed sequence tag (EST)-simple sequence repeats (SSRs) in silico candidates of resistance to *Meloidogyne* spp. in common bean. Seven DNA sequences from soybean associated with genetic resistance have been identified and obtained in the NCBI database. Its homology in common bean genome was verified using the BLAST tool. The cellular processes involved were also checked using the Blast2GO program. The identification of microsatellite markers and design of the primer pairs was performed using the SSRLocator and Primer3 programs, respectively. The transferability rate of common beans the target sequences identified was 86%, demonstrating the power of success of this method. All the cellular processes involved in the original DNA sequences were verified from EST on beans, with E-value between 0 and 2.9×10^{-168}. Fifteen EST-SSRs candidates for common bean resistance were identified, which have proved to be suitable for their amplification by PCR. The transferability analysis of ESTs related to resistance to *Meloidogyne* spp., especially among soybeans and common beans is efficient. Based on this study, 15 EST-SSRs candidates are available for validation and later use.

Key words: simple sequence repeats (SSRs) markers, assisted selection, ontology, pre-breeding.

INTRODUCTION

The common bean (*Phaseolus vulgaris*) is a leguminous vegetable cultivated in 113 tropical and subtropical countries, which has a high economic value and market acceptance due to its balanced chemical composition, including various proteins, complex carbohydrates, minerals and vitamin B complex (Broughton et al., 2003). Brazil is the world leader in the production and consumption of beans, although it is not yet self-sufficient

*Corresponding author. E-mail: ivan.menezes@ifgoiano.edu.br. Tel: +55 (64) 3461 1900, +55 (64) 9279 9708.

in the production of the same (Wander, 2005). The bean is cultivated in most Brazilian states, in a variety of soils, climates, growing season and farming systems (Silva and Wander, 2013). The adversities which the beans have been exposed contribute to high diversity found in the culture, enabling the selection, that is, genetic variation for breeding (Burle et al., 2010).

This availability of variability generates demand of detailed information about their genetic resources for use, which will be required to meet a process of expansion and growing interest in improved cultivars increasingly productive and adapted to adverse conditions of development. Another point required and of greatest interest to breeders is the introduction of desired genetic variation in the Active Germplasm Bank (AGB) on which lineages are selected, reducing the segregation of characters already improved. The assisted selection allied to the DNA markers is a useful method for this purpose.

Over the past decades, bean culture is going through several changes in the form of management, production systems and breeding. Coupled with the landscape changes for planting, the various plant health problems arise, making diseases, caused by plant pathogens, to cause a strong deterrent to obtain better yields in the country in diverse cultures, like the beans (Vieira et al., 1998). These changes contributed to the introduction of emerging pathogens, and old diseases that were not considered problems for the culture started to be highlighted. An important example is the root-knot nematodes, considered bean pests only in some producing regions of Brazil. Though the diseases caused by nematodes are not widely distributed in the Brazilian states, their damage lead to the Brazilian production not meeting the supply demand of the domestic market (Yokoyama, 2007).

An important form of management of nematodes is the use of resistant varieties, which, despite having been found for *Meloidogyne* spp. some find themselves still not very exploited in bean crop (Carneiro et al., 1992; Walber et al., 2003), a fact that underscores the importance in the development of tools that help produce detailed information for using these varieties, in addition define preventive recommendations. In this context, DNA markers are biotechnological tool of quick evaluation that can help design strategies of use of available genetic resources.

The DNA markers, such as Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP), have often been used in bean breeding programs (Müller et al., 2015; Müller et al., 2014). Its use has involved studies of quantification and determination of gene diversity (Cardoso et al., 2014); characterization of Value for Cultivation and Use (VCU) (Cardoso et al., 2013), assisted selection (Alzate-Marian et al., 2005), among others. The SSRs have been widely used in recent years and had selected population studies and use of genetic resources in plants for breeding purposes because of their hypervariability and ease polymorphism detection (Li et al., 2015a; Li et al., 2015b; Kaur et al., 2015; Zhai et al., 2015).

Obtaining selective microsatellite markers developed from databases, get easier, faster and economic compared to the ones developed by conventional methods (Buso et al., 2006). Thus, the recent full genomic sequencing advances and the increase in banks expressed sequence tag (EST) of different species, has facilitated studies of genetic similarity between phylogenetically related species (Schmutz et al., 2014; Wang et al., 2014). With this study we propose to identify and characterize *in silico* microsatellite markers candidates in *P. vulgaris* associated with genes that confer resistance to root-knot nematode (*Meloidogyne* spp.). The product generated from this study could be used as a preventive polymorphism identification tool related to the phenotypic behavior of bean caused by parasitism.

MATERIALS AND METHODS

Initially this study used secondary source materials to find articles covering genes or DNA sequences identified that confer a phenotypic behavior of resistance to nematodes belonging to *Meloidogyne* spp. in soybean (*Glycine max*), within the following databases: NCBI (National Center for Biotechnology Information), Scopus, Science Direct and CAPES Journals. The search was conducted from 09/2014 to 03/2015 and used the following indexing terms in various combinations: (1) Resistance; (2) *Glycine*; (3) *Meloidogyne*; (4) Gene; (5) QTL; (6) Marker; all descriptors were searched in Portuguese and English languages. Articles, published between 1990 and 2014, were found to be related to the subject. From the first articles read other potential were identified according to the methodology "snowball" (Bernard, 2011).

In the selected articles, the #id sequence or genes related to a phenotypic behavior of resistance to root-knot nematode were transcribed. Based on these #id or genes sought to access their DNA target sequences in their entirety using the NCBI database. Then the "FASTA" file format of each target sequence was used separately for the identification of homologous regions in the common bean genome by the "BLAST" tool (Basic Local Alignment Search Tool) available on the online program PHYTOZOME 10.3. Homologous sequences in common beans were chosen by the value E-value <1e-50 with sizes above 1000 bp. Then, the functional annotation of target sequences identified in beans was performed using the program BLAST2GO (Conesa et al., 2005).

To identify putative markers SSRs in the identified target sequences in common bean was used the program SSRLocator (Maia et al., 2008). Only with microsatellite repeats larger than three motifs were used for the design of the foward (F) and reverse (R) primers using the program Primer 3 (Rozen and Skaletsky, 2000). The designed primer pairs for the localized SSRs were used on an *in silico* PCR test model by primer- BLAST tool available in NCBI. This tool is used to check specificity of the pair primers against a database of interest, which at the time was the genome of *P. vulgaris*.

To verify the presence of dimers between the forward and reverse sequences of each primer was used AutoDimer, a program that makes use of a similar slide algorithm capable of comparing two overlapping strands of DNA (Vallone and Butler, 2004). Then the pairs designed EST-SSR primers were named "IFRTXX" being

IFRT relative to 'Federal Institute Goiano - Campus Urutaí' and XX the SSR primer number (Table 1).

RESULTS AND DISCUSSION

Seven genes described in the literature which confer resistance to root-knot nematode (*Meloidogyne* spp.) in Soybean (*Glycine max*) were found namely: Rhg1, Rhg4 (Concibido et al., 2004), Rmi1 (Luzzi et al., 1994), Extensin 1, Extensin 2, Pectin Esterase 1 and EREBP (Pham et al., 2013) (Table 1). From the sequences of the selected genes were identified regions of about 70% homology in common beans, except for the Pectin Esterase 1 gene, generating a sequence transferability rate of 87%. This success of genomics synteny between the two species is already well reported in the literature, which is due to the fact that they are phylogenetically sister groups, favoring the high occurrence of orthologous genes between them (Schmutz et al., 2014). A result that confirms the story of divergence between species from a complete duplication of the genome event approximately 56.5 million years ago (Lavin et al., 2005).

Using homologous target sequences in common bean was possible to recover the functional processes checked in selected genes in soybeans, with the exception of Ext1 gene, averaging 82.5% similarity and E-value values ranging from 0 to 2.9 and e-168. In general, the functional processes involved five categories: Cell wall structure, organization of the cell wall, integral membrane component, protein kinase activity and transcriptional regulation (Table 2). This comparison confirms the functional completeness homology, obtained using the transferability of soybean ESTs in common beans.

The identification of heterologous EST through *in silico* approach on comparative studies of intraspecific genes evolutionarily has become an easy and low-cost strategy, not practiced before the Post-Genomic Era (Zane et al., 2002; Pandey and Sharma, 2012). This understanding has allowed advances in the development of efficient functional SSRs markers (Sterky et al., 1998; Kaliswamy et al., 2015), as this work and others (Gupta et al., 2010; Victoria et al., 2011; Wang et al., 2014). Demonstrating that ESTs are good sources of sequences for prospecting functional microsatellite markers (Andersen and Lubberstedt, 2003), in this study, and associated with resistance to root-knot nematodes in bean.

Twenty-six different SSRs were identified for the ESTs found in common beans from genes of genetic resistance to root-knot nematodes in soybean. Of those twenty-six only fifteen were considered because present a number of repetitions larger than three. For each of the six genes have been identified SSRs with at least two different repeat motifs, with the exception of Rhg1, Rhg4 and Rmi1 genes identified for which only one SSR (Table 1). The length of the pairs of designed primers was suitable, ranging from 18 to 26 base pairs (bp), whereas the approximate length of 20 bp defined as optimal (Wang et al., 2014). The expected size of amplification product by PCR ranged between 182 and 357 pb, with a range of melting temperature (Tm) from 59 to 61ºC, which allows to estimate an average value approximately to 60°C among the sequences (forward and reverse) and primer pairs.

The small average difference in Tm between the sequences facilitates adjustment of the annealing temperature (Ta) of the F and R primers defined by "Ta = Tm ± 5", which should be similar. Defining Ta as the temperature at which pairing occurs between the primer and the flanking region of the target in the DNA of interest. The wide temperature range and length of the sequences of SSR primers >18 bp gives to the designed markers a high stringency (Bornet and Branchard, 2001). This increased reliability and reproducibility provides the certainty to be evaluating specific loci, and to facilitate the comparison of data by different researchers.

We expect that the fifteen EST-SSRs show a high amplification rate, since there was no competition between F and R sequences of each primer, either the formation of self-dimers. It was still possible to recover ESTs from 87% of the designed primers using the PrimerBLAST tool, allowing us to perform better thorough pre-selection, seeking to increase this amplification rate (Table 1). The success of this rate transferability studies using ESTs has been high, reported in literature values from 60 to 90% in different species (Wang et al., 2014; Luro et al., 2008; Saha et al., 2004).

Considering the SSRs classification based on the motifs identified, was verified the occurrence almost exclusively of trinucleotide markers (n = 13) and only two (02) dinucleotide. As the number of tandem repeats found a range from four to seventeen repetitions. Of the 15 SSRs, eight were classified as perfect, five compounds and one discontinued. The perfect SSRs are those with uninterrupted sequence motif; the interrupted show a sequence of discontinuous motif for a small and different sequence; and the compounds are those formed with at least two distinct motifs (Oliveira et al., 2006).

The high abundance of di- and trinucleotide and small amplitude in the length of motifs in the SSR target structure are related to the sequence of EST and its mutational dynamic (Schlötterer, 2000). In expressed plant sequences, the prevalence of these repetitions has been widely reported (Gupta et al., 2010; Wang et al., 2014; Hong et al., 2015; Boccacci et al., 2015). The regions of ESTs are transcriptional targets rich in dimers in the untranslated regions (UTR) and in the predominance of trimers in the open reading frame (ORF) (Ellegren, 2004). These regions exhibit less mutational rate compared to the others repeating regions of the genome not associated with genes (Li et al., 2002), thus supporting a lower amplitude of motifs, since abrupt changes in ORFs were being selected negatively (Metzgar et al., 2002). This indicates that the reduced size is due to the action selection designed to preserve

Table 1. Description of 26 EST-SSRs candidate to nematode resistance in beans with their Forward (F) and Reverse (R) sequences,

No.	Primer ID	Gene	Cr.	TM (°C)	ES	Primer Sequence (5' - 3')	Motifs	*NCBI* access number
1	IFRT02	EREBP	9	59 59	206	F: GGGTGGTAACCTCACCTTCA R: GGCGAGGAAAACAGACACTC	$(ATT)_3(ATT)_3$	XM_007133308.1
2	IFRT03	EREBP	9	59 60	197	F: TCGTTCATGGAGTTCATAGCA R: AATCCACAGAGCCATCCTTG	$(CTT)_4$	No match
3	IFRT06	Ext1	3	60 59	200	F: CACCTCCTTCCACCACAAAC R: TCTGGGGTAGTTTCCATTGC	$(CGG)_3(CTA)_3(CCG)_4(CTA)_3$	XM_007154277.1
4	IFRT08	Ext1	3	59 59	242	F: CCTACTACTCACCACCTCCACC R: AAGCAAGAACACCAGAAAGGAG	$(CCG)_4$	XM_007154277.1
5	IFRT09	Ext1	3	59 60	291	F: CAAGAAACGAGAAAAGAAGGGA R: GGTGCTGACCAAATAAGACTCAC	$(AT)_5$	XM_007154277.1
6	IFRT10	Ext2	8	59 59	198	F: AGTCTCCTCCTCCACCATCA R: GAGACTTGTAGTAGTAGGGAGGTGGT	$(CTA)_3(CAC)_3$	XM_007139060.1
7	IFRT11	Ext2	8	59 60	197	F: CATCACCACCACCACCATAC R: TGATGGATCAGGTGGAGGA	$(TAC)_3(CCA)_3(TAC)_6(CTA)_3$	XM_007139060.1
8	IFRT12	Ext2	8	59 60	211	F: CACCACCACCCCCATACTAC R: TGGTGAGGGTGAGGATAAGC	$(CTA)_3(CCA)_3(CAC)_4(CTA)_3(CTC)_3$	XM_007139060.1
9	IFRT13	Ext2	8	61 59	182	F: TCCTCCTCCACCTTCTCCAT R: AGTAGGGTGGTGGTGGTGAT	$(CTA)_3(CCA)_3(CAC)_3$	XM_007139060.1
10	IFRT14	Ext2	8	59 60	394	F: ATCACCACCACCACCCTACTAC R: ACCGACAACCTTAACGATCAAT	$(CTC)_4$	XM_007139060.1
11	IFRT15	Ext2	8	60 60	190	F: GTCGCTTATCCTCACCCTCA R: GTCGAAGCATCAGCATCAGA	$(ATT)_5$	XM_007135485.1
12	IFRT16	Ext2	8	61 60	208	F: ACCTCCTCCACCTGATCCAT R: AGATGGTGGTGGTGGTGACT	$(CAC)_4$	XM_007139060.1
13	IFRT20	Rhg1	1	59 59	302	F: ATGCTCCTCAATGGTTTCAACT R:AGGTTGGTTTTCTCCACTACCA	$(TC)_8(TC)_5$	XM_007163524.1
14	IFRT25	Rhg4	1	59 59	212	F: AACCCTACCAAAGGCCAGAT R: TGCAGGAATGCTTGATTGAG	$(GAA)_4$	XM_007163741.1
15	IFRT26	Rmi1	2	60 61	352	F: AAAATTCCCATTGTCCTCTCCT R: GAAACAACTTTTGGCTTTGGTG	$(CGA)_4$	XM_007159942.1

the structure of the transcript because abrupt changes easily affect the corresponding gene product.

Conclusion

With this early work, fifteen (15) EST-SSRs candidate markers in six different genes that give soybean root-knot nematode resistance are available for validation in common bean.

Table 2. Cellular processes linked to homologous sequences.

Gene/sequence	Protein	Cellular processes
EREBPm	Ethylene-responsive transcription factor rap2-12	transcription regulation
Ext1	Leucine-rich repeat extensin-like protein 6	-
Ext2	Pollen protein ole e i-like protein	Structure and organization of the cell wall
Rhg1	Receptor-like kinase	Activity of kinase protein
Rhg4	Receptor-like kinase tmk4	Integral component of membrane
Rmi1	Recq-mediated genome instability protein 1	Formation of floral organs

Moreover, it was possible to demonstrate that transferability between different species, but close evolutionarily following the example soybean and common bean, can increase the efficiency in the transfer of genomic information by homology ESTs gene of interest. Finally, it is noteworthy the promising results of this herein study, once validated the genetic link by phenotypic evaluation essays of hospitability to root-knot nematode in beans and association of polymorphism amplified by suggested markers studies, they can help programs improvement, outlining their crosses directed to the phenotype of interest, in this case the resistance to root-knot nematode. Yet it is noteworthy that these markers will facilitate the development of superior genotypes with multiple sources of resistance.

Conflict of Interests

The authors have not declared any conflict of interests.

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academicJournals

Vol. 11(23), pp. 2013-2023, 9 June, 2016
DOI: 10.5897/AJAR2015.10512
Article Number: C2B9D2C58898
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Poultry manure influenced growth, yield and nutritional quality of containerized aromatic pepper (*Capsicum annuum* L., var 'Nsukka Yellow')

Paul Kayode Baiyeri[1]*, Grace Taiwo Otitoju[2], Ngozi Eucharia Abu[3] and Simon Umeh[1]

[1]Department of Crop Science, University of Nigeria, Nsukka, Enugu State, Nigeria.
[2]Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Received 12 October, 2015; Accepted 6 May, 2016

'Nsukka Yellow' pepper is an aromatic pepper (*Capsicum annuum*) grown in specific rural locales in southeastern Nigeria. Ripe fruits are predominantly yellow and are exported to Nigerian cities where they command premium price. The need to develop appropriate technologies for adaptation and cultivation of 'Nsukka Yellow' pepper in cities where there are scarcity of agricultural lands motivated evaluation of the pepper as a potherb using three poultry manure (PM) rates (0, 5 and 10 t/ha). Three-week old seedlings were transplanted into 11 L containers and laid out in a completely randomized design replicated 10 times. Growth, yield and nutritional quality of fruits (at different ripening stages) were determined in response to applied manure. Plant height, number of leaves and number of branches increased with increasing level of PM, which translated to increased number of fruits and fruit weight per plant. Plants that received 10 t/ha of PM produced the highest fruit yield. Mature green, half ripe and fully ripe fruits were analyzed for ash, carbohydrate, fat, crude fiber, moisture, protein, alkaloid, flavonoid, tannin and volatile oil contents using standard methods. Both yellow fruits and red fruits (off-type from mutated branches) were harvested and analyzed for nutritional quality. Fruits harvested from 10 t/ha PM had the highest percent fat, crude fiber, moisture content, alkaloid, flavonoid, tannin and volatile oil. However, 5 t/ha produced fruits with the highest ash and protein contents; fruits from plants without manure had the highest percent carbohydrate. Fully ripe fruits had the highest percent carbohydrate, crude fiber, alkaloid, flavonoid, tannin and volatile oil, but green fruits had higher values for moisture and protein. Red fruits had the highest values for fat, carbohydrate, protein, alkaloid, flavonoid and volatile oil while the yellow type had the highest percent values for ash, crude fiber and tannin. Data on growth, yield and nutritional quality had significant positive response to the PM rates, suggesting that 10 t/ha is recommendable for growing the pepper as a potherb.

Key words: Aromatic pepper, containerization, manure rates, nutritional quality.

INTRODUCTION

Pepper (*Capsicum annuum*) originated in South America and spread into Asia and Africa (George, 1985). It is among the most commonly grown crops throughout Africa because of its utilization in soup, stews and salads (Heiser, 1995). There are five cultivated species of *Capsicum*, which are *Capsicum annuum, Capsicum*

Plate 1. Fruits of ripe 'Nsukka Yellow' pepper.

baccatum, Capsicum pubescens, Capsicum chinense and *Capsicum frutescens* (Heiser and Pickersgill, 1969; Bosland and Votava, 2000). Agusiobo (1976) and Uguru (1996) noted that *C. annuum* and *C. frutescens* are grown on commercial scale in most countries of the world.

Nigeria is the largest producer of pepper in Africa covering about 50% of total Africa production (Adetula and Olakojo, 2006). Nigeria was named 12th among top producing countries of chillies and peppers in the world (Hays, 2009). Idowu-Agida et al. (2012) reported that Nigeria has more than 200 selections of pepper. Ten genotypes of aromatic pepper types have also been characterized in the derived savanna ecology of southeastern Nigeria (Abu and Uguru, 2006; Abu et al., 2011).

In almost every tropical country, pepper has become the most popular condiment used to add zest and flavor to otherwise dull foods (Kochhar, 1986; Alabi, 2006). It has extensive culinary uses. It is used in pickles, ketchup and sauce seasoning dishes and in sausages (Purseglove, 1968). Pepper is rich in vitamins especially ascorbic acid and vitamin A. Bosland and Votava (2000) reported that pepper contains more vitamin C than tomato. Uzo (1982) reported that pepper is the major source of capsaicin, an alkaloid, which is used in medicine as a digestive stimulant. Peppers also contain various phenolics, flavonoids and carotenoids (Amakura et al., 2002; Materska and Perucka, 2005). Carotenoids are important colorants in vegetables, which often impart orange and red colors (Delgado-Vargas and Paredes-Lopez, 2003). Carotenoids in peppers include capsanthin, β-carotene and capsorubin (Howard et al., 2000; Guzman et al., 2010). The yellow-orange color of peppers is formed by *α*- and *β*-carotene, zeaxanthin, lutein and *β*-cryptoxanthin and these compounds are antioxidants and can reduce harmful oxidation reactions in human body; thus consumption of peppers may prevent various diseases associated with free radical oxidation, such as cardiovascular disease, cancer and neurological disorders (Howard et al., 2000).

The group of pungent components peculiar to the fruits of *Capsicum* plants is called capsaicinoids. The environment, especially the climate, light intensity, soil type, moisture level, fertilization and temperature during plant growth, is considered to have an impact on capsaicinoid levels, as does the age of the fruit (Estrada et al., 2002).

Asiegbu and Uzo (1984) reported that 'Nsukka yellow' pepper of the species *C. annuum* is a flavor pepper with a distinctive aroma and predominantly yellow color (Plate 1), which enhances its acceptability, thereby making it to attract higher prices than other peppers in groceries. Ripe 'Nsukka Yellow' pepper fruits are predominantly yellow (Plate 1) and are usually exported to Nigerian cities where they command premium price. It is widely speculated that the use of inorganic manure has resulted in dis-flavor of the 'Nsukka yellow' pepper, and it has also been argued that the use of organic manure is the best option in the cultivation of the pepper (Onugha, 1999).

Optimum organic manure needs for containerized 'Nsukka Yellow' pepper has not been reported in literature. The main bulk of pepper production in southeastern Nigeria is in the hands of small-scale farmers. They have limited resources to buy inorganic fertilizer because of the high cost. Organic manure that is more available as homestead wastes is a popular alternative to inorganic fertilizer and may be more affordable (Ndukwe et al., 2010, 2012; Ndubuaku et al., 2014). There is a need to work out the optimum manure rate for container-grown pepper, and that was addressed in this paper.

*Corresponding author. E-mail: paul.baiyeri@unn.edu.ng. Tel: +234(0)8039281834.

MATERIALS AND METHODS

The field experiment was conducted in the research farm of the Department of Crop Science, University of Nigeria, Nsukka, Nigeria located at latitude 06° 25[1] N, longitude 07° 24[1] S and at an altitude of 477.2 m above sea level.

Nursery operation

A ground nursery measuring 1.8 x 3 m was used for raising the seedlings. The nursery site was cleared of grasses and hoed to a fine tilt. Cured poultry manure was thoroughly mixed with the topsoil and watered to field capacity. Seeds were drilled in rows spaced at about 10 cm apart to a planting depth of about 2 cm, a day after nursery bed preparation. The seeds were covered with fine sand, watered lightly and mulched; furthermore, routine nursery maintenance practices were performed until seedlings were transplanted with ball of earth to the experimental pots.

Field experimentation

Thirty 11-L calibrated plastic pots were perforated at three points at the bottom to ensure drainage and were filled with 13.5 kg (dry weight) of soil each. The thirty pots were randomly allotted to the three experimental treatments, that is, 0, 5 and 10 t/ha cured poultry manure, giving 10 replications per treatment laid out in completely randomized design. Seedlings were watered, mulched and capped immediately after transplanting to provide temporary shade, reduce excessive moisture loss through evapo-transpiration, and to ensure ease of seedling establishment. Application of manure as top-dressing was done two weeks after transplanting the seedlings.

Plant growth and yield

Data were collected on plant height (cm), number of leaves and branches per plant over time. Fruits were harvested as ripening sets in and cumulative harvest per week was recorded as yield (grams) per plant. Harvest lasted for about three months; cumulative number of fruits harvested and the total yield (grams) per pot were recorded. Fruits weight loss under ambient laboratory condition was determined over time.

Soil and manure analyses

Soil samples were collected from representative spots on the experimental site from where soil was collected for potting using soil auger to a depth of 20 cm, the samples were made into a composite sample. A sub-sample was taken, air-dried, crushed and sieved with 2-mm mesh sieve after which physical and chemical analyses were carried out. A sample of the poultry manure used for experiment was also collected and analyzed for percentages of NPK. Standard laboratory procedures (Bouyoucos 1951; AOAC, 2005) were followed for the determination of physicochemical properties of the soil and manure used. Phosphorus was determined using phospho-molybedate method as described by Pearson (1976). 5 ml of the dissolved ash sample was pipetted into a test tube and 5 ml of molybdate solution was added. The standard calibration curve was prepared using phosphate salt. The absorbance was read at 470 nm in a spectrophotometer (Spectrolam 21) and the concentration calculated from the standard curve. Analyses were carried out at the laboratory of the Department of Soil Science, University of Nigeria, Nsukka, Enugu State, Nigeria.

Laboratory experimentation on nutritional quality of fruits

Nutrient contents of 'Nsukka yellow' pepper as influenced by poultry manure rate and ripening stages were evaluated at the Department of Crop Science Analytical Laboratory. This was a follow-up study to the field experimentation outlined above. Fruits were harvested at three different ripening stages and notable sport mutations were utilized. Thus, the experiment was a 3 x 3 x 2 factorial in completely randomized design, comprising the initial three levels of poultry manure (0, 5 and 10 t/ha), three ripening stages of fruits (Green, half-ripe and fully ripe) and two colour types of the pepper (the conventional yellow type, and off-type from spot mutations of branches, colored red). Mature fruit samples were washed, dried with paper towel, ground and finely diced for subsequent analyses. All analyses were prepared in triplicate and percent ash, carbohydrate, fat, crude fiber, moisture, protein, alkaloid, flavonoid, tannin and volatile oil contents were determined using standard methods. Moisture was determined by gravimetric method.

Nutritional and phytochemical analyses

Proximate composition of respective treatment samples was performed to determine percent ash, crude fiber, protein, fats, carbohydrate and moisture contents of fruits using the standard methods by association of official analytical chemist (AOAC, 2005). Similarly, total alkaloids, total flavonoids, tannin and volatile oil were determined. Two grams of the ground sample put in silica dish was heated in a muffle furnace at 600°C for 3 h and allowed to cool in a desiccator and weighed to determine percent ash. Crude protein was determined by the micro-Kjeldahl method while percent crude fat was by Soxhlet extraction method. Following AOAC (2005) standard method, crude fiber was determined, however moisture content of samples were determined using 2 g of samples dried to constant weight at 60°C in a hot air circulating oven for 24 h.

Determination of total alkaloids, total flavonoids and tannin was by spectrophotometric method but volatile oil was determined using direct steam distillation with petroleum spirit at 40°C (AOAC, 2005).

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using Genstat release 7.22 DE (GENSTAT, 2009). Test of significance of treatments means was by Fisher's least significant difference (F-LSD) at 5% probability level, as outlined by Obi (2002). Second order interaction involving manure rate, ripening stage and pepper type was further modeled with genotype-genotype-environment (GGE) biplot analysis (Yan, 2001).

RESULTS

The textural class of the potted soil was sandy clay loam; the soil was acidic and generally low in essential plant nutrients. Meanwhile, the poultry manure utilized was high in pH, organic carbon and organic matter; besides, the exchangeable minerals were relatively high (Table 1).

Significance tests of variance components shown in Table 2 revealed that manure rate (MR), fruit type (FT) and ripening stage (RS) were very highly significant ($p \leq 0.001$) for most traits, although FT did not significantly influence percent fat. First and second order interactions of MR, FT and RS were significant for most traits except flavonoids, tannins and volatile oils.

Table 1. Physical and chemical characteristics of soil and poultry manure used.

Physio -chemical properties	Soil	Poultry manure
Clay (%)	25	-
Silt (%)	15	-
Find sand (%)	25	-
Coarse sand %	35	-
Textured class	Sand clay loam	
Soil $_PH$ (in H_2O)	6.0	8.9
Soil $_PH$ (in KCL)	5.0	8.6
Total carbon %	0.47	46.08
Total organic matter (%)	0.82	79.45
Total nitrogen (%)	0.098	0.476
Exchangeable sodium (meq/100 g of soil)	0.211	0.63
Exchangeable potassium (meq /100 g of soil)	0.21	1.83
Exchangeable calcium (meq /100 g of soil)	2.60	116.00
Exchangeable magnesium (meq /100 g of soil)	1.60	98.00
Exchangeable Aluminum (meq /100 g of soil)	-	-
Exchangeable Hydrogen (meq /100 g of soil)	1.20	-
Cation exchange capacity (meq /100 g of soil)	15.60	-
Base saturation (%)	29.62	
Available phosphorus (ppm)	4.66	1.06

Table 2. Test of significance of variance components showing degree of freedom and probability of significance.

Variances	df	Ash (%)	Fat (%)	CHO (%)	Crude fiber	Protein	Moisture	Alkaloid	Flavonoid	Tannin	Volatile oil
MR	2	***	*	***	*	***	***	***	***	***	***
FT	1	***	NS	***	***	***	***	***	***	***	***
RS	2	***	***	***	***	***	***	***	***	***	***
MR x FT	2	***	NS	***	NS	***	***	***	*	NS	*
MR x RS	4	***	**	*	***	***	***	***	NS	***	NS
FT x RS	2	***	NS	***	***	***	***	***	NS	NS	NS
MR x FT x RS	4	***	*	***	*	*	***	***	NS	NS	NS
Residual	36										
Total	53										

Variances: Variance components; df: degree of freedom; MR: manure rate; FT: fruit type; RS: ripening stage; NS: non-significance at 5% probability level.

Growth and yield data

Table 3 shows that physiologically timed events such as (time to first, 50 and 100%) anthesis, fruiting and harvesting responded to the rate of manure. In all cases, there were inverse responses of days to specific event as manure rate increased from zero to 10 t/ha.

As expected, plant growth traits, that is, plant height, number of branches and number of leaves per plant, increased as plant age and rate of poultry manure increased (Table 4). Significant ($p \leq 0.05$) treatment effect was obtained for plant height from the 10^{th} week after transplanting, however, number of branches and leaves per plant varied significantly as from the sixth week. These growth characters increased progressively with increased poultry manure.

Poultry manure significantly ($p \leq 0.05$) influenced the cumulative number of fruits and weight of fruits harvested per plant (Table 5). The number and weight of fruits harvested when plants received poultry manure were several multiples of the number and weight harvested from plants not fertilized. Application of 10 t/ha of poultry manure outperformed the 5 t/ha application rate. Frequency of fruit harvest was not statistically significant

Table 3. Days to first, 50 and 100% anthesis, fruiting and harvesting as influenced by rate of poultry manure.

Manure rate t/ha	1st	50%	100%
Anthesis			
0	48	63	72
5	41	57	64
10	33	50	58
Mean ± SD	40.7±7.5	56.7±6.5	64.7±7.0
Fruiting			
0	58	75	88
5	53	68	86
10	42	63	82
Mean ± SD	51±8.2	68.7±6.0	85.3±3.1
Harvesting			
0	84	120	141
5	82	109	127
10	73	99	120
Mean ± SD	79.7±5.9	109.3±10.5	129.3±10.7

SD: Standard deviation.

Table 4. Effect of manure rates on plant height (cm), number of branches and number of leaves per plant over time.

Manure rates (t/ha)	2 WAT	6 WAT	10 WAT	14 WAT
Plant height				
0	10.5	15.5	23.1	27.4
5	9.9	15.0	28.6	36.8
10	11.9	17.7	41.4	46.6
$LSD_{(0.05)}$	NS	NS	10.6	6.3
Number of branches				
0	0.0	1.6	2.6	3.9
5	0.3	2.4	4.9	7.8
10	0.2	2.9	6.0	10.7
$LSD_{(0.05)}$	NS	0.6	1.3	2.5
Number of leaves				
0	8.6	15.9	32.8	47.3
5	7.8	21.6	60.5	107.5
10	9.6	28.3	94.8	126.7
$LSD_{(0.05)}$	NS	9.1	17.2	27.6

WAT: Weeks after transplanting; NS: non-significance at 5% probability level.

(Table 5).

Evaluation of fruits weight loss under tropical ambient laboratory condition revealed that the quantity of poultry manure applied significantly ($p \leq 0.05$) affected the percent moisture loss over the period of study (Table 6). In all cases, no application of manure increased the percent moisture loss while the least loss of moisture was from fruits that received the highest dosage of manure. Correlative responses of yield components to plant growth were determined to ascertain the strength of

Table 5. Effect of manure rate on components of yield of 'Nsukka Yellow' pepper.

Manure rate (t/ha)	Number of fruits	Fruits weight (g)	Number of harvest
0	10.2	38.0	10.0
5	29.1	127.0	8.9
10	43.4	204.0	11.3
$LSD_{(0.05)}$	10.4	62.5	NS

Table 6. Percent fruit weight loss of 'Nsukka Yellow' pepper over time (days) as influenced by poultry manure rates.

Manure rate (t/ha)	Loss at 8 DAS (%)	Loss at 12 DAS (%)	Loss at 16 DAS (%)	Loss at 20 DAS (%)
0	11.1	25.5	45.0	73.4
5	8.8	20.8	34.4	58.2
10	6.6	14.6	26.7	43.7
$LSD_{(0.05)}$	2.9	6.5	11.6	18.5

DAS: Days after storage.

Table 7. Relationship between plant growth parameters and yield components.

Growth parameters	Correlation coefficients	
	Number of fruits harvested	Weight of harvested fruits
Plant height (cm):		
6 WAT	0.54*	0.63**
10 WAT	0.74**	0.82**
14 WAT	0.85**	0.87**
Number of branches:		
6 WAT	0.54*	0.46*
10 WAT	0.73**	0.65**
14 WAT	0.62**	0.49**
Number of leaves:		
6 WAT	0.78**	0.73**
10 WAT	0.85**	0.79**
14 WAT	0.77**	0.61**

WAT: Weeks after transplanting. *, **: Correlation coefficient significant at 5 and 1% probability level, respectively.

relationships between growth (plant height, number of branches and number of leaves) at different growth ages and the eventual fruit yield (Table 7). Plant height, number of branches and number of leaves at 6, 10 and 14 weeks after transplanting (WAT) had significant ($p \leq 0.05$) and positive relationships with number of fruits harvested and fruit weight. In some cases, growth traits of older plants had higher correlation coefficient with yield components (Table 7).

Data on proximate and phytochemical qualities

The proximate quality traits were significantly affected by ripening stage (RS) across manure rates (MR) (Table 8). Irrespective of MR, ripe fruits had higher percent crude fat, fiber and carbohydrate, meanwhile unripe (green) fruits harvested from plants fertilized with 10 t/ha contained the highest percent protein. All the phytochemicals were higher in ripe fruits regardless of the manure rate.

Combined effects of MR and FT showed that yellow fruits contained higher ash, crude fiber and moisture across the MR, but the red fruit type had higher carbohydrate and slightly higher protein (Table 9). Red fruits contained more alkaloids, flavonoids and volatile oils irrespective of the quantity of manure earlier applied to the crop. However, tannin was slightly higher in yellow fruits. It is noteworthy that increasing manure rate increased the quantity of phytochemicals in both the red

Table 8. Combined effects of manure rate and ripening stage of fruits on nutritional quality of Nsukka Yellow pepper.

Manure rate (t/ha)	Ripening stage	Ash (%)	Fat (%)	CHO (%)	Crude fiber (%)	Protein (%)	Moisture (%)	Alkaloid (mg/100 g)	Flavonoid (mg/100 g)	Tannin (mg/100 g)	Volatile oil (mg/100 g)
0	FR	1.55	1.42	24.21	2.00	3.12	67.71	21.36	0.57	1.48	0.57
0	HR	1.73	0.45	23.24	1.48	3.65	69.44	6.70	0.39	0.76	0.39
0	GN	1.20	0.15	21.96	0.27	3.92	72.51	0.46	0.45	0.58	0.45
5	FR	2.10	1.43	22.17	2.55	3.73	68.01	20.56	0.59	1.45	0.59
5	HR	1.55	0.45	22.17	1.25	4.55	69.87	18.56	0.46	0.86	0.46
5	GN	1.42	0.20	20.28	0.23	4.20	73.67	0.45	0.44	0.67	0.44
10	FR	1.55	1.65	20.30	2.38	3.73	70.39	35.22	0.95	1.46	0.95
10	HR	1.58	0.47	19.49	1.42	3.78	73.67	21.51	0.51	0.91	0.51
10	GN	1.37	0.17	18.57	0.37	4.93	74.60	1.83	0.57	0.79	0.57
$LSD_{(0.05)}$		0.09	0.10	0.59	0.16	1.00	0.58	0.67	0.16	0.05	0.06

CHO: Carbohydrate; FR: fully ripe; HR: half ripe; GN: green (unripe).

Table 9. Combined effects of manure rate and pepper type (yellow and red) on nutritional quality.

Manure rate (t/ha)	Pepper type	Ash (%)	Fat (%)	CHO (%)	Crude fiber (%)	Moisture (%)	Protein (%)	Alkaloid (mg/100 g)	Flavonoid (mg/100 g)	Tannin (mg/100 g)	Volatile oil (mg/100 g)
0	Red	1.28	0.70	22.59	1.04	70.84	3.54	10.11	0.51	0.91	0.51
0	Yellow	1.71	0.64	23.69	1.46	68.92	3.58	8.90	0.43	0.97	0.43
5	Red	1.50	0.70	22.73	1.22	69.62	4.23	15.55	0.52	0.98	0.52
5	Yellow	1.88	0.69	20.49	1.44	71.41	4.09	10.83	0.48	1.01	0.48
10	Red	1.37	0.47	20.51	1.21	71.90	4.27	24.71	0.80	1.01	0.80
10	Yellow	1.63	0.78	18.57	1.58	73.58	4.03	14.33	0.54	1.09	0.54
$LSD_{(0.05)}$		0.07	0.08	0.48	0.13	0.48	0.55	0.08	0.13	0.04	0.13

CHO: Carbohydrate.

and yellow fruits.

Table 10 presents variations in fruits biochemical qualities due to ripening stages of the yellow and red fruit types. Fully ripe fruits of both types contained higher proportion of ash, fat, carbohydrate and crude fiber, but protein and moisture were higher in unripe (green) fruits. Regardless of fruit types fully ripe fruits had higher alkaloids, flavonoids, tannin and volatile oils. The second order interaction involving manure rate (MR), fruit type (FT) and ripening stage (RS) showed how the combined effects of the three factors affected the phytochemicals in pepper fruit (Figure 1).

The biplot explained 95.1% of the total existing variation suggesting that deductions from it are reliable. Ripe fruits of the red type that were harvested from 10 t/ha poultry manure (M10rfr) contained higher quantity of phytochemicals especially flavonoid and volatile oils. It was evident from the biplot that application of manure tended to increase quantity of phytochemicals in the fruits especially when the fruits were fully ripe or half ripe before harvesting (Figure 1).

Table 10. Combined effects of pepper type (yellow and red) and ripening stage on nutritional quality.

Pepper type	Ripening stage	Ash (%)	Fat (%)	CHO (%)	Crude fiber (%)	Protein (%)	Moisture (%)	Alkaloid (mg/100 g)	Flavonoid (mg/100 g)	Tannin (mg/100 g)	Volatile oil (mg/100 g)
Red	FR	1.41	1.50	23.36	1.92	68.01	3.67	31.49	0.83	1.43	0.83
Red	HR	1.41	0.47	22.60	1.30	69.87	4.07	17.59	0.48	0.81	0.48
Red	GN	1.32	0.18	19.86	0.26	73.67	4.31	1.29	0.52	0.65	0.52
Yellow	FR	2.06	1.50	21.09	2.70	70.39	3.39	19.94	0.57	1.50	0.57
Yellow	HR	1.83	0.44	20.80	1.46	73.24	3.92	13.59	0.43	0.86	0.43
Yellow	GN	1.33	0.17	20.69	0.32	74.60	4.39	0.54	0.46	0.71	0.46
$LSD_{(0.05)}$		0.07	0.08	0.48	0.13	0.48	0.08	0.55	0.13	0.04	0.13

CHO: Carbohydrate; FR: fully ripe; HR: half ripe; GN: green (unripe).

Proximate quality traits responded significantly to the combined effects of MR, FT, and RS as shown in the biplot in Figure 2, which explained 81.8% of the total variation. Yellow pepper fruits grown under 5 t/ha or 10 t/ha manure regimes and harvested green contained more protein and moisture. Percent carbohydrate was highest in ripe red fruits that were not fertilized (M0rfr). Similarly, quantity of fat was higher in ripe yellow fruit type that manure was not applied (M0yfr). There were higher values of crude fiber and ash in ripe yellow fruits grown under either 5- or 10- t/ha manure (M5yfr or M10yfr).

DISCUSSION

The physicochemical analysis of the soil sample showed that the soil was low in essential plant nutrient elements and was acidic following Ibedu et al. (1988) classification of soils in the zone. However, the poultry manure applied was sufficiently high in pH and essential elements to bridge putative nutrient deficiency gap of the soil used.

The quality of growth of potted plants is influenced to a large extent by the quality of the growth substrate provided for growing the plants. The adequacy of the essential nutrient elements in the container certainly will influence growth performance of the plants. Thus, it was appropriate that the quantity (rate) of the poultry manure used, which invariably influenced the adequacy of essential nutrient elements in the container, influenced growth, yield and nutrient quality of ‘Nsukka Yellow’ pepper.

Plant growth characters increased as the organic manure level increased, which is attributable to greater supply of plant nutrients with incremental application of poultry manure since the un-amended soil was low in nutrient content. Asiegbu and Uzo (1984) reported a high response of eggplant and onion to incremental application of organic manure in a similar soil at Nsukka. Similarly, these authors reported that farmyard manure (FYM) benefitted fruit set in eggplant and generally enhanced size characters in both onion and eggplant.

The positive response of physiological-time events to manure rates suggested that phenological changes (anthesis, fruiting and fruit maturity) in ‘Nsukka Yellow’ pepper could be influenced by the quantity of nutrient elements (determined by the quantity of manure applied) available for growth. This is corroborated by the significant increase in growth traits (plant height, number of branches and number of leaves) as manure rate increased.

For most crops there is a direct relationship between growth and yield. This was evident in this study as plant height, number of branches and number of leaves were positively and significantly correlated with number of fruits and weight of fruits harvested. Abu et al. (2013) reported a similar finding for field grown aromatic peppers.

In their study, fruit yield could be predicted from the combined effects of number of nodes, number of leaves and fruits per plant, with above 80% predictive accuracy. In the current study, it was notable from the correlative responses that yield could be predicted from 6-week old plants although 10-week old plants were stronger estimator), the positive relationship suggest that the better the crop performance at this stage, the more the yield, thus allowing for effective nutrient management in the case of early season poor crop performance. The result (suggested that, providing adequate growth environment for

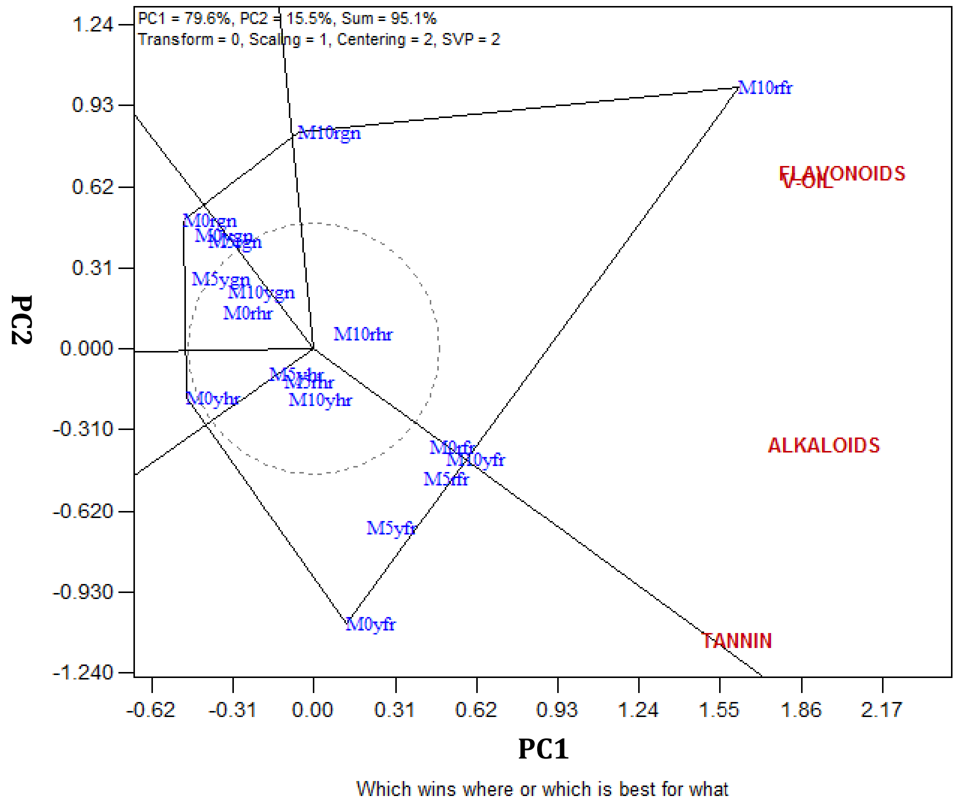

Figure 1. Biplot analysis of combine effects of poultry manure rate, different ripening stages and pepper type on the phytochemical qualities of 'Nsukka pepper' (yellow and red).

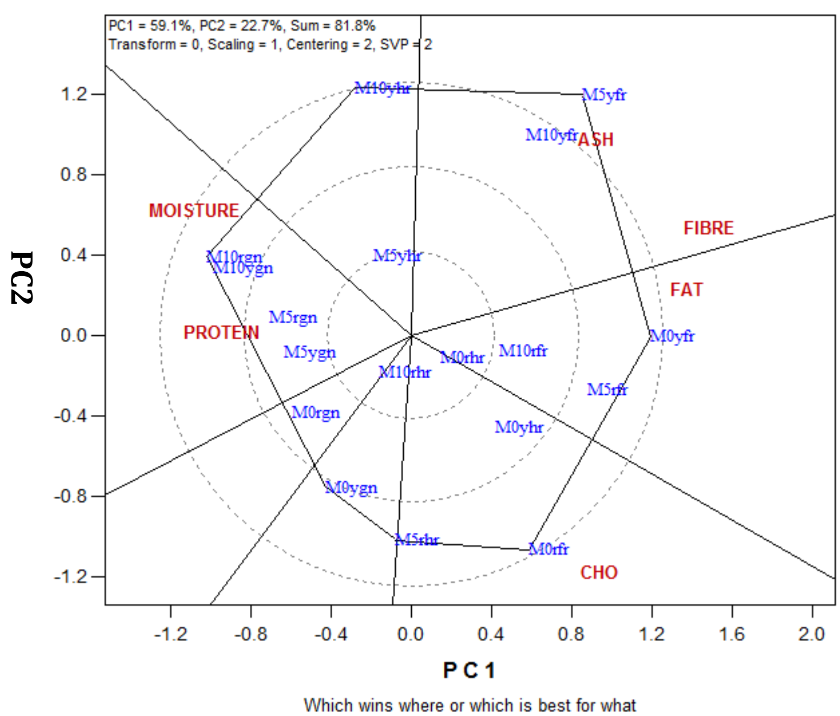

Figure 2. Biplot analysis of combined effects of poultry manure rate, different ripening stages and pepper type on the proximate qualities of 'Nsukka pepper' (yellow and red).

containerized plants will enhance its yield capacity.

The use of organic manure especially from poultry source has become increasingly important in southeastern Nigeria because of its role in increasing the nutritive value of most fruit crops and leafy vegetables (Ndukwe et al., 2010, 2012; Ndubuaku et al., 2014). In the current study, application of poultry manure increased most of the nutritional properties of the 'Nsukka Yellow' pepper. Significant effect of poultry manure rate on the nutrient quality of pepper is also supported by an earlier study on passion fruits (in the same location), which showed that nutritional quality of the juice varied with poultry manure rate (Ani and Baiyeri, 2008).

Nutritional quality of pepper fruits varied significantly with ripening stage, suggesting that ripening may have enhanced biosynthesis of these nutrients. Noteworthy is that green (unripe) pepper fruits had higher percent protein, although alkaloids, flavonoids and volatile oils increased with ripening. Earlier studies on plantain fruits reported that ripening increased the quantity of most of the nutrient elements assayed for (Baiyeri and Unadike, 2001; Baiyeri et al., 2011).

Conclusion

Growth and yield performances and nutritional quality of pepper could be manipulated positively by the application of poultry manure. 'Nsukka Yellow' pepper could be cultivated in pots without compromising both yield and quality attributes of the pepper. Application of 10 t/ha poultry manure is recommended for growing the crop.

Conflict of Interests

The authors have not declared any conflict of interests.

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Vol. 11(23), pp. 2024-2031, 9 June, 2016
DOI: 10.5897/AJAR2016.11126
Article Number: 7A905CB58900
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Sources of phosphorus for the establishment of palisade grass (*Urochloa brizantha* (Hochst. ex A. Rich.) R.D Webster)

Amilton Alves Filho* Regina Maria Quintão Lana, Reginaldo de Camargo, Marina Alves Clemente and Isabel Dayane de Souza Queiroz

Federal University of Uberlândia, Institute of Agricultural Sciences, CEP: 38.400-902 Uberlândia, Minas Gerais, Brazil.

Received 13 April, 2016; Accepted 20 May, 2016

In Brazil, about 80% of the 60 million hectares of pasture are in some state of degradation. Among the causes of this degradation is the lack of investment in liming and fertilization at the time of planting. Phosphorus is especially critical because it stimulates root growth in the establishment phase of forage growth. The objective of the study was to evaluate different sources of phosphorus in the formation of Marandu and Xaraés forages. An experimental design of randomized blocks in a factorial arrangement, 2 x 4, with 5 repetitions was used. Treatments consisted of two cultivars of *Urochloa brizantha*: Marandu and Xaraés and three sources of phosphorus: triple superphosphate (41% P_2O_5), natural reactive phosphate (28% P_2O_5) and organic mineral (4- 14-8) plus the absence of phosphate fertilizer (control). Plant height, stem diameter, chlorophyll A and B content and fresh and dry matter mass were evaluated 60 days after germination. Leaf content was also examined for the following mineral nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). The Marandu cultivar was superior to the Xaraés cultivar in terms of height. Among the sources of phosphorus examined, there were no significant differences among organic mineral, triple superphosphate and reactive phosphate, for any of the characteristics considered. All the sources, however, differed from the control. The maximum extraction of soil macronutrients by *Urochloa brizantha* was, in descending order: the macronutrients K>N>Ca>Mg>P>S and micronutrients Mn>Fe>Zn>Cu. These nutrients, thus, needed to be replaced as they were removed through grazing, to replenish the soil. The sources of phosphorus evaluated were not associated with differences in the leaf contents of Cu, Zn and S. The leaf content of the Xaraés cultivar had higher values of N, P, K, Ca, Mg, S, Mn and Zn in relation to Marandu.

Key words: Forage, degraded area, mineral nutrients, fertilization.

INTRODUCTION

The Brazilian cattle industry plays an important role in the national economy. Almost 20% of the total land area is used for pasture. Cattle production, however, has been characterized by years of exploitation. Pastures are usually of low soil fertility and mineral nutrients extracted by the animals are not restored, leading to degradation. Dias-Filho (2012) mentioned that one of the main causes of grassland degradation is the absence of periodic restoration of soil fertility. Pasture degradation is associated with reduced animal carrying capacity and reduced productivity of meat and milk.

According to Freire et al. (2005), following nitrogen, phosphorus is the most important element for forage. It is important in the early development of roots and tillers. The lack of phosphorus in forage grasses results in stunted plants without side tillers and a high concentration of nitrogen in the dry matter. According to Maciel et al. (2007), the efficiency of phosphorus fertilizer is influenced by several factors including soil type and the source of P used. Pastures planted on sandy or clay soils require more phosphorus than those planted on other types of soil. For Novais et al. (2007), the high reactive phosphate is the most used in Brazilian agriculture because of its agronomic efficiency in the short term. However, the strong competition for absorption, between soil and plant, results in this form of phosphorus having an elevated cost per unit of available mineral. Rezende (2013) has reported that an alternative to reduce the cost phosphate fertilizers for the maintenance and restoration of pastures is the use of less soluble phosphate sources such as natural reactive phosphate.

The use of organic sources of phosphate in Brazilian agriculture has increased because of the high cost of soluble phosphate fertilizers as well as an increase in the supply of soluble organic fertilizer. Caione et al. (2011) reported that organic sources of P play an important role in the life of microorganisms, increasing the cation exchange capacity (CEC) and phosphorus mobility in soil.

The *Urochloa brizantha* cultivars: Marandu and Xaraés are options for beef and dairy farmers to diversify existing pastures. Macedo (2005) reported that *Urochloa* totals 85% of the forage grasses grown in the Brazilian savannah. *Urochloa* is therefore of fundamental importance in the production of meat and milk in Brazil. It makes these activities possible in poor and acid soils, predominately in the savannah. However, one of the main problems related to the establishment and maintenance of pastures in Brazilian Oxisols, is the extremely low levels of available phosphorus. In addition to the deficiency of this element in a form available to plants, there is the problem of the high phosphorus adsorption capacity of acid soils as well as those with high levels of iron and aluminum (Macedo, 2004).

Urochloa brizantha cv. Marandu is resistant to leafhopper damage and is productive when well managed and fertilized properly (Macedo, 2005). Xaraés is adapted to humid tropical regions and is recommended for medium fertility, well-drained soils of medium texture. It is not as well adapted to low fertility, acid soils as Marandu but responds well to liming and fertilization (Jank et al., 2005). These cultivars have similar performances in the production of meat and milk. However, Xaraés is more productive than Marandu due to leaf elongation rates and density of tillers (Lara and Pedreira, 2011).

The objective of this study was to evaluate different sources of phosphorus in the formation of Marandu and Xaraés pastures and examine the leaf contents of the two cultivars for macro and micronutrients under greenhouse conditions.

MATERIALS AND METODS

The experiment was conducted in a greenhouse of the Institute of Agricultural Sciences, Federal University of Uberlândia - UFU, MG, during the period of May to July 2013. The Oxisol (clay) soil was collected to a depth of 30 cm in a degraded pasture area of the experimental farm of the University. After drying in the shade and screening, a sample was taken for chemical characterization: water pH = 4.6; Mech = P-1.6 mg dm^{-3}; S-SO_4- = 16 mg dm^{-3}; K^+ = 0.15 cmolc dm^{-3}; Ca^{2+} = 1.0 cmolc dm^{-3}; Mg^{2+} = 0.3 cmolc dm^{-3}; Al^{3+} = 0.0 cmolc dm^{-3}; H + Al = 2.7 cmolc dm^{-3}; organic matter (OM) = 2.7 kg dag^{-1}; CTC at pH 7 (T) = 4.15 cmolc dm^{-3} and base saturation (V) = 35%. An experimental design with randomized blocks was used, depending on the greenhouse lighting gradient in a factorial arrangement: 4 x 2 with five repetitions. Treatments consisted of the two cultivars of *Urochloa*: Marandu and Xaraés and four phosphorus sources: absence of phosphate fertilizer (control), triple superphosphate (41% P_2O_5), Reactive Phosphate Natural (28% P_2O_5) and organic mineral (4-14-8). The experiment was conducted in 5 dm^3 vessels, adjusting with liming and fertilization with nitrogen and potassium, since the organic source had levels of these nutrients. Different sources were added to the soil at doses of 70 kg ha^{-1} of P_2O_5 according to recommendation (Cantarutti et al., 1999). Each pot was prepared with 20 *Urochloa* seeds which were thinned after germination, leaving six plants per pot. At 20 days after germination, plants were top-dressed with urea and potassium chloride at doses of 60 kg ha^{-1} of N and K_2O.

Irrigation was provided whenever necessary to keep moisture at 60% of field capacity. The evaluations made after 60 days included: height of the last fully expanded leaf, measured with a graduated scale; stem diameter, measured with a digital caliper; chlorophyll A and B, with electronic measurement for chlorophyll content ClorofiLOG; fresh weight of the above ground part of the plant and dry weight, prepared in a forced air circulation oven at 65°C for 72 h. Samples of leaves were washed with distilled water and a solution of 0.1 mol L^{-1} HCl and deionized water. After washing, samples were dried and ground in a Willy mill type (2 mm sieve) and examined to determine levels of N, P, Ca, Mg, Cu, Fe, Mn and Zn. To determine the total nitrogen content, a sulfuric acid

*Corresponding author. E-mail: amiltonaf@yahoo.com.br. Tel: 34 32182225.

Table 1. Foliar macronutrient contents of *U. brizantha*, c.v Marandu and Xaraes, grown in a greenhouse with varying sources of phosphorus.

Sources of P	N	P	K	Ca	Mg	S
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
NP	28.20^{b}	2.43^{b}	41.05^{b}	5.64^{b}	3.03^{b}	2.06^{a}
OM	31.59^{a}	3.41^{a}	42.95^{ab}	6.15^{ab}	3.48^{a}	1.75^{a}
TS	30.44^{a}	3.46^{a}	43.55^{a}	7.31^{a}	3.43^{ab}	1.84^{a}
RP	31.76^{a}	3.44^{a}	42.45^{ab}	6.15^{ab}	3.37^{ab}	1.87^{a}
Means	30.49	2.93	42.5	6.31	3.32	1.88
CV	5.55	9.83	4.56	17.97	10.89	16.29
MMD	2.13	0.28	2.36	1.38	0.44	0.37
Cultivars						
Xaraes -2	32.77^{a}	2.54^{a}	45.02^{a}	7.14^{a}	3.76^{a}	1.98^{a}
Marandu -1	30.08^{b}	2.16^{b}	39.71^{b}	5.39^{b}	2.82^{b}	1.76^{b}
Means	31.42	2.35	42.36	6.26	3.29	1.87
MMD	1.13	0.15	1.25	0.73	0.23	0.37
			F			
Sources of P (P)	5.63*	0.09*	11.36*	3.85*	0.39*	0.17^{NS}
Forages (F)	71.74*	1.43*	281.6*	30.61*	8.80*	0.45*
Interaction P x F	5.04^{NS}	0.04^{NS}	2.48^{NS}	1.93^{NS}	0.15^{NS}	0.04^{NS}

Means followed by the same letters in columns are not statistically different from each other at the 5% probability by the Tukey test. *Significant at 5% probability and NS = not significant, respectively. The F. test CV = coefficient of variation. MMD = minimum meaningful difference. Sources NP - no phosphate; OM - organic mineral; TS- triple superphosphate; RP - reactive phosphate; CV = coefficient of variation.

digestion Kjeldah method was used (EMBRAPA, 2009). To determine levels of P, K, Ca, Mg, Cu, Fe, Mn and Zn, leaf samples were subjected to nitro-perchloric digestion. Subsequently, an atomic absorption spectrophotometer was used with an air/acetylene flame for readings of the nutrients.

The data were submitted to tests of the homogeneity of variances, normality of residuals and non-additivity blocks using the SPSS 17.0 program. The analysis of variance was performed using the SISVAR program (Ferreira, 2008) and the means were compared using the Tukey test at 0.05 significance.

RESULTS AND DISCUSSION

The analysis of variance showed no significant interaction (P > 0.05) between phosphorus sources and the two forage cultivars for all of the foliar primary and secondary macronutrients studied (N, P, K, Ca, Mg, S) (Table 1). The maximum concentration of mineral nutrients in the fresh forage was observed in the following order (K> N> Ca> Mg> P> S) at 60 days after germination. Examining the Marandu cultivar, Primavesi et al. (2006) found the same descending order of extraction of macronutrients. For this reason, it is crucial to restore mineral nutrients via fertilization as forage is grazed.

The Xaraés leaves had higher nitrogen contents (32.77 g kg^{-1}), phosphorus (2.54 g kg-^{1}), potassium (45.02 g kg^{-1}), calcium (6.31 g kg^{-1}), magnesium (3.32 g kg^{-1}) and sulfur (1.98 g kg^{-1}). According to Monteiro (2004), the concentrations of nutrients in plant tissues are closely related to the production of forage. For Euclides et al. (2009), Xaraés has greater carrying capacity than Marandu.

The average nitrogen content in the fresh forage ranged from 31.76 to 28.20 g kg^{-1}. The variances were influenced by the different phosphorus sources (Table 1). Increases in the nitrogen contents were observed regardless of the source of phosphorus evaluated. Silva et al. (2004) observed an increase of 1.52 g kg^{-1} in leaf nitrogen content after applying 100 kg ha^{-1} of soluble phosphorus in the form of mineral fertilizer. According to Werner et al. (1996), adequate nitrogen for *Urochloa* is 13-20 g kg^{-1}. Study performed by Mattos and Monteiro (2003) showed that the concentration of nitrogen in the newly expanded leaves of the first cutting of *Urochloa* ranged from 12.2 to 30.0 g kg^{-1} between the lowest and the highest nitrogen doses applied. Silva et al. (1994) observed a linear increase in the concentration of nitrogen in Marandu leaves with an increasing nitrogen rate and found a concentration of 30.2 g kg^{-1} following a dose of 60 kg N ha^{-1}. An equal amount of nitrogen was used for top-dressing. Prado (2008) reported that nitrogen is the most abundant anionic macronutrient in the plant and is also the nutrient most required, being a constituent of a series of compounds essential for plant growth and development.

The phosphorus content of the above ground parts of the forages was influenced by phosphorus sources. Treatments that received any source of P showed higher concentrations as compared to the control (no phosphorus treatment, Table 1). Similar results have been reported by Oliveira et al. (2012) who observed a greater concentration of phosphorus when using phosphorus sources including soluble reactive phosphate. Phosphorus omission in forages, on the other hand, promotes the reduction of an accumulation of this nutrient in the above ground part of the plant (Avalhaes et al., 2009).

Prado et al. (2011) studied the performance of *Panicum maximum* cv. Tanzania grown in nutrient solution and found that phosphorus omission stunted plants and reduced the numbers of side tillers, leading to a lower production of dry matter. Marschner (1995) mentioned that the P content of 3 to 6 g kg^{-1} is ideal to optimize growth during the vegetative stage.

Regarding potassium reported in Table 1, only triple superphosphate differed from the control (no phosphorus). No differences among treatments with natural and organic or reactive phosphate were observed. Silva et al. (2004) observed an increase of 12.25 g kg^{-1} of foliar potassium with the use of 100 kg ha^{-1} of P_2O_5 in the form of superphosphate. Werner et al. (1996) suggested that the appropriate concentration of K in newly expanded leaves of Marandu is in the range of 15 to 30 g kg^{-1}.

For foliar calcium levels (Table 1) it was observed that treatment with triple superphosphate differed from the control but showed no difference as compared to alternative treatments with organic mineral or natural reactive phosphate.

Phosphorus in its various sources influenced the foliar concentration of magnesium. Treatment with the organic mineral had the highest average value (3.48 g kg^{-1}) of magnesium. This was superior to no treatment (3.03 g kg^{-1}), but no differences were observed among treatment with triple superphosphate or reactive phosphate. Therefore, the use of a phosphorus source in the establishment phase of both forages was associated with higher absorption of calcium and magnesium.

The analysis of variance showed no significant differences ($P > 0.05$) for the interaction between P sources and forage content of the micronutrients (Cu, Fe, Mn and Zn). However, there was significance, for P sources and the cultivars of *Urochloa,* for Mn and Zn (Table 2). The reductions of several micronutrients: manganese, iron, zinc and copper were higher than for others. Similar reductions were observed by Costa et al. (2010). These results demonstrate the need to replace the micro-nutrients in the soil as forage plants are grazed.

For manganese (Table 2), treatment with reactive phosphate differed from the control, but showed no significant difference from treatments with triple superphosphate or organic mineral. Carvalho et al. (2003) reported that the average foliar manganese content in *Urochloa* cultivars is 166 mg kg^{-1}, a value exceeded by both cultivars: Marandu 207.25 and 255.00 mg kg^{-1} Xaraés.

The average sulfur content (S) ranged from 1.98 g kg^{-1} in Marandu to 1.76 g kg^{-1} in Xaraés (Table 2). These values were within the critical level (between 1.0 to 2.0 g kg^{-1}) of S in tropical forages. The extraction of S for the production of 20 t ha^{-1} $year^{-1}$ dry weight is approximately 50 kg ha^{-1} $year^{-1}$ (Oliveira et al., 2008). According to Corsi et al. (2007), excess sulfur in the soil is harmful because it reduces the absorption of molybdenum (Mo) by the plants. This results in losses in quality and productivity of the forage.

The sources of phosphorus examined did not influence the level of copper (Cu) in the above ground parts of the two cultivars of *Urochloa*. Increases were not expected in copper content because it was not included in the micronutrients in the formulation of the sources of phosphorus of the research. Similarly, there was no difference in the concentration of copper between Marandu and Xaraes. Silva et al. (2011) found no increase in the copper content of the leaves of *Urochloa decumbens* using mineral fertilizer at a dose of 60 kg ha^{-1} N, 90 kg ha^{-1} P_2O_5 and K_2O 100 kg ha^{-1}. The organic source of phosphorus also did not affect the copper content of the leaves of the *Urochloa* cultivars evaluated. The fact that the organic mineral fertilizer did not yield an increase in copper is probably due to the low phosphorus dosage: 70 kg ha^{-1} P_2O_5.

The sources of phosphorus examined also did not affect the iron (Fe) content of the leaves (Table 2). For Carvalho et al. (2003), the average iron content in *Urochloa* leaves is between 100 and 487 mg kg^{-1}. For Werner et al. (1996), the ideal range for iron in forage is between 196 - 239 mg kg^{-1}. In the present study, Marandu had a mean value of 202.42 mg kg^{-1} and Xaraes: 182.51 mg kg^{-1}. Both values are within the range reported by Carvalho et al. (2003).

For zinc (Table 2), the Tukey test found no significant differences in the leaves among the control, treatments with organic mineral, reactive phosphate, or superphosphate. Zinc content ranged from 28.02 to 28.96 mg kg^{-1}. The observed values were close to those reported by Silva et al. (2011) for *Urochloa decumbens*. They did not observe significant influence between organic and mineral fertilizers (N, P and K) in the zinc content of the plants. Gallo et al. (1974) related that the critical level for zinc in forage is 27.3 mg kg^{-1}, a value exceeded in all the treatments of the present research. Silva et al. (2011) discovered that the highest zinc content in *Urochloa* leaves is in the first cutting (35 days). They reported a mean value of 35.67 mg kg^{-1}. In the present study, the leaves of the Xaraés cultivar had a higher concentration of zinc (31.16 mg kg^{-1}) than Marandu.

Table 2. Foliar micronutrient contents in *U. brizantha*, c.v Marandu and Xaraés, grown in a greenhouse with varying sources of phosphorus.

Sources of P	Cu	Fe	Mn	Zn
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
NO	9.48^{a}	208.92^{a}	217.01^{b}	28.02^{a}
OM	8.86^{a}	195.48^{a}	238.95^{ab}	28.50^{a}
TS	9.55^{a}	187.21^{a}	221.64^{ab}	28.96^{a}
RP	9.34^{a}	180.24^{a}	252.08^{a}	28.73^{a}
Means	9.30	147.90	232.42	28.55
CV	8.43	27.31	12.24	10.02
MMD	0.95	64.37	34.75	4.06
Forages				
Xaraes -2	9.26^{a}	202.42^{a}	255.19^{a}	31.16^{a}
Marandu -1	9.35^{a}	182.51^{a}	207.25^{b}	30.05^{b}
Means	9.30	192.46	231.22	30.60
MMD	0.50	34.17	18.45	2.16
		F		
Sources of P (P)	0.96^{NS}	1520.14^{NS}	2609.31*	27.98*
Forages (F)	0.07^{NS}	3951.41^{NS}	22930.7*	33.59*
Interaction P x F	0.43^{NS}	1761.10^{NS}	524.43^{NS}	5.87^{NS}

Means followed by the same letters in columns are not statistically different from each other at the 5% probability by the Tukey test. *Significant at 5% probability and NS = not significant, respectively. The F. test CV = coefficient of variation. MMD = minimum meaningful difference. Sources NP - no phosphate; OM - organic mineral; TS - triple superphosphate; RP- reactive phosphate; CV = coefficient of variation.

Marandu was taller than Xaraés (Table 3). However, there was no difference between the two in relation to the stem diameters. According to Rezende (2013), the average height of *Urochloa* is an important factor in decisions regarding stocking levels, the time of entry and exit of the animals from pastures and also the length of rest periods and the method of occupation of pastures, including possible rotational grazing.

The heights and diameters of the forage plants were not found to be affected by the different sources of phosphorus used in the study. There were no significant differences among the organic mineral fertilizer, triple superphosphate and reactive phosphate for either height or diameter of the forage plants (Table 3). Dias et al. (2012) reported that fertilization of Marandu with phosphorus using reactive phosphate, superphosphate or a combination of both in Oxisol, at a dose of 70 kg P_2O_5 ha^{-1} increased phosphorus levels in a similar manner whether examining dry matter production of roots or grass height.

Although, few significant differences were observed among the sources of phosphorus with the micronutrients, all tests of phosphorus use differed from the micronutrient levels of the control. This indicated that the two cultivars did respond to fertilization. Positive responses from the use of phosphate sources in the production of forage have been reported by several authors (Dias et al., 2012; Franzini et al., 2009; Guedes et al., 2009; Ieiri et al., 2010; Oliveira et al., 2012).

The green and dry mass yields of both cultivars showed no significant differences (P> 0.05) (Table 4) but there was an increase due to the addition of any of the sources of phosphorus. However, significant differences were not observed among the sources of phosphorus. Similar results have been reported by Dias et al. (2015). They noted that there were no differences in the dry matter production of *Urochloa* leaves among various sources of phosphate fertilizer (superphosphate, reactive phosphate from Algeria, natural reactive phosphate from Algeria and simple superphosphate), at 70 kg ha^{-1} P_2O_5. Costa et al. (2008) evaluated the response of different sources of phosphorus applied in Haplortox latosol using *Urochloa brizantha* and found that the total dry mass production and phosphorus accumulation were more efficient with sources of higher solubility among these reactive forms of phosphate rock.

Soluble sources (organic mineral and triple superphosphate) were not found to be more effective than the reactive phosphate in the production of fresh and dry biomass. Production was lower than expected. Guedes et al. (2009) reported that less soluble phosphorus sources have lower efficiency than soluble phosphates in the short term but in the long term the residual effects are generally higher. Probably, the rapid solubility of P

Table 3. Height (cm) and stem diameter (mm) of Urochloa *brizantha* cv. Marandu and Xarés grown in a greenhouse using different phosphorus sources.

Source of P	***Urochloa***					
	Height			**Stem diameter**		
	Xaraes	**Marandu**	**Mean**	**Xaraes**	**Marandu**	**Mean**
NP	15.06	16.16	15.61^{B}	1.76	1.68	1.72^{B}
OM	18.65	25.66	22.16^{A}	2.34	2.44	2.39^{A}
TS	19.80	26.53	23.16^{A}	2.72	2.57	2.64^{A}
RP	19.47	26.23	22.85^{A}	2.36	2.27	2.32^{A}
Mean	18.25^{b}	23.64^{a}		2.30^{a}	2.24^{a}	
	CV: 18.39%			CV: 18.76%		

Means followed by the same capital letters in columns and small letters in lines are statistically different from each other at the 5% probability level by the Tukey test. P sources: NP - no phosphate; OM - organic mineral; TS - triple superphosphate; RP - reactive phosphate; CV = coefficient of variation.

Table 4. Fresh and dry matter (g) of the above ground parts of *U. brizantha* c.v Marandu and Xaraes cultivated in a greenhouse using different sources of phosphorus.

Source of P	***Urochloa***					
	Fresh matter			**Dry matter**		
	Xaraés	**Marandu**	**Mean**	**Xaraés**	**Marandu**	**Mean**
NP	10.39	9.85	10.12^{B}	1.74	1.40	1.57^{B}
OM	22.86	33.10	28.00^{A}	3.52	4.50	4.01^{A}
TS	24.99	30.69	27.84^{A}	3.85	4.23	4.04^{A}
RP	23.46	30.98	27.22^{A}	3.70	4.11	3.91^{A}
Means	20.42^{a}	26.16a		3.20^{a}	3.56^{a}	
	CV: 42.69 %			CV: 42.82%		

Means followed by the same capital letters in columns and small letters in lines are statistically different from each other at the 5% probability level by the Tukey test. P Sources: NP - No Phosphate; OM - Organic Mineral; TS- Triple Superphosphate; RP- Reactive Phosphate; CV = Coefficient of variation.

present in triple superphosphate and organic mineral interacted with the clay Oxisol used in the experiment, reducing the absorption of P and the resulting biomass of two cultivars. Loganathan and Fernando (1980) discovered that when adding a soluble source of phosphorus to a particular soil, more than 90% of the total quantity applied is absorbed in the first hour of contact with the soil.

Chlorophyll A and B levels (fresh and dry matter) did not differ between Marandu and Xaraés cultivars or among any of the three sources of phosphorus evaluated (Table 5). However, the chlorophyll contents A and B were significantly less in the control treatment. Therefore, it was understood that there was an increase in the production of chlorophyll A and B among all the sources of phosphorus. According to Cruz et al. (2007), a reduction in the amount of chlorophyll may be due to a negative effect of nitrogen deficiency on photosynthetic rate. According to Rezende (2013), phosphorus fertilization on forage generates an increase in the production of chlorophyll, suggesting that phosphorus absorption favors the absorption of nitrogen by forage.

Irving (2015) believes that the photosynthetic rate varies among species but leaf nitrogen content and light intensity are the determining factors. Carbon is one of the principal components of the photosynthetic system. Malavolta (2006) reported that the indirect effect of chlorophyll is due to the role of phosphorus in plant nutrition since its part in the ATP molecule benefits the active process of nitrogen absorption. Martins and Pitelli (2000) observed an increase in levels of chlorophyll A and B in soybeans with liming. Therefore, the chlorophyll A and B contents are not only associated with nitrogen levels but also with the nutritional status of forage plants.

Conclusions

The two cultivars of *U. brizantha*, c.v Marandu and Xaraés, were responsive to the phosphate fertilization of

Table 5. Chlorophyll A and B levels in *U. brizantha* c.v Marandu and Xaraés cultivated in a greenhouse using different sources of phosphorus.

Source of P	*Urochloa*					
	Chlorophyll A			Chlorophyll B		
	Xaraes	Marandu	Mean	Xaraes	Marandu	Mean
NO	18.9	20.1	19.5[B]	6.9	6.6	6.8[B]
OM	29.5	32.0	30.7[A]	9.4	10.0	9.7[A]
TS	32.5	32.7	32.6[A]	10.6	9.6	10.1[A]
RP	30.1	30.1	30.1[A]	8.9	8.4	8.7[A]
Means	27.75[a]	28.72a		9.0[a]	8.6[a]	
	CV: 11.33%			CV: 16.77%		

Means followed by the same capital letters in columns and small letters in lines are statistically different from each other at the 5% probability level by the Tukey test. P Sources: NO- no phosphate; OM- organic mineral; TS- triple superphosphate; RP- reactive phosphate; CV = coefficient of variation.

all of the sources tested. The three sources of phosphorus used in the experiment promoted increases of height and stem diameter for both cultivars, when compared with the control plants. There was also an increase in the levels of chlorophyll A and B with the use of all sources of phosphate fertilizer. The two cultivars had increased quantities of fresh and dry, regardless of source when compared with the control. Leaf contents of S, Fe and Cu, however, were not affected by phosphorus. The *U. brizantha* c.v Xaraés had higher levels of N, P, K, Ca, Mg, Mn and Zn in the leaves.

Conflict of Interests

The authors have not declared any conflict of interests.

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academicJournals

Vol. 11(23), pp. 2032-2042, 9 June, 2016
DOI: 10.5897/AJAR2015.10649
Article Number: F7A1B3C58902
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Fractal analysis in the description of soil particle-size distribution under different land-use patterns in Southern Amazonas State, Brazil

José M. Cunha[1]*, Milton C. C. Campos[1], Denilton C. Gaio[2], José S. Nogueira[2], Marcelo D. R. Soares[3], Douglas M. P. Silva[1] and Ivanildo A. de Oliveira[4]

[1]Federal University Amazonas, Institute of Education, Agriculture and Environment, 69800-000, Humaitá, Amazonas State, Brazil.
[2]Federal University Mato Grosso, Physics Institute, 78060-900, Cuiaba, MatoGrosso State, Brazil.
[3]University of Campinas, School Agricultural Engineering, 13083-875, Campinas, Sao Paulo State, Brazil.
[4]Estadual University Paulista - UNESP, Faculty of Agriculture and Veterinary Sciences, Department of Soils and Fertilizers, 14870-900, Jaboticabal, SP, Brazil.

Received 13 November, 2015; Accepted 23 April, 2016

Understanding the variability of soil properties is crucial to identify areas susceptible to physical degradation. The soil degradation is often determined by the current state of the soil structure, that is, the aggregate size distribution. Therefore, this article suggested evaluating aggregate sizes' distribution by using the fractal theory. The goals were: (i) to calculate the fractal mass dimension of soil aggregates in areas under agroforestry, forestry, sugarcane, cassava and pasture in Southern Amazonas state, Brazil, showing correlations with soil properties; (ii) to compare the means of fractal dimension mass of the distribution of particle sizes on different soil under the different uses; and (iii) to investigate spatial variability of such fragmentation for each management system. Fragmentation was determined from fractal mass dimension. Aggregates were sampled within a depth range of 0.00 to 0.10 m, over a regular sample grid of 70 × 70 m, with georeferenced sample points, and regular spacing each 10 m, totaling 64 points per mesh. Higher mean values of fractal mass dimension were found in agroforestry use system and the lowest under native forest and pasture, with no statistical difference fractal mass dimension, when assessed in relation to the type and land use. The degree of fragmentation of soil aggregates was found to be influenced by the type of soil and strongly correlated with fine particles, higher in Red-Yellow Oxisols with better physical quality when compared with other areas. It showed a strong spatial dependence and the exponential model that got the best adjustment of the semivariogram.

Key words: Soil physics, geostatistics, fractional dimension.

INTRODUCTION

Changes in vegetation composition of natural ecosystems associated with management practices, which are attributed to factors such as farm, livestock, bring consequences not only in relation to biodiversity, but also when analyzing the damage caused to the soil and its ability reuse and / or storage (Chaves et al., 2012). The quality of the soil structure is a good indicator of sustainability management systems, which in turn is influenced by pedogenetic conditions. On the other hand, the use and comprehensive management of soil can result in irreparable consequences for the physical soil quality, reducing it in their productive potential. Researchers have investigated soil use quality, assessing the relationships between direct and indirect measures over the soil (Usowicz and Lipiec, 2009; Campos et al., 2012; Oliveira et al., 2013). In contrast, soil management and crop type would change the aggregates and, consequently, its structure.

The soil is considered a complex system resulting from the interaction of geological, topographical and climatic factors, among others, which together form indicators (variables) that characterize (Freitas et al., 2014). Compositions difference of the soil particles sizes exhibit fractal features with irregular shapes and structures of self-similarity (Tyler and Wheatcraft, 1992). This aroused the academic community to use the fractal as an effective descriptive tool to characterize the size of the soil particles' (Prosperini and Perugini, 2008; Xia et al., 2015), structure, aggregation and soil erodibility (Ahmadi et al., 2011; Xu et al., 2013; Tang et al., 2013; Xiao et al., 2014).

Thus, the fractal theory has contributed to characterize the size distribution of soil particles as a way to assess the impacts of the soil use and quantify relationships between the use the soil and physical and chemical properties of the soil. Accordingly, the mass fractal dimension of size distribution of particles is useful parameters able to monitor the degree of degradation of the soils (Su et al., 2004).

Studies have revealed significant correlations of soil attributes with the dimension of fractal mass. Gui et al. (2010) found that the fractal dimension increases with small particles and decreases to larger particles in addition to positive correlation with soil organic matter. Different soil uses and plant communities or vegetation revealed that the size of the soil particles differed significantly between the different systems of management and land use, influencing also the values of the fractal mass dimension of soil (Wang et al., 2006, 2008; Zhao et al., 2006; Liu et al., 2009; Xu et al., 2013).

Overall, soil properties have a high degree of spatial variation due to pedogenic features (Oliveira et al., 2013; Aquino et al., 2014). A large number of studies have made use of geostatistics to characterize and compare the various soil attributes and search for a corresponding statistical correlation (Allaire et al., 2012; Usowicz and Lipiec, 2009; Millán et al., 2012). Castrignanó and Stelluti (1999) used fractal geometry and geostatistics to describe the importance of clay variability on soil clustering. Carvalho et al. (2004) ascertained the fragmentation of soil aggregates through the fractal theory, assessing the spatial dependence under varied treatments. Millán et al. (2012) used the multivariate spatial analysis for some soil physical attributes that have correlations to physically degraded areas.

Despite of the great contributions related to physical and chemical properties, a better understanding is required about its use in soil science; thus, this research is a way to use such attribute as a tool for empirical analysis that can be added to other research of this nature.

Therefore, the goals of this research were: (i) to calculate the fractal mass dimension of soil aggregates in areas under agroforestry, forestry, sugarcane, cassava and pasture in Southern Amazonas state, Brazil, showing correlations with soil properties; (ii) to compare the means of fractal dimension mass of the distribution of particle sizes on different soil under the different uses; and (iii) to investigate spatial variability of such fragmentation for each management system.

MATERIALS AND METHODS

The study was performed in the counties of Humaitá and Manicoré, which are located in Southern Amazonas state, in Brazil, under different management systems and soil types. The treatments were areas under agroforestry on Red-Yellow Latosol (Oxisol), sugarcane and cassava on Haplic Cambisol (Inceptisol), and areas under natural forest and pasture on Yellow Argisol (Ultisol). Both municipalities are placed near BR 364 and 230 road (also called "Transamazônica"), toward Apuí city in Amazonas state (Figure 1). The studied areas are situated under the same climatic zone, which belong to group A (rainy tropical climate) and Am (monsoon type climate) according to Köppen's classification, with a short dry season. Rainfall ranges from 2.250 and 2.750 mm and rainy season starting in October up to June. Annual mean temperatures alternate between 25 and 27°C and air relative humidity from 85 to 90%.

Five management systems were selected under different traditional uses. A squared sample grid was set on each soil use area, which has an area of 0.49 ha and soil samples were collected from each mesh cross point (grid point), whose regular spacing was each 10 m, totaling 64 sample points for each grid. These points were georeferenced by means of a GPSMAP 76CSx Garmin device (Garmin Ltd., Taipei, Taiwan).

Samples were collected within August to October of 2012. Soil deformed samples were taken from a depth range of 0.00 to 0.10 m at each grid point, keeping a preserved structure within soil clod to determine the stability of aggregates via wet sieving, totalizing 320 soil samples for all five sample grids.

Soil clods were slightly crushed, manually, and passed through a 9.51 mm sieve being retained on a 4.76 mm one; then, they were shade dried for stability analysis. Aggregate separation and stability were determined by method proposed by Kemper and Chepil (1965), with modifications in the following diameter classes: 4.76 to 2.0; 2.0 to 1.0; 1.0 to 0.50; 0.50 to 0.25; 0.25 to 0.125; 0.125 to 0.063 mm. 20 g of aggregates retained in the sieve of 4.76 mm were used and placed in contact with water in sieve 2.0 mm and

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

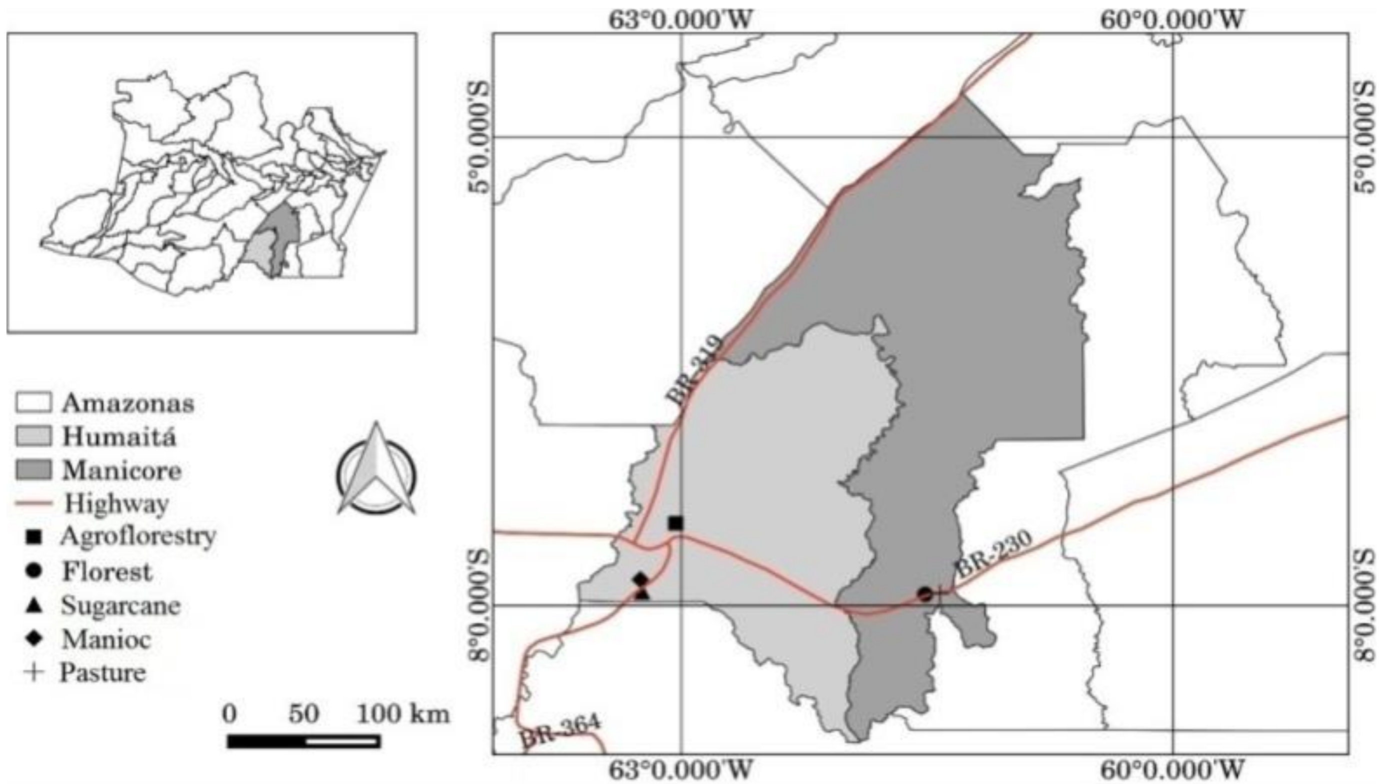

Figure 1. Location of the studied area.

subjected to vertical shaking in Yoder device (SOLOTEST, Bela Vista, São Paulo, Brazil) for 15 min. The content retained on each sieve size was then placed in an oven at 105°C for further weighting on digital scale.

Results expressed in aggregate mass retained at each sieve size and analyzed for spatial variability determination of soil aggregation by means of fractal geometry and geostatistics.

Fractal definition in soil physics can be based on the relationship of aggregate number and particle size distribution as the following equation (Mandelbrot, 1982; Turcotte, 1986):

$$N(X > x_i) = k{x_i}^{-D_f}, \quad (1)$$

wherein N (X > x_i) is the number of accumulated particles (objects) at a certain size that is bigger than x_i, established by the sieve size, k is the number of elements in a size unit set and D_f is the fractal dimension, which ascertains the fragmentation of soil aggregates (Carvalho et al., 2004). Equation 1 cannot be used directly to study soil aggregates, because it would be unfeasible to count the number of particles of each size. Therefore, a method which estimates the number of particles retained by a sieve size using the fractal mass model, developed by Tyler and Wheatcraft (1992), can be used and it is defined by the equation:

$$\frac{M(r < R_i)}{M_T} = \left(\frac{R_i}{R_{máx}}\right)^{3-D_f}, \quad (2)$$

wherein M (r <R_i) is the accumulated mass of particles of an r size lower than sieve size (R_i), M_T is the total mass of the particles, $R_{máx}$ is a parameter that determines the diameter of the largest aggregate and D_f is the fractal mass dimension of the aggregates. This dimension has as superior and inferior limits the values within 0 to 3. This way, such limitation demonstrates a physical mismatch of the accumulated mass to surpass the total mass within a system when D_f < 0 and D_f > 3.

Aggregate stability data for each sample point were transformed into log-log scale for accumulated mass $\left(\frac{M(r<R_i)}{M_T}\right)$ and particle size ($\frac{R_i}{R_{máx}}$), being set in the Equation 2. They show that a function of power law index is required to describe soil aggregates' fragmentation, based on sieve fragmentation. The amounts retained at each sieve were measured in percentage, which is the ratio between the mass of aggregates of each sieve by the mass of aggregates used in the Yoder (20 g) multiplied by one hundred. These proportions related to the diameter of sieves, following a power law scale set to the sieve diameters, as raised by the fractal theory (Mandelbrot, 1982). The D_f and $R_{máx}$ parameters were determined by fitting the log-log transformation curve from Equation 2, using the *nls* function of the R software (R Core Team, 2013) to set the parameters.

The values of means, maximum and minimum differences, variance, standard deviation, asymmetry, kurtosis and variation coefficients were calculated via data exploratory analysis. Comparisons of aggregate stability means, GMD, WMD and fractal mass dimension for all soil management systems were analyzed by the Tukey test at 5% significance. Pearson correlations were carried out between the size of fractal mass and granulometric parameters of the soils. The statistical analyses were performed by Statistica 7.0 version (StatSoft, 2005).

The fractal mass dimension was characterized spatially by

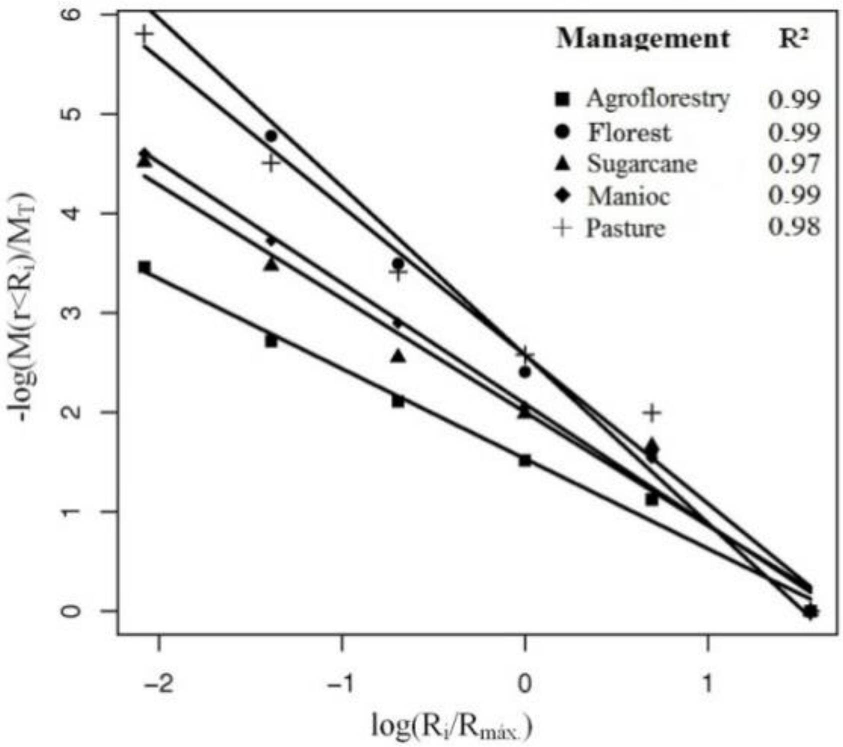

Figure 2. Cumulative mass log-log fit according to particle diameter. The symbol represents experimental data and the straight line stands for the fitted model.

means of geostatistics (Carvalho et al., 2004; Millán et al., 2012). The experimental semivariogram was estimated under an intrinsic hypothesis using the following equation:

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(x_i) - Z(x_i + h)]^2, \qquad (3)$$

wherein $\hat{y}(h)$ represents the variance at an h distance, N(h) is the number of pairs in semivariance calculation, Z(x_i) is the value of a Z attribute at an x_i position and Z(x_i+h) is the value of Z at an h distance from x_i.

From a mathematical model fitting to $\hat{y}(h)$ calculated values, theoretical model coefficients are defined for the semivariogram: nugget effect (C_0), structural variance (C), sill (C_0 + C) and range (a_o). The intersection of semivariance and y-axis is the nugget effect and stands for the attribute variability in a shorter spacing than the sampled one (Siqueira et al., 2008). In the spatial dependence analysis of soil properties under study, we used the classification proposed by Cambardella et al. (1994), in which spatial dependence will be strong if the ratio $\left[\frac{C_0}{C_0+C}\right]$ is ≤ 25. If the ratio is between 26 and 75%, spatial dependence will be considered moderate; and when it is from 75 to 100%, approximately, the dependence can be classified as weak. The range (a_o) represents the distance within which attributes are correlated, under a uniform area with an estimated radius. Semivariogram analysis was carried in October using GS + 7.0 software (Robertson, 1998) to check variability presence or absence. The best-fitted semivariogram model was defined in terms of the highest coefficient of determination (R^2), the lower nugget effect and highest coefficient of variation of cross validation.

Once checked, the spatial dependence of soil attributes, semivariance values were obtained and interpolated with ordinary kriging data. It enabled building contour maps of each attribute, helping in their interpretation.

RESULTS AND DISCUSSION

Figure 2 shows a linear trend of logarithmic transformation for aggregate mass and sieve sizes, being in accordance with findings of previous studies such as Liu et al. (2009), Parent et al. (2011) and Xu et al. (2013). Yet Menéndez et al. (2005) and Prosperini and Perugini (2008) had found a multiple linear trend for such parameters, indicating different arrangements for different particle sizes (sieve diameters).

Soil aggregation is strongly influenced by some physical and chemical soil attributes. Soil use and management provide changes on these attributes, since they modify its structure and aggregate stability. Furthermore, other environmental and anthropic factors may alter soil aggregation. In this sense, Table 1 shows some of these chemical properties in agroforestry (AF), natural forest (NF), sugarcane (SC), cassava (CA) and pasture (Pt) systems.

Table 2 presents the descriptive statistics of the

Table 1. Soil chemical properties and texture under different management systems.

Soil management	pH in H_2O	P	H + Al	Ca	Mg	K	V	C_{org}	Sand	Silt	Clay
		mg kg^{-1}	cmol$_c$ kg^{-1}				(%)	g kg^{-1}	g kg^{-1}		
AF	3.8	8.2	18.9	0.2	0.1	0.1	2.5	11.9	220.8	230.1	549.1
NF	4.0	6.1	7.5	0.5	0.2	0.2	10.7	10.8	358.8	313.2	327.9
SC	4.4	6.4	9.0	1.5	0.8	0.1	20.5	17.7	240.6	474.5	284.9
CA	3.9	6.0	15.8	0.2	0.1	0.1	3.2	16.1	158.9	557.6	283.4
Pt	4.3	4.6	6.1	1.0	0.5	0.2	21.4	15.9	410.8	227.6	361.6

AF: Agroforestry; NF: natural forest; SC: sugarcane; CA: cassava; Pt: pasture.

Table 2. Descriptive statistics of the variables aggregate size, geometric mean diameter, weighted mean diameter and fractal mass dimension within the studies areas.

Soil management	Variable	SW	Δx	Var	SD	C_s	C_k	VC (%)
AF		ns	40.26	87.3	9.3	-0.60	-0.40	11.4
NF		ns	37.10	87.4	9.4	-0.52	-0.63	12.2
SC	4.76-2.00 (mm)	ns	23.79	32.5	5.7	-0.68	-0.10	6.4
CA		ns	30.46	48.6	7.0	-0.66	-0.14	8.2
Pt		ns	22.32	27.7	5.3	-0.74	-0.13	5.8
AF		ns	8.29	4.5	2.1	0.84	-0.09	56.7
NF		0.29	13.09	10.1	3.2	0.32	-0.59	34.8
SC	2.00-1.00 (mm)	ns	5.71	1.8	1.4	0.64	-0.20	66.7
CA		ns	7.82	4.0	2.0	0.58	-0.16	57.1
Pt		ns	8.64	4.1	2.0	0.59	-0.36	64.5
AF		ns	18.58	17.5	4.2	0.92	0.70	50.6
NF		ns	28.68	49.1	7.0	0.71	-0.30	50.3
SC	< 1.00 (mm)	ns	18.93	19.5	4.4	0.71	-0.02	53.7
CA		ns	23.70	28.0	5.3	0.79	0.20	47.3
Pt		ns	16.65	12.4	3.5	1.02	1.01	62.5
AF		0.12	2.89	0.36	0.6	0.30	0.37	28.6
NF		ns	1.58	0.16	0.4	-0.40	-0.77	17.4
SC	GMD (mm)	ns	1.39	0.12	0.34	-0.47	-0.59	13.6
CA		0.19	1.73	0.14	0.38	-0.55	0.08	16.5
Pt		ns	1.21	0.08	0.28	-0.60	-0.21	9.7
AF		ns	1.24	0.07	0.27	-0.67	-0.16	10.3
NF		ns	0.92	0.05	0.23	-0.62	-0.48	8.2
SC	WMD (mm)	ns	0.58	0.02	0.14	-0.69	-0.11	4.5
CA		ns	0.77	0.03	0.18	-0.71	-0.03	6.0
Pt		ns	0.45	0.01	0.12	-0.60	-0.54	3.7
AF		0.74	0.53	0.013	0.114	0.144	-0.431	5.78
NF		0.24	0.57	0.019	0.136	-0.141	-0.704	9.36
SC	D_f	0.71	0.48	0.011	0.106	-0.046	-0.234	6.03
CA		0.52	0.39	0.008	0.088	-0.019	0.018	4.90
Pt		0.15	0.48	0.013	0.113	0.092	-0.777	8.07

SW: Shapiro-Wilk's normality test; Δx: difference between maximum and minimum value; Var: variance; SD: standard deviation; C_s: asymmetry coefficient; C_k: kurtosis coefficient; VC: variation coefficient; ns: not significant; GMD: geometric mean diameter; WMD: weighted mean diameter.

Table 3. Aggregate size distribution and mean test of the variables geometric mean diameter, weighted mean diameter and fractal mass dimension in areas under different uses in Southern Amazonas.

Soil management	Aggregate classes%			GMD (mm)	WMD (mm)	D_f
	4.76-2.00	2.00-1.00	<1.00			
AF	80.79^c	4.04^b	15.17^a	2.1^c	2.9^c	1.97^a
NF	76.38^d	9.44^a	14.17^{ab}	2.3^c	2.8^c	1.45^c
SC	89.64^a	2.12^c	8.24^c	2.5^b	3.1^a	1.75^b
CA	85.07^b	3.59^b	11.34^b	2.3^c	3.0^b	1.79^b
Pt	90.52^a	3.24^{bc}	6.25^c	2.9^a	3.2^a	1.40^c

AF: Agroforestry; NF: natural forest; SC: sugarcane; CA: cassava; Pt: pasture. Means followed by the same letter in the first column do not differ from each other by the Tukey's test (p< 0.05).

variables aggregate size, geometric mean diameter (GMD), weighted mean diameter (WMD) and fractal mass dimension (D_f). Only aggregate size between 4.76 and 2.00 mm, GMD, WMD and D_f had a reasonable standard deviation, resulting in low variance data with regards to mean values. Data showed variance homogeneity ($p < 0.05$); however, mean tests (Table 3) of soil physical attributes displayed significant alterations ($p < 0.05$) for all variables when comparing all evaluated systems. All variables had asymmetry and kurtosis values close to zero, which characterizes a symmetric distribution and justify mean and median similar values, although most variables have no normal distribution, it is important to highlight that the distribution has no long tails.

Regarding the variation coefficient (Table 2), fractal mass dimension was low in all management uses, with the lowest values found for cassava (VC = 4.90 %) and the highest one for native forest (VC = 9.36%). It demonstrates a low variation in soil fragmentation degree, what denotes uniformity in mean values. Still, range was larger in NF ($\Delta x = 0.57$) and smaller in cassava fields ($\Delta x = 0.39$), which might be related to soil chemical or physical features. The range (Δx) between maximum and minimum values of the attributes reinforces data suitability to assess spatial patterns of variables. Kurtosis and asymmetry coefficients are compared to a normal distribution with values near zero (Table 2), showing either a symmetric and platykurtic distribution ($C_k < 3$) for all areas. In these, soil fragmentation degree (D_f) presented low values for standard deviation.

However, Table 3 shows a higher percentage of 4.76 to 2.00 mm aggregate size, highlighting pasture and sugarcane fields which have significant differences when compared with the others. This physical feature is owned to fasciculate roots of grasses and sugarcane that favors soil clustering. Such result gives soil a greater resistance to degradation (Oliveira et al., 2013), since roots are responsible for soil restructuring and reduction of erodibility (Neves et al., 2006).

Both GMD and WMD were higher in pasture and sugarcane fields, filling soil with greater contents of organic carbon (Table 1), which also plays an important role in soil aggregation (Rozane et al., 2010). This result also corroborates the Matos et al. (2008), when evaluating the stability of aggregates on the impact of organic and mineral fertilizers to the soil. Actually, a larger amount of dry matter is found on such environments, increasing as consequence soil organic matter. Grain size is another element with great importance to soil aggregation; Table 1 shows that agroforestry soil has higher contents of clay. Yet for sugarcane soil, there is predominance of silt fraction and for pasture, it is sand.

Contrarily, fractal mass dimension has been pointed to describe qualitatively distribution of particle size, aggregates and the degree of soil fragmentation (Carvalho et al., 2004; Filgueira et al., 2006; Prosperini and Perugini, 2008). The greatest fractal mass dimension was seen in agroforestry areas ($D_f = 1.971$), highlighting soils with greater physical stability as cited by Xu et al. (2013).

There was no significant difference of fractal mass dimension between sugarcane and cassava ($D_f = 1.758$ and $D_f = 1.796$, respectively). Millán et al. (2012) found values similar to sugarcane in a Vertisol in Bayamo, Cuba. These areas seem to have a lower quality compared to agroforestry areas, which might be related to machinery use (soil compaction, pedogenic aspects and crop burnings). In addition, native forest and pasture had no differences among each other ($D_f = 1.453$ and $D_f = 1.401$, respectively), which had the smallest fractal mass dimension, what denotes a lower quality compared to the others. This result is inverse to the aggregate stability for pasture system (Table 3), having a greater percentage of grain sizes >2.00 mm. It may be related to cattle stamping and machinery use on grazing areas, what could have favored soil compaction, favoring with that in denser clusters, but under lower structural resistance. Another fact is that both pasture and forest environments have similar chemical and textural characteristics (Table 1).

Comparing the soil types, characterized by the size of

Table 4. Aggregate size distribution and mean test of the variables geometric mean diameter, weighted mean diameter and fractal mass dimension at different soil types in Southern Amazonas.

Soil types	Aggregate classes			GMD	WMD	D_f
	4.76-2.00	**2.00-1.00**	**<1.00**	**(mm)**	**(mm)**	
Latosol (Oxisol)	80.79[b]	4.04[b]	15.17[a]	2.1[b]	2.9[b]	1.97[a]
Argisol (Ultisol)	83.72[b]	6.21[a]	7.21[b]	2.5[a]	3.0[a]	1.78[b]
Cambisol (Inceptisol)	87.53[a]	2.69[b]	5.61[c]	2.4[a]	3.0[a]	1.41[c]

Means followed by the same letter in the first column do not differ from each other by the Tukey's test ($p < 0.05$).

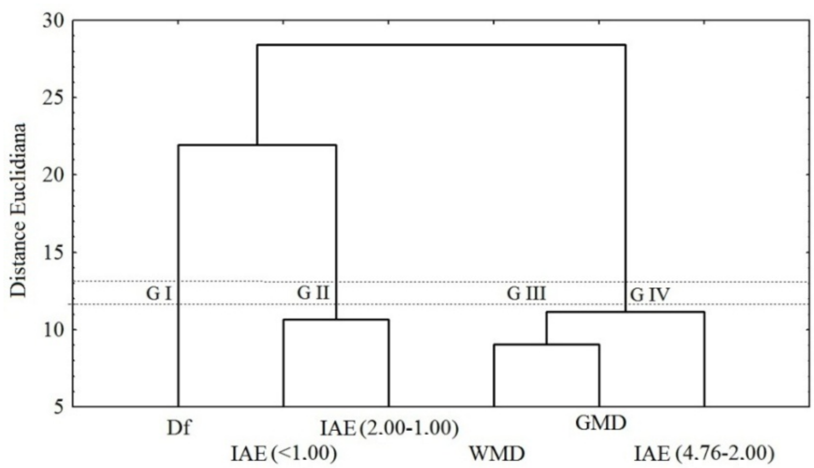

Figure 3. Dendrogram of hierarchical cluster analysis showing the clustering of the analyzed variables in Southern Amazonas. Df = Fractal mass dimension; IAE (%) = aggregate stability index; GMD = geometric mean diameter; WMD = weighted mean diameter.

the soil particles, Red-yellow Latosol (agroforestry), Yellow Argisol (forest and pasture) and Haplic Cambisol (sugarcane and cassava), a greater percentage of aggregates with 4.76 to 2.00 mm were seen in the Cambisol (Table 4), which differed from the other types. The attributes GMD and WMD had no significant differences in comparing Argisol against Cambisol;
however, both differed from Latosol.

Nevertheless, regarding the size class of <1.00 mm, we observed a higher percentage in Latosols, which showed the highest value for the fractal mass dimension. Recent research has identified strong correlation of the fractal mass dimension with fine particles (Gui et al., 2010; Xia et al., 2015), found that the mass fractal dimension increased with clay content, but decreased with sand content. This explains the fact that D_f is more correlated to <1.00 mm classes, in which the hierarchical cluster analysis identifies the separation of groups (Figure 3) assuring such statement, with D_f strongly correlated with aggregate stability index, IAE <1.0 mm and IAE between 2.00 and 1.00 mm. From Figure 3, it can be observed a group formation G I and II (D_f and 2.00 to 1.00 mm and <1.00 mm). However, GMD and WMD (G III) have a strong correlation with the size class of 4.76 to 2.00 mm (G IV), which substantiates its greater influence on estimated values of GMD and WMD.

Another relevant point is that D_f did not differ among management systems (Table 3) and soil types (Table 4). It may be related to strong correlations between fractal mass and some physical and chemical attributes that characterize the soil types.

Thus, it was observed in Latosols positive correlation (r_{cor} = 0.45) of the mass fractal dimension with the sand content and a negative (r_{cor} = -0.35) with the clay content; in Argissolos positive correlation (r_{cor} = 0.18) the fractal dimension of the mass with silt content and negative (r_{cor} = -0.22) with the soil organic matter. There was no evidence of correlation of fractal mass dimension with the soil properties of the order of Cambissolos. It is possible that the mass fractal dimension is more sensitive to different types of soils which uses the ground. Soil quality is closely related to its chemical properties, but also has a strong relation as the particle size and content of soil organic matter, these in turn favoring the a good ground support structure and drainage of water and nutrients. Still, different land uses and communities of plants and vegetation (Wang et al., 2006; Wang et al., 2008; Zhao et al., 2006; Liu et al., 2009; Xu et al., 2013) revealed that

Table 5. Geostatistical analysis of fractal mass dimension in the studied areas.

Study area	Model	Nugget effect (Co)	Sill (Co+C)	Range (m)	$\left[\frac{C_0}{C_0+C}\right] \times 100$ *	R^2 **
AF	Exp.	0.00029	0.01428	47.7	2.03	0.93
NF	Exp.	0.00001	0.02922	18.9	0.03	0.84
SC	Exp.	0.00001	0.01162	30.3	0.09	0.85
CA	Exp.	0.00001	0.00869	17.5	0.11	0.89
Pt	Exp.	0.00089	0.01238	29.4	7.19	0.98

Exp: Exponential; *Spatial dependence degree; **Determination coefficient.

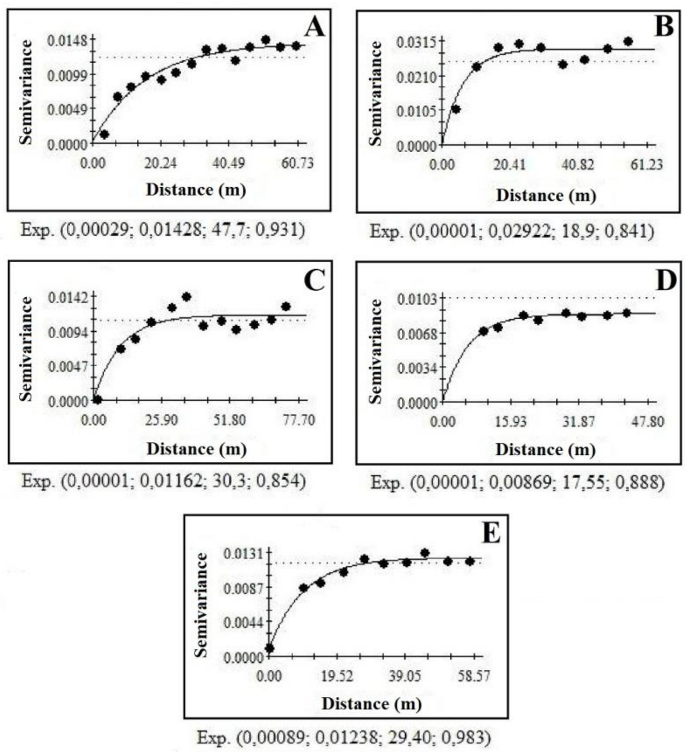

Figure 4. Experimental semivariogram of fractal mass dimension Df of the areas. A) Agroforestry; B) Natural Forest; C) Sugarcane; D) Cassava; E) Pasture. Below the pictures, it follows information as: Model (nugget effect; sill; DSD; range; R^2). DSD: Degree of Spatial Dependence; R^2: coefficient of determination.

the size of the soil particles differed significantly between the different management and land use systems, influencing also the values of fractal dimension of soil mass.

From geostatistics, Figure 4A to E semivariogram fits of fractal mass dimension for each studied area. It was observed that soil fragmentation degree has strong spatial dependence for all management systems, which may be expressed in terms of semivariogram model fits (Table 5 and Figure 4). The exponential model was the one that best fit the experimental semivariogram in the areas, which is based on determination coefficient that reached values higher than 84% (Table 5 and Figure 4).

Each spatial pattern of fractal mass dimension presents different spatial dependence ranges (Table 5 and Figure 4). The agroforestry had the highest average value of the range, showing less variability of the data, justifying the fact that this management system causes less impact to the soil and thus the mass fractal dimension has lower variability. It can be stated that the higher the range, the lower the variability. The range is the distance at which the sampling points are correlated, that is the points

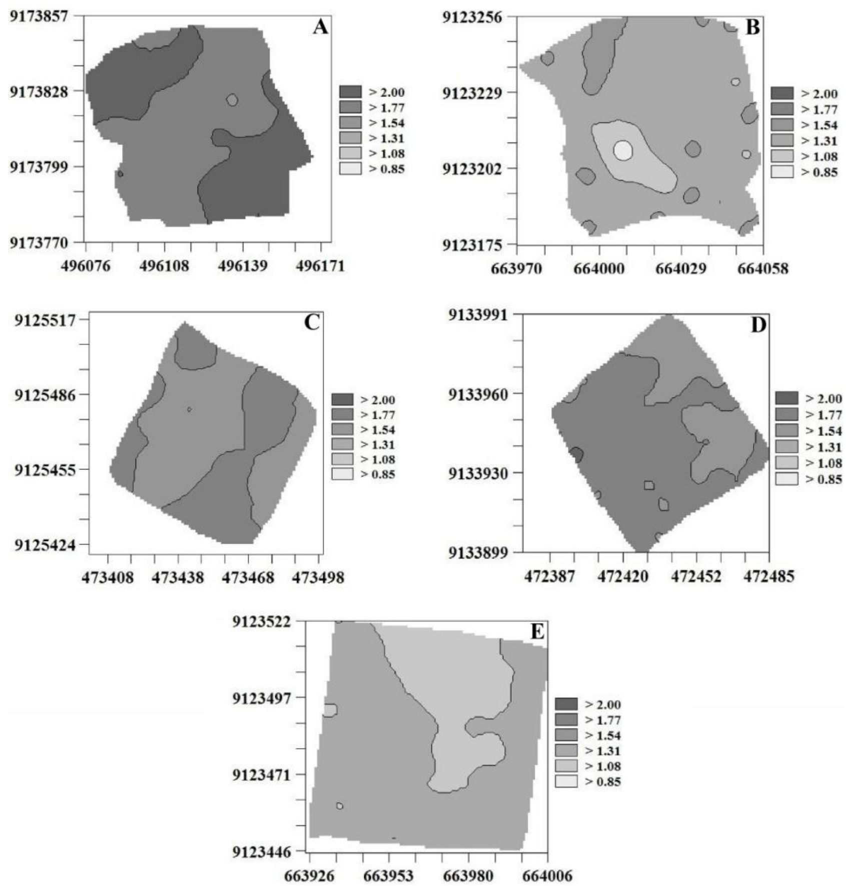

Figure 5. Kriging maps of fractal mass dimension Df in the studied areas: A) Agroforestry; B) Natural Forest; C) Sugarcane; D) Cassava; E) Pasture.

located a distance equal to the range area are more homogeneous with each other (Marques, Jr. et al., 2008). The areas of sugarcane and pasture also presented high and very similar values, perhaps by presenting a very similar radiculare system, even belonging to different soil classes (Cambisol and Ultisol, respectively). Millan et al. (2012) in a study to quantify the spatial structure of physical properties, they found scope of spatial dependence fractal mass dimension greater than 24 m in a Vertissols dedicated to sugarcane cultivation in the last sixty years.

The spatial dependence range of fractal mass dimension apparently has greater influence on the use and management of soil, since the soil structure is strongly affected by inappropriate land use, the use of agricultural machinery, livestock trampling, tickler soil, excessive land use without replenishment of nutrients, among others. The range of areas of native forest and cassava were very similar, also belong to different soil classes, but with lower values to other areas, with higher data variability. Under native forest, due to lack of agricultural practices, justified the fact that the fractal mass dimension present greater independence of spatial correlation, which in turn the soil is kept in conditions conducive to vegetation development and guarantee its own sustainability.

Interpolated values of the semivariogram models were used to build two-dimensional contour maps, whose kriging maps of soil fragmentation are represented in Figure 5A to E. These maps show an overview of the soil fragmentation under cultivated areas, identifying spots with most degraded physical structures.

Figure 5 shows that the fractal mass dimension in native forest (Figure 5B) had a heterogeneous behavior compared to the other areas and, likewise pasture, got the lowest values; therefore, it is a less fragmented soil area.

We have noticed that in the upper left and lower right corners of the agroforestry system (Figure 5A) regions with greater soil fragmentation, in which a best soil physical quality is encountered. Moreover, although there was no difference of fractal mass dimension between sugarcane and cassava areas (means of 1.758 and 1.796, respectively), Figure 5D demonstrates larger and more extensive mean values in cassava field; thus, having a better physical quality.

Conclusions

Agroforestry environments have greater mean values of fractal mass dimension, while native forest and pasture have the lowest ones. There was no difference between fractal mass dimension when evaluated between soil type and land use.

Comparing soil physical attributes among the management systems and soil types, fragmentation degree was influenced by the second, with greater fractal mass dimension in Red-Yellow Latosols, presenting a better physical quality compared to the others. Thus, the fractal mass dimension was found to be strongly correlated with fine particles, that is, IAE between 2.00 and 1.00 mm and IAE <1.00 mm.

Soil aggregate fragmentation described by fractal mass dimension had strong spatial dependence and the exponential model obtained a semivariogram best fit.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank the FAPEAM and FAPESP for financial support.

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academicJournals

Vol. 11(23), pp. 2043-2049, 9 June, 2016
DOI: 10.5897/AJAR2016.10833
Article Number: 7449A8F58904
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Propolis extract coated in "Pera" orange fruits: An alternative to cold storage

Flávia Regina Passos*, Fabrícia Queiroz Mendes, Mariana Crivelari da Cunha, Marília Crivelari da Cunha and André Mundstock Xavier de Carvalho

Institute of Agricultural Sciences, Federal University of Viçosa Campus of Rio Paranaíba – UFV CRP, Rio Paranaíba, Minas Gerais, Brazil.

Received 22 January, 2016; Accepted 10 March, 2016

Orange (*Citrus sinensis*) is a non-climacteric fruit that can be stored for long periods. However, the development of physiological and phytopathological disorders limits its postharvest storage. The objective of this study was to evaluate the effects of coating orange with propolis extract on the physicochemical characteristics of the 'Pera' orange during storage under ambient temperature. The fruits were selected and submitted to five postharvest treatments, three different forms of dip coating (70% alcohol, hydroalcoholic extract of propolis to 2.5%, hydroalcoholic extract of propolis to 5%), and two controls (one uncoated and one uncoated fruit kept under refrigeration). The variables weight loss, firmness, total soluble solid (TSS), titratable acidity (TA), ratio TSS/TA, and hydrogenic potential (pH) were evaluated at 0, 10, 18, and 25 days of storage. Treatment with propolis extract coating reduced the weight loss until the 18th day of storage. The fruit coated with propolis extract remained firmer up to 25 days of storage; this result is not significantly different from that of other postharvest treatments. The postharvest "refrigerated" treatment showed lesser weight loss and firmness during the storage period as a result of chilling injury. Coating with propolis extracts resulted in significant alterations of small magnitude in the variables TSS, TA, TSS/TA, and pH in oranges at the end of the storage period.

Key words: *Citrus sinensis*, coating, storage, quality, weight loss.

INTRODUCTION

Orange (*Citrus sinensis*) is a non-climacteric fruit that features respiratory activity and relatively low ethylene production, which declines slightly after harvest. The physical and chemical parameters in fruits can change during postharvest period (El-Ramady et al., 2015; Volpe et al., 2002). Therefore, some problems related to long storage conditions of oranges, including physiological and biochemical losses are able to improve disorders both in the skin and pulp, besides phytopathological agents that result in deterioration of fruit (Chitarra and Chitarra, 2005).

Postharvest conservation during storage at low

*Corresponding author. E-mail: flaviapassos1@yahoo.com.br.

temperatures allows reduction on cell metabolism during physiological process during maturation. However, it can cause physiological disorders such as chilling injury if the storage temperature is less than the Security Minimum Temperature (SMT) (Kluge et al., 2007). To orange storage any authors have already described optimal temperature changing of 0 to 9°C at 85 to 90% relative humidity (RH), at which the fruit can be stored by 3 to 13 weeks, depending on the cultivar and climatic conditions (El-Ramady et al., 2015; Chitarra and Chitarra, 2005). However, prolonged exposure to low temperatures than SMT is critical for orange fruit, and symptoms of chilling injuries are characterized by the appearance of necrotic superficial depressions in the bark (Kluge et al., 2006). Under this problematic with fruits, principally with characterized summer fruits, a technique of coating procedure has been used successfully (Aquino et al., 2015; Cissé et al., 2015; Ali et al., 2015a; Daiuto et al., 2012).

The application of coatings is a technique that is commonly practiced in postharvest orange conservation (Alleoni et al., 2006; Contreras-Oliva et al., 2011; Kouassi, et al., 2012; Pereira et al., 2014; Vieites et al., 1996). This procedure provides protection against water loss and allows increase postharvest conditions on shelf life (Pereira et al., 2014). However, thickness and coating type can lead to improper way to improve shelf life (Chitarra and Chitarra, 2005). Normally, these coating are prepared with wax and resins dissolved by apolar solvents. The excess of coating under the tissue can lead to an excessive production of ethanol and acetaldehyde, which are associated with anaerobic conditions (Contreras-Oliva et al., 2011). Overall, these substances when produced change fruit flavor.

The biopolymers used in the coating formulations include polysaccharides, proteins, lipids, and resins (Song and Cheng, 2014; Chitarra and Chitarra, 2005). Coatings with hydroxypropyl methylcellulose and beeswax provided weight loss control and maintenance of firmness and nutritional quality of ‘Valencia’ oranges (Contreras-Oliva et al., 2011). These coating materials containing essential oils (Du Plooy et al., 2009), which have fungistatic effect on oranges. Alleoni et al. (2006) observed a lower weight loss ‘Pera’ orange coated based protein concentrate of whey, associated with two types of plasticizers (glycerol and sorbitol) after 11 days of storage. In a few years, propolis has also been evaluated to improve quality of coatings. The hydrophobic compounds of propolis, as waxes and essential oils, acts as a barrier to water vapor and gas exchange (Ali et al., 2015b, 2014; Carvalho et al., 2013; Zahid et al., 2013) and possess broad spectrum of antimicrobial activity (Ali et al., 2015b, 2014).

Propolis is a resinous substance produced by Africanized bees *Apis mellifera* L. by the action of its enzymes on plant exudates (Sforcin, 2007). Its extracts is a good option of coating material with respect to its origin, and it is presumably safer for both the consumers and the environment when used as a substitute to synthetic materials commonly used in postharvest conservation of fruit.

Therefore, this study aimed to evaluate the coating effects with propolis extract on the physicochemical characteristics of ‘Pera’ oranges during storage under ambient temperature condition.

MATERIALS AND METHODS

Propolis, brown type, extracted by Africanized bees *Apis mellifera* L., was picked up from apiaries located in the southern state of Paraná, Brazil, with bee pasture typical mixed Ombrophylous forest ecosystem. The crude propolis was subjected to pre-cleaning, washing with cold water, and drying at 60°C for 10 h. The dried propolis was then packed in polyethylene bags and stored in a freezer at -5°C for 12 h. Then, 100 g of the material was triturated in a blender mix, packaged in an amber glass-bottle container, and the volume was made to 1 L with 70% ethanol (1th dilution). The suspension was kept to stand for 5 days at room temperature (25 ± 1°C). After this period, the homogenate was filtered by quantitative filter paper (JP 42; Quanty®; blue strip). The solution was used as stock solution to be used to obtain final concentrations of 2.5 and 5.0% (2th dilution) (Carvalho et al., 2013). In all dilutions, ethanol was used as diluent. Samples of ‘Pera’ oranges were acquired in a commercial market place and transported until laboratory analyses. The fruits were screened by shape uniformity, color, ripening index maturity and absence of pathogens. For the experimental setup, a group of 200 fruits were separated in five treatments as follows:

Treatment 1: Control - fruit without coating
Treatment 2: Alcohol - fruit coated with 70% (v/v) ethanol
Treatment 3: 2.5% propolis - fruit coated with 2.5% (w/v) of propolis hydroalcoholic extract;
Treatment 4: 5% propolis - fruit coated with 5% (w/v) of propolis hydroalcoholic extract;
Treatment 5: Refrigerated - uncoated fruit refrigerated at 9 ± 1°C.

The coatings were applied by immersion of the fruit in solutions mentioned individually for 5 s. After application, the fruit were placed horizontally on a nylon screen to drain the excess fluid for approximately 5 min. The fruit of the postharvest treatments “control”, “alcohol”, “2.5% propolis”, and “5% propolis” were placed on a workbench in a completely randomized design under the following storage conditions: 25 ± 3°C at 68 ± 10% RH. The fruit of the postharvest treatment “refrigerated” were stored under refrigeration at 9 ± 1°C at 70 ± 5% RH, based on the retail market conditions in Brazil that usually does not control the temperature and relative humidity during storage.

‘Pera’ oranges were obtained from the local market of Rio Paranaíba, Minas Gerais, Brazil and selected based on the of their peel color with no more than 15% of the surface area of the yellow peel. Evaluation of the orange fruit was performed before the application of postharvest treatment (time 0) and at 10; 18; and 25 days of storage, with three different periods of evaluation. The experimental units were subjected to analyses of weight loss (non-destructive group), firmness, total soluble solid (TSS), titratable acidity (TA), ratio TSS/TA, and hydrogen potential (pH) (destructive group), according to methods described by Adolfo Lutz Institute (2008).

For the weight loss analyses (non-destructive group), each fruit

Table 1. Weight loss (%) of 'Pera' orange coated and uncoated with propolis extract during the storage period.

Treatments	0 day	10 days	18 days	25 days	Marginal means	Adjusted model	R^2
Control	0	8.74^{B}	14.80^{B}	21.82^{A}	15.12	$\log(\hat{y}+1) = 0.025x + 0.745$	0.998
Alcohol		10.32^{A}	17.27^{A}	25.58^{A}	17.72	$\log(\hat{y}+1) = 0.022x + 0.847$	0.985
2.5% propolis		7.75^{BC}	12.05^{C}	21.41^{A}	13.74	$\log(\hat{y}+1) = 0.027x + 0.653$	0.985
5% propolis		6.88^{C}	12.88^{BC}	20.26^{A}	13.34	$\log(\hat{y}+1) = 0.029x + 0.613$	0.998
Refrigerated		5.65^{D}	9.15^{D}	14.68^{B}	9.83	$\log(\hat{y}+1) = 0.025x + 0.573$	0.998
Marginal Means		7.87	13.23	20.75		C.V.: 3.7%	

Means followed by the same letter in the column are not different at 5% significance level, by the SNK test.

was weighed at the beginning of the experiment (time 0) and at 10; 18; and 25 days of storage. The weight loss analysis was arranged in a completely randomized design in a split plot factorial 5 × 3, with the plots postharvest treatments (control, alcohol, 2.5% propolis, 5% propolis, and refrigerated) and in subplots evaluation times (10; 18; and 25 days of storage), with six replications. The analysis was performed by using a semi-analytical electronic balance (BL-320H model; Splabor), with 0.001 g sensitivity. Final weight was subtracted from the initial weight of the fruit and the results were expressed as percentage.

Analyses of firmness, TSS, TA, TSS/TA, and pH (destructive group) were determined in an entirely randomized design in a factorial arrangement of subdivided plots 5 × 3 + 1, and the plots postharvest treatments (control, alcohol, 2.5% propolis, 5% propolis, and refrigerated) and at subplots evaluation times (10; 18; and 25 days of storage), with the addition of the analyses performed at time zero, with six replications. For the determination of firmness, a digital penetrometer (PTR-300 model; Instrutherm®) with a probe diameter of 5 mm was used. The firmness was measured at two opposite points of the fruit in the equatorial region of the fruit, and a small portion of the peel was removed using a blade. The results were expressed in Newton (N). The TSS was measured directly by using a digital refractometer (PAL-1 model; Atago®), with automatic temperature compensation to 20°C, and the results were expressed as percentage (m/m). The TA was determined by titration of the sample with NaOH 0.01 mol L^{-1}, using 1% phenolphthalein as an indicator and expressed as percentage of citric acid, the dominant acid in orange fruit. The TSS/TA was calculated by dividing the TSS and TA, and the results were expressed by the resultant absolute value. The pH values were obtained by direct reading using the digital pH meter (MPA-210 model; Tecnopon® - Piracicaba, Brazil), calibrated with pH 4.0 and 7.0 buffer solutions, and the results were expressed by the resultant absolute value.

The data were checked by homogeneity of variances (Hartley test) and normality of residuals (Jarque - Bera test). The variables weight loss and TSS/TA were subjected to log and square root transformations, respectively, to suit analysis of variance (ANOVA) assumptions, using the F test at 5% significance. The influence of factors (postharvest treatments and storage period) and their interactions on the responses were submitted to factor analysis of split plot. After the split of analysis of variance (ANOVA), average postharvest treatments were compared by Student-Newman-Keuls (SNK) with $p < 0.05$. The mean of period analysis were submitted to regression analysis with $p < 0.05$, and the adjustment of the data was fetched to models with up to two dependent factors. When the interaction between the factors was not significant ($p > 0.05$), the marginal averages were used to compare treatments. When the interaction was significant, however, it proceeded to the unfolding of the interaction.

RESULTS AND DISCUSSION

There was influence of storage period (F = 608.29; $p < 0.001$), the types of postharvest treatment (F = 29.42; $p < 0.001$), and the interaction between factors (F = 2.43; $p = 0.037$) on the percentage weight loss (Table 1). A significant increase in weight loss was noted for all postharvest treatments during the storage period. This loss can be attributed to the metabolic reactions such as respiration and transpiration of the product, which reduces the amount of water present in the plant tissue (Chitarra and Chitarra, 2005).

The fruit coated only with alcohol showed greatest loss of weight, which was significantly higher than that for other types of postharvest treatments, until the 18th day of storage. Oranges concerning the postharvest treatment showed wrinkling and dehydration bark visually perceptible. The propolis extract coatings showed lower permeability to water vapor between the fruit and the medium until the 18th day of storage, thereby reducing the weight loss of oranges. Ali et al. (2015b) also observed an highest percentage weight (19.1%) in peppers coated with ethanol under refrigeration conditions at 13°C and RH 90%, while control fruits and coated with 1% and 5% ethanolic extract propolis did not differ (17.2, 18 and 16.85%, respectively). However, pitaya coated with 0.5% ethanol extract of propolis showed a 13% weight loss when stored at 20 ± 2°C and 80 ± 5% RH for 20 days of storage (Zahid et al., 2013).

Only the postharvest "refrigerated" treatment showed significant difference, with smaller loss of weight during the 25 days of storage. As at low temperatures, the metabolic respiration processes gets reduced, and consequently, the weight loss of the fruit is also reduced (Chitarra and Chitarra, 2005). However, this procedure may not be effective for long storage periods, as it has risks of cold disorders (Kluge et al., 2007, 2006). Citrus fruit can develop a disorder called "brown stem", manifesting dryness, discoloration, and wrinkling of the shell around the peduncle. These characteristics could be observed in fruit under refrigeration in the present study from the 18th day of storage onwards. According to Chitarra and Chitarra (2005), this disorder causes the

Table 2. Firmness (N) of 'Pera' oranges coated and uncoated with propolis extract during the storage period.

Treatments	0 day	10 days	18 days	25 days	Marginal means	Adjusted model	R^2
Control	8.05	10.60^{A}	8.56^{B}	9.75^{AB}	9.64	*	
Alcohol		9.87^{A}	9.19^{B}	12.33^{A}	10.46	$\hat{y} = 0.144x + 7.958$	0.729
2.5% propolis		8.64^{A}	11.98^{A}	10.82^{AB}	10.48	$\hat{y} = 0.142x + 7.988$	0.688
5% propolis		9.50^{A}	9.14^{B}	11.06^{AB}	9.90	$\hat{y} = 0.103x + 8.068$	0.797
Refrigerated		8.60^{A}	9.02^{B}	8.98B	8.87	$\hat{y} = 8.867$	
Marginal means		9.44	9.58	10.59	C.V.: 13.9%		

Means followed by the same letter in the column are not different at 5% significance level, by the SNK test.*Models with two dependent factors could not be fitted.

death of epidermal cells and the collapse of oil glands, and it is considered as a transpiration phenomenon associated with the ability of a fruit to control its weight loss. The RH of the refrigerator (70 ± 5%) may have contributed to the development of the disorder in the postharvest treatment "refrigerated". In general, fruit stored under refrigeration with RH of 85 TO 95% suffer reduced skin dissection, reduced distortion, lower degreening, and cold disorder (Chitarra and Chitarra, 2005).

The percentage of weight loss of 'Pera' oranges was higher than the reported data. Pereira et al. (2014) studied 'Valencia Delta' oranges coated with carnauba wax and found 26% weight loss for control fruit and 14% for coated fruit stored for 28 days under the ambient temperature condition. Alleoni et al. (2006) reported weight loss of 4.70 to 8.96% for 'Pera' oranges coated with protein concentrate whey and plasticizers (glycerol and sorbitol) during the 11 days of storage under 22 ± 1°C at 80 ± 2% RH.

The firmness variable also suffered significant influence of the postharvest treatment (F = 4.03; p = 0.020) and storage period (F = 3.75; p = 0.034) as well as interaction between the factors (F = 2.77; p = 0.019). An increase in the firmness value was noted during the storage period for the postharvest "alcohol", "2.5% propolis", and "5% propolis" treatments, as evidenced by the adjusted linear models. The orange fruit subjected to postharvest treatment "refrigerated" showed a relatively constant firmness value during the 25 days of storage (Table 2).

The hardening of oranges during the storage period after the postharvest "alcohol", "2.5% propolis", and "5% propolis" treatments can be justified by the presence of water-alcohol solution that can be given to the insolubility pectic material, which inhibits the degradation of pectin by pectinmethylesterase (PME) and polygalacturonase (PG) (enzymes responsible for softening of fruit). The propolis extract coating enhances firmness retention and concurs with the results of Ali et al. (2014) and Zahid et al. (2013). The chilli and pitaya, respectively, appeared firmer than the postharvest treatment control during the duration of storage.

The enzymes PME and PG act on the hydrolysis of glycosidic α-1-4 galacturonic acid when de-esterified, triggering depolymerization and solubilization of pectic (Ali et al., 2004). The inhibitory substances of these enzymes such as sucrose, maltose, and glucose by non-competitive inhibition and some peptides compete for the binding sites of PME, which can explain failure of demethoxylation of pectin chains that decrease the action PG (Wakabayashi, 2000). The TSS of 'Pera' oranges were stable during the storage period (F = 0.64; p = 0.532). The different types of postharvest treatments influence the TSS content (F = 3.55, p = 0.031). The interaction of factors on the TSS content was not significant (F = 1.01; p = 0.445) (Table 3). The stability of the TSS values was consistent with that reported by Pereira et al. (2014) and Vieites et al. (1996). Salunkhe and Desai (1984) stated that oranges, being non-climacteric fruit, do not suffer rapid changes in TSS values immediately after harvest. Only the "refrigerated" postharvest treatment showed lower TSS values that those after "control" postharvest treatment, due to the reduction of metabolic process and respiration activity. The TA values varied significantly among the postharvest treatments (F = 3.26; p = 0.041) and during the storage period (F = 14.46; p < 0.01), showing a significant interaction between the factors (F = 2.47; p = 0.031) (Table 4). The TA did not present significant differences between the postharvest treatments until the 18th day of storage. However, after 25 days of storage, the values for postharvest "control" treatment differed significantly from those for other treatments. It was also observed that the acidity was constant over the course of 25 days of storage for the postharvest "5% propolis" treatment.

The fruit of the postharvest "control", "alcohol", and "2.5% propolis" treatments showed increased TA content over the 25 days of storage; this effect was much more pronounced in the postharvest "control" treatment. Also noted by Pereira et al. (2014) and Vieites et al. (1996), this increase in acidity can be attributed to the degradation of pectins PME and PG and the possible formation of galacturonic acid, as well as associated with

Table 3. Total soluble solid (%) of 'Pera' oranges coated and uncoated with propolis extract during the storage period.

Treatments	0 day	10 days	18 days	25 days	Marginal means
Control	8.23	9.83	9.43	10.80	10.02^{A}
Alcohol		9.78	8.36	9.18	9.10^{AB}
2.5% própolis		10.18	9.23	8.25	9.22^{AB}
5% propolis		9.63	9.45	9.16	9.41^{AB}
Refrigerated		8.43	9.25	8.23	9.63^{B}
Marginal means		9.57	9.14	9.12	C.V.: 13.6%

Means followed by the same letter in the column are not different at 5% significance level, by the SNK test.

Table 4. Titratable acidity (%) of 'Pera'oranges coated and uncoated with propolis extract during the storage period.

Treatments	0 day	10 days	18 days	25 days	Marginal means	Adjusted model	R^2
Control	0.45	0.46^{A}	0.68^{A}	0.90^{A}	0.68	$\hat{y} = 0.001x^2 - 0.006x + 0.442$	0.991
Alcohol		0.40^{A}	0.58^{A}	0.63^{B}	0.54	$\hat{y} = 0.008x + 0.402$	0.693
2.5% propolis		0.52^{A}	0.47^{A}	0.67^{B}	0.55	$\hat{y} = 0.007x + 0.433$	0.735
5% propolis		0.53^{A}	0.41^{A}	0.52^{B}	0.49	$\hat{y} = 0.486$	
Refrigerated		0.30^{A}	0.53^{A}	0.56^{B}	0.46	*	
Marginal means		0.44	0.53	0.66		C.V.: 26.1%	

Means followed by the same letter in the column are not different at 5% significance level, by the SNK test. *Models with two dependent factors could not be fitted.

Table 5. Ratio of total soluble solid and titratable acidity for 'Pera' oranges coated and uncoated with propolis extract during the storage period.

Treatments	0 day	10 days	18 days	25 days	Marginal means	Adjusted model	R^2
Control	18.23	23.73	13.83	12.17	16.58	$\sqrt{\hat{y}} = -0.004x^2 + 0.057x + 4.359$	0.737
Alcohol		25.54	14.91	14.89	18.45	$\sqrt{\hat{y}} = 3.834 + 0.828/(1+e^{(x-15.378)})$	0.672
2.5% propolis		15.20	20.25	12.87	16.11	$\sqrt{\hat{y}} = 3.996$	-
5% propolis		18.91	24.43	18.48	20.61	$\sqrt{\hat{y}} = 4.530$	-
Refrigerated		29.33	17.56	14.78	20.56	$\sqrt{\hat{y}} = -0.006x^2 + 0.128x + 4.373$	0.710
Marginal means		22.54	18.20	14.64	C.V.: 14.0%		

Means followed by the same letter in the column are not different at 5% significance level, by the SNK test.

the fermentation process. The TSS/TA value differed significantly for fruit subjected to different postharvest treatments and time of storage (F = 10.70; $p < 0.001$), as well as presented significant interaction between the factors (F = 3.37; $p = 0.021$). While the postharvest treatments showed no significant difference (F = 2.31; $p = 0.106$) (Table 5).

The fruit coated with hydroalcoholic extract of 2.5% propolis and 5% propolis showed no significant differences in the TSS/TA values during storage. For postharvest "control" and "refrigerated" treatments, a decrease in this index was perceived during storage. The variation TSS/TA value is indicative of the fruit ripening stage, which determines the balance of sweet and sour flavors in the fruit (Chitarra and Chitarra, 2005). Values of TSS/TA between 23.50 and 29.68 were found in "Valencia Delta" orange coated with carnauba-based wax stored under ambient conditions (Pereira et al., 2014).

Table 6. Hydrogenionic potential (pH) for 'Pera' oranges coated and uncoated with propolis extract during the storage period.

Treatments	0 day	10 days	18 days	25 days	Marginal means
Control	3.84	3.96	3.97	3.88	3.94
Alcohol		4.13	4.11	4.04	4.09
2.5% propolis		3.92	4.20	3.97	4.03
5% propolis		3.88	4.17	4.33	4.13
Refrigerated		4.47	4.13	4.30	4.30
Marginal means		4.07	4.12	4.10	C.V.: 6.3%

The evolution of TSS/TA oranges can be explained by the relationship rootstock/eating area, age of trees, flowering, and productivity, as well as variation in the climate over the years (Volpe et al., 2002). The author also reported differences in the TSS/TA values within the fruit of the same variety grown in terms of producing region, climate, time of harvest, and soil, among others. The postharvest treatment (F = 2.30; p = 0.052) and storage time (F = 0.16; p = 0.850) did not influence the pH values (Table 6).

According to Chitarra and Chitarra (2005), in a concentration range of acids between 2.5 and 0.5%, the pH may increase and the acidity may decrease, indicating the use of organic acids in the cell vacuole during the breathing process, since they constitute an excellent energy reserves to support the maturation of the fruit. However, in this study, an increase in acidity was noted for postharvest "control", "alcohol", and "2.5% propolis" treatments along with maintenance of pH for all postharvest treatments, regardless of whether or not there was variation in TA (Tables 4 and 6). The acid formed during storage are weak acids that are not deprotonated at low pH values, not contributing to change this.The pH value obtained in this study was lower than that found by Pereira et al. (2014) in "Delta Valencia" orange. The authors verified pH between 4.51 and 4.41 for fruits control and 4.51 to 4.37 for the fruits coated with carnauba-based wax stored under ambient conditions.

Conclusion

Coating with hydroalcoholic extract of propolis is effective in reducing weight loss in 'Pera' oranges until the 18th day of storage. Fruit coated with propolis hydroalcoholic extract showed, in general, similar behavior to the uncoated fruit kept at room temperature. Oranges coated with ethanol-water solutions maintained the firmness during the 25 days of storage. Changes in the TSS content, TA, TSS/TA, and pH promoted by coatings with hydroalcoholic extract of propolis were small in magnitude during the storage period. The values of TSS, TA, TSS/TA, and pH for "Pera" orange do not change with refrigeration conditions and with coated with hydro-alcoholic extract of propolis.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGMENT

The authors thank CAPES-Brazil for granting the research fellowship.

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academicJournals

Vol. 11(23), pp. 2050-2057, 9 June, 2016
DOI: 10.5897/AJAR2016.10936
Article Number: F4B772558906
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Rooting of African mahogany (*Khaya senegalensis* A. Juss.) leafy stem cuttings under different concentrations of indole-3-butyric acid

Rodrigo Tenório de Vasconcelos, Sérgio Valiengo Valeri*, Antonio Baldo Geraldo Martins, Gabriel Biagiotti and Bruna Aparecida Pereira Perez

Department of Vegetable Production, Universidade Estadual Paulista Julio de Mesquita Filho, Prof. Acess Road Paulo Donato Castellane, s/n, Jaboticabal,SP, Brazil.

Received 24 February, 2016; Accepted 23 April, 2016

Vegetative propagation were studied in order to implement *Khaya senegalensis* A. Juss. wood production, conservation and genetic improvement programs. The objective of this research work was to establish the requirement as well the appropriated concentration of indolbutiric acid (IBA) in the *K. senegalensis* leafy stem cuttings to produce new plants. The basal end of the leafy stem cuttings were immersed, at first subjected to the so called slow method, in a 5% ethanol solution with 0, 100, 200 and 400 mg L^{-1} of IBA for 12 h and, as another procedure, the so called quick method, to a 50% ethanol solution with 0, 3000, 6000, 9000 and 12000 mg L^{-1} of IBA for 5. The leafy stem cuttings were transferred to plastic trays filled with 9.5 L of medium texture expanded vermiculite in which the cuttings had their basal end immersed to a depth of 3 cm in an 8.0 x 8.0 cm spacing. The experimental units were distributed according to a completely random design with five replications. Each experimental unit consisted of twelve cuttings. The experiment was conducted in a greenhouse under a 50% of shade condition during the period from January to June of 2012. The quick method, in comparison with the slow one, gave the best results in terms of percentage of rooted stem cuttings and height of the sprouted plantlets. In the quick method, no differences between IBA concentrations were observed. The slow and the quick methods did not differ as to stem cuttings survival, total root length per leafy stem cutting, number of roots per stem cutting, plantlets survival and percentage of sprouted plantlets. It was concluded that *K. senegalensis* may be propagated by leafy stem cuttings taken from plantlets of seminal origin without the use of IBA.

Key words: *Khaya*, Senegal mahogany, vegetative propagation, auxin, seedlings.

INTRODUCTION

The genus *Khaya*, a member of the Meliaceae family, is composed of four important species of commercial wood- *Khaya ivorensis, Khaya grandifolia, Khaya anthotheca* and *Khaya senegalensis*; all of them are commonly

*Corresponding author. E-mail: sergio.valeri1@gmail.com. Tel: +55(16)992075633.

known as African mahogany. None of those species are substantially different from mahogany species of Tropical America (*Swietenia macrophylla, Swietenia mahagoni*) either as to physiognomic aspects or wood quality (Lamprecht, 1990).

K. senegalensis natural distribution is verified to occur from Mauritania and Senegal east region up to Uganda northern region, where rain precipitation varies from 650 to 1300 mm up to 1800 mm, a year (Nikiema and Pasternak, 2008), where, under conditions of fertile soils, trees may reach up to 35 m of height, diameter of 1.5 m, and trunks 8 to 16 m long (Joker and Gaméné, 2003). The species was introduced in Australia, China, Vietnam, Malaysia, Indonesia, Sri Lanka and Tropical America for wood production (Arnold et al., 2004).

K. senegalensis wood is hard, heavy, durable and displays very nice graphic delineations. It is used in furniture manufacture and in internal decoration of civil engineering constructions (Lamprecht, 1990). Reforesting with *K. senegalensis* in Africa is restricted to areas where the species is of natural occurrence since it is susceptible to cedar tip moth (*Hypsipyla robusta*). In northern Australia, the species was well succeeded due to the trees growing rapidly and because of the absence of *H. robusta* (Arnold et al., 2004; Nikles et al., 2008). In Brazil, the *K. senegalensis* plantations are young (they started in 2000), *H. robusta* does not occur and they are resistant to the cedar tip moth occurring in the Tropical America (*Hypsipyla grandella* Zeller) and they grow more than the mahogany of the genus *Swietenia.* Cultivating mahogany trees of the *Swietenia* genus in the American continent is economically unviable since they are susceptible to cedar tip moth (*H. grandella*). Thus, cultivating *K. senegalensis* in the Tropical America is a promising investment considering periods between 12 and 20 years. Studies on the vegetative propagation of *K. senegalensis* are just beginning thus demanding further work to reach a desirable level of efficiency in the production of seedlings (Pinheiro et al., 2011).

The sexual propagation of *K. senegalensis* is limited by the long time (between 15 and 20 years) it takes for a tree to be formed and to produce commercially satisfactory volumes of wood and also because its seeds loose viability in a very short period of time– under natural conditions, 2 to 3 weeks after harvest (Arnold et al., 2004). The International Union for the Conservation of Natural Resources (IUCN) has classified the species as a vulnerable one due to the loss of its natural habitat, to the selective cutting of its wood, and the large scale harvest of its bark and branches for medicinal purposes and of the leaves as fodder (Nikiema and Pasternak, 2008). The high costs and bureaucratic problems for the importation of seeds as well as the inconveniences of becoming dependent on international seed stocks make sexual propagation of *K. senegalensis* a very difficult option. These are reasons important enough to justify opting for vegetative propagation in order to implement *K. senegalensis* wood production, conservation and genetic improvement programs (Opuni-frimpong et al., 2008).

Although, a protocol for the in vitro propagation of K. senegalensis has already been established (Hung and Trueman, 2011), the use of stem cuttings is still one of the most important techniques for the vegetative propagation of selected clones viewing silvicultural purposes due to its technical applicability, operationality and competitive cost as compared to other asexual propagation techniques.

Indole-3-butyric acid (IBA) is one of the common growth regulators used to stimulate rooting cuttings, it is a localized action photostable substance, but less sensitive to biological degradation as compared to other synthetic auxin. Increasing the concentration of exogenous auxin stimulates the rooting of stem cuttings to a maximum value, from which any concentration can increase inhibitory effect. The appropriate content of exogenous auxin that stimulates rooting depends on the species and the auxin concentration in the tissue (Fachinello et al., 2005).

The objective of this research work was establish the requirement as well the appropriated concentration of IBA in the *K. senegalensis* A. Juss. stem cuttings to produce new plants.

MATERIALS AND METHODS

The stem cuttings used in this experiment were taken from seedlings developed from seeds imported from the African village of Tiakane, in Burkina Faso (11° 11' 10'' N and 1° 12' 14'' W). The seedlings were produced at the 'São Gabriel' nursery in Monte Alto, state of São Paulo, Brazil (21° 15' 39'' S and 48° 29' 45'' W), during the period of November of 2011 to January of 2012. The seedlings grew in plastic containers with a volume of 120 cm^3 filled with Plantmax® substratum whose structural composition was of 60% of pine bark, 15% of granulometrically fine vermiculite, 15% of granulometrically superfine vermiculite, and 10% of humus. To the substratum in each container , 1.2 g of Osmocote Plus® (slow action fertilizer with a NPK composition of 15-09-12 and 1.0% of Mg, 2.3% of S, 0.012% of B, 0.05% of Cu, 0.45% of Fe, 0.06% of Mn, 0.02% of Mo, and 0.05% of Zn) were added.

The seedlings were 80 days old when the leafy stem cuttings were taken from them. The basic plant material was formed by stem cuttings taken from 540 seedling sprouts. From each one of the 540 seedlings, a stem cutting was taken from the apical branch. The preparing of the leafy stem cuttings consisted in the discarding of the first pair of apical leaves to avoid dehydration keeping three whole subsequent leaves.

The leafy stem cuttings had a mean diameter of 4 mm, 8 cm of length and three whole leaves with a leaf area of 48 cm^2, using the LI-3100C Area Meter - LI-COR Biosciences. The leafy stem cutting base was shaped to a bevel just below the bud so as to increase the contact surface between the plant tissue and the ethanol/indole-3-butyric acid- IBA solutions.

At first, the leafy stem cuttings were submitted to the quick immersion method meaning that they were immersed for 5 s in hydroalcoholic (50% of ethanol) solutions containing 0, 3000, 6000, 9000, and 12000 mg L^{-1} of IBA. In the other procedure (the slow method), the stem cuttings were immersed for 12 h in a 5% ethanol solution containing 0, 100, 200 and 400 mg L^{-1} of IBA. After those periods, the stem cuttings were transferred to plastic trays (internal dimensions of 49.5 cm of length, 30.5 cm of width and 11 cm of height) filled with 9.5 L of medium texture expanded vermiculite in which the cuttings had their basal end immersed to a depth of 3 cm in an 8.0 x 8.0 cm spacing.

The experimental units were distributed according to a completely random design with five replications. Each experimental unit consisted of twelve cuttings. The experimental units were placed in a greenhouse under a 50% of shade condition made possible by a screen, during the period of January to June of 2012. The cuttings were irrigated by means of a nebulization system which provided the plants with water for 20 s at 40 s intervals. A thermo hygrometer was set in the greenhouse near the experimental units. The mean low and the maximum temperatures were, respectively 17 (night) and 35°C (day). The mean low and maximum relative humidity were, respectively, 45 and 95%, using a digital thermometer-hygrometer.

45 days after the cuttings had been planted, non-destructive evaluations of cutting survival, number of rooting cuttings, adventitious roots total length using a graduated ruler, and number of adventitious roots per cutting were evaluated. Following those measurements, the rooting cuttings were transferred to plastic vases with 1500 cm^3 of the substratum Plantimax®. To each vase, 15 g of Osmocote Plus® were added and the vases taken to the growth area where illumination underwent a reduction of 30%. The vases stayed in that area for additional 120 days. During that period, the plants were irrigated by micro aspersion for 15 min at 45 min intervals. Percentage of surviving plants, sprouted plants and sprout height were evaluated 165 days after the cuttings had been planted.

The statistical analyses of the data were performed considering two factors: forms by which the cuttings were immersed in the solution (slow and quick) and IBA concentrations in each form of immersion, in a hierarchical scheme. The effects of IBA concentration in each form of immersion were determined by a polynomial regression analysis, using a program AgroEstat (Barbosa and Maldonado Júnior, 2015).

RESULTS AND DISCUSSION

Survival

Leafy steam cuttings survival 45 days after rooting started was of 100%.The treatments had no effect on plant survival. This is thought to be due to the presence of three whole leaves in the cuttings. Leaves contain carbohydrates, growth regulators, and other components essential for rhizogenesis (Alfenas et al., 2004).

Ky-Dembele et al. (2011) verified that leafless *K. senegalensis* cuttings taken from 1 year old plants showed mortality larger than those where leaves were present. These results indicate that leaves are of fundamental importance for *K. senegalensis* cuttings survival. Results published by Faria et al. (2007) indicated that the survival of cuttings of grape IAC 572-Jales was highly dependent on the presence of leaves.

Work by Ferriani et al. (2008) with stem cuttings of *Piptocarpha angustifolia* Dusén showed that the presence of leaves represents the photosynthetic apparatus needed for root initiation.

Rooting

Rooted cuttings 45 days after planting was 95.74%. This good rooting rate is partially due to the presence of three whole leaves attached to the cuttings. Leaves represent an essential stimulus for root initiation (Hartmann et al., 2002). This is an effect related to the translocation of carbohydrates to the cutting basal end, in addition to auxin and other important rooting cofactors. Data published by Ky-Dembele et al. (2011) showed that the rooting of *K. senegalensis* cuttings were of 80, 50 and 57% when the cuttings at planting had leaf areas of 22-28, 12-16, and 6-8 cm^2, respectively.

The age (80 days) of the plantlets at which the cuttings were taken is also considered an important factor for the rooting percentage presented by them. The successful rooting of the cuttings may be ascribed to the juvenile characteristics of the mother plant. When a plant passes from the juvenile to the adult phase it undergoes several changes the main ones being related to the growth habit, the shape and retention of leaves, leaf and stem anatomy, cuttings rooting capacity and formation of roots, and growth vigor (Wendling and Xavier, 2001). Among the factors affecting root formation in cuttings, age of the mother plant is of high importance. Cuttings taken from young plants root is more easy as a result of a higher number of rooting cofactors and lower inhibitors content (Fachinello et al., 2005).

Ky-Dembele et al. (2011) evaluated the effects of mother plants age and IBA concentration (0, 2500, 5000 and 10000 mg L^{-1}) using the quick method on the rooting of cuttings taken from 3 month old seedlings, 100 year old trees and 5 to 15 year old trees. Cuttings taken from the seedlings rooted significantly more (90%) than those taken from the 100 year old trees (11%) and from those 5 to 15 year old trees. The authors concluded that the age of the mother plant affects the vegetative propagation of *K. senegalensis*.

The cuttings whose basal end was treated by the quick method gave the best results as to rooting percentage in comparison with the slow method (Table 1). In the slow method, the amount of absorbed phytohormones depends on the environmental conditions surrounding the place where the treatment is applied, the type of cutting and the species (Fachinello et al., 2005). Due to the long period of time the cuttings remain in the solution, results become dependent on those environmental conditions (Hartmann et al., 2002). On the other hand, some species, such as *Caesalpinia echinata*, respond better to the slow method in comparison with the quick one (Valeri et al., 2012).

Tofanelli et al. (2003) verified that the quick method gave better results to root stem cuttings of peach cultivars. According to these authors, a possible explanation for these results is that the absence of an artificial irrigation by nebulization system may have forced the cuttings to absorb too high an amount of the IBA solution which, instead of a stimulatory, had an inhibitory effect on root development.

Number of roots per cutting did not differ when both

Table 1. *Khaya senegalensis* rooting percentage, number of adventitious roots per leafy steam cutting, and total length of adventitious roots per cutting 45 days after planting as influenced by the way of immersion and indole-3-butyric acid (IBA) concentrations.

Sources of variation	Means		
	Rooting percentage	**Root number**	**Root length (cm)**
Quick Immersion	98.33[a]	5.96[a]	57.24[a]
Slow Immersion	92.50[b]	6.41[a]	53.09[a]
	Significance F-Test		
	Rooting percentage	**Root number**	**Root length (cm)**
Immersion Forms	9.80**	2.05[ns]	2.07[ns]
[IBA]/Quick Immersion	0.54[ns]	3.68*	2.12[ns]
[IBA]/ Slow Immersion	1.56[ns]	8.37**	5.42**
Coefficient of variation (%)	6.49	17.05	17.37

Means, in the same column, followed by the same small case letter, are not significantly different (*P > 0.05*). ([ns]) = non significant; (* and **) = Significant differences, respectively, at P < 0.05 and P < 0.01.

Table 2. *Khaya senegalensis* rooting percentage, number of adventitious roots per leafy steam cutting, and total length of adventitious roots per cutting 45 days after planting as influenced by IBA concentrations applied by the quick immersion method.

IBA concentrations mg L^{-1}	Means		
	Rooting percentage	**Root number**	**Root length (cm)**
0	100.00	6.28	63.87
3000	98.33	6.96	63.78
6000	95.00	4.89	50.93
9000	98.33	5.15	51.92
12000	100.00	6.53	55.70
Sources of variation	**Significance F-Test**		
	Rooting percentage	**Root number**	**Root length (cm)**
Linear Regression	0.00[ns]	0.78[ns]	4.29*
Quadratic Regression	1.64[ns]	4.56*	1.80[ns]
Cubic Regression	0.00[ns]	6.82*	1.30[ns]

([ns]) = Non-significant; (*) = Significant differences at *P < 0.05.*

immersion methods were compared (Table 1). In the quick immersion method, the number of roots per cutting varied with growing doses of IBA, this variation being more clearly described by a third degree equation (Table 2). This variation results from small differences between the means, which varied between 4.89 and 6.96 and that close to a straight line with a mean number of 5.96 roots per cutting.

Ky-Dembele et al. (2011), using the quick method (the IBA concentrations were 0, 2500, 5000 and 10000 mg L^{-1}) to root *K. senegalensis* cuttings verified that when the cuttings were taken from 100 year old trees, the higher doses of IBA caused the number of secondary roots to increase. On the other hand, when the cuttings were taken from 3 month old seedlings, IBA had no effect on the number of roots per cutting. The results found for the quick immersion method may be explained by the degree of juvenility of the plant material– in this case, the endogenous hormone balance is favorable to rooting. This material may show negative response to exogenous applications of plant hormones (Souza Júnior et al., 2008).

Increasing IBA concentration in the slow immersion method caused a linear increment in the number of roots per cutting (Table 3 and Figure 1). Similar results were reported by Vale et al. (2008) when increasing doses of IBA (0, 100, 200, and 300 mg L^{-1}) were applied to guava 'Paluma' cultivar cuttings- the higher the dose, the larger the number of roots per cutting. Similar results were also reported by Pinto and Franco (2009), when *Lippia alba* cuttings were immersed in IBA solutions with 500 and 1000 mg L^{-1} in the slow immersion method– the larger IBA concentration resulted in higher numbers of roots per cutting.

Table 3. *Khaya senegalensis* rooting percentage, number of adventitious roots per leafy steam cutting, and total length of adventitious roots per cutting 45 days after planting as influenced by indole-3-butyric acid (IBA) concentrations applied by the slow immersion method.

IBA concentrations mg L^{-1}	Means		
	Rooting percentage	Root number	Root length (cm)
0	91.67	5.27	41.43
100	88.33	5.54	51.65
200	96.67	6.57	53.41
400	93.33	8.27	65.86
Sources of variation	**Significance F-Test**		
	Rooting percentage	**Root number**	**Root length (cm)**
Linear Regression	0.83^{ns}	24.48**	15.61**
Quadratic Regression	0.28^{ns}	0.35^{ns}	0.07^{ns}
Cubic Regression	3.57^{ns}	0.29^{ns}	0.58^{ns}

(^{ns}) = Non-significant; (**) = Significant difference at *P < 0.01.*

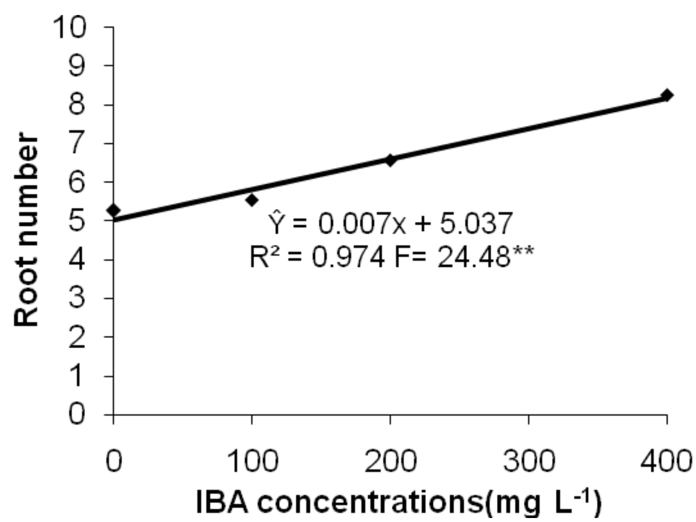

Figure 1. Number of adventitious roots per leafy steam cutting of *Khaya senegalensis* 45 days after planting as influenced by indole-3-butyric acid concentrations applied by the slow immersion method.

The cuttings whose basal end were treated with the quick immersion method showed a linear reduction in the roots total length as the IBA concentration increased (Table 2 and Figure 2). Souza Júnior et al. (2008), evaluating the effects of IBA concentration on the rooting of *Grevillea robusta* cuttings, and verified that roots total length was reduced with IBA doses higher than 2000 mg L^{-1}. IBA exogenously applied may stimulate root initiation but it may be toxic to certain types of cuttings (Hartmann et al., 2002).

Ky-Dembele et al. (2011), applying growing doses of IBA (0, 2500, 5000 and 10000 mg L^{-1}) to *K. senegalensis* cuttings verified that the length of the longest root in cuttings taken from 3 month old seedlings did not differ from that of cuttings taken from 100 year old trees, although the length of the longest root of both types of cutting was larger than that of cuttings taken from 5 year old trees. These results show that young propagules, including sprouts taken after pruning 100 year old trees, are favorable for the rooting of *K. senegalensis* cuttings.

In the cuttings whose basal end had been treated by the slow immersion method, the roots total length showed a linear increment as IBA concentration was increased (Table 3 and Figure 3). Tonietto et al. (2001), working with two cultivars (Reubennel and Puma 7) of *Prunus domestica* verified that IBA increases rooting, the number

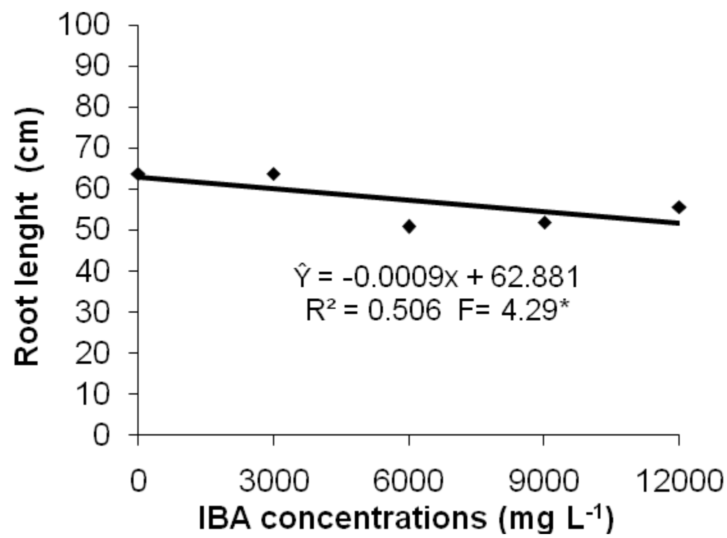

Figure 2. Total length of adventitious roots per leafy steam cutting of *Khaya senegalensis* 45 days after planting as influenced by IBA concentrations applied by IBA concentrations applied by the quick immersion method.

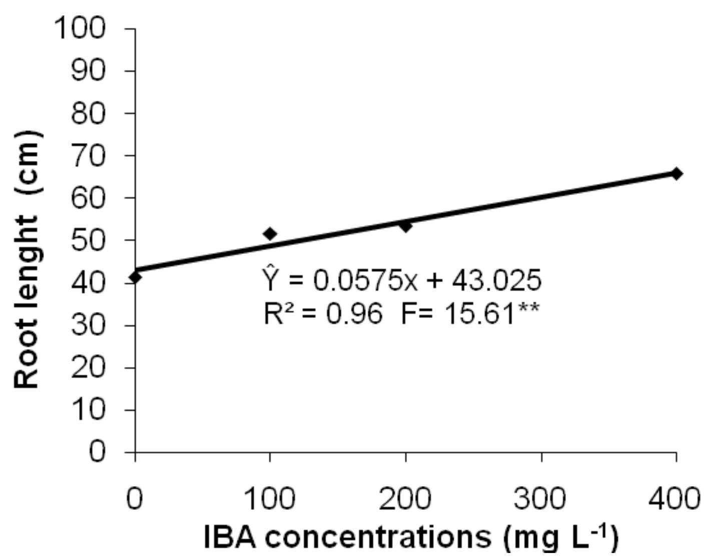

Figure 3. Total length of adventitious roots per leafy steam cutting of *Khaya senegalensis* 45 days after planting as influenced by IBA concentrations applied by the slow immersion method.

and length of roots of both cultivars. IBA, according to the authors, because it facilitates root formation, gave the roots more time to grow thus reaching higher lengths which is an indirect effect of IBA. Similar observations were reported by Cunha et al. (2004) in a research work with *Sapium glandulatus* cuttings- their length increased with IBA doses.

Seedling survival

New plants survival was 100% 165 days after the cuttings had been planted. The presence of leaves in the cuttings is thought to be the main factor determining such high survival percentage. In addition to that, optimal temperature and humidity conditions to which the

Table 4. Percentage of *Khaya senegalensis* plants bearing sprouts and sprouts height 165 days after planting as influenced by the way of immersion and indole-3-butyric acid (IBA) concentrations.

Sources of variation	Means	
	Plants bearing sprouts (%)	sprouts height (cm)
Quick Immersion	96.94[a]	8.04[a]
Slow Immersion	95.88[a]	6.47[b]
	Significance F-Test	
Immersion Forms	0.44[ns]	8.02**
[IBA]/ Quick Immersion	0.89[ns]	0.36[ns]
[IBA]/ Slow Immersion	0.52[ns]	0.73[ns]
Coefficient of variation (%)	5.45	25.27

Means, in the same column, followed by the same small case letter, are not significantly different ($P > 0.05$). ([ns]) = non-significant; (**) = Significant difference at $P < 0.01$.

seedlings were exposed are also considered as very important for new plants survival. Xavier et al. (2003) ascribed the survival of vegetative *Cedar fissilis* propagules to the adequate control of greenhouse environmental conditions. Wendling and Xavier (2003) attributed the high survival percentages observed in their experiment to good environmental conditions under which *Eucalyptus* clones were submitted. They also pointed the importance of the genetic makeup of their plant material.

New plants characteristics

There was no difference between the immersion methods as to percentage of sprout bearing plants (Table 4). Even when the data were submitted to regression analysis, no differences were found between IBA concentrations in both immersion methods.

Dias et al. (2011), in a study with *Prunus cerrulata* cuttings, verified that IBA concentrations of 0, 1000 and 2000 mg L^{-1} did not influence the results of number of plants bearing sprouts. In *Prunus mume*, IBA concentrations of 0 and 2000 had no significant effect on the percentage of plants bearing sprouts (Mayer et al., 2001).

The quick method resulted in higher sprout growth (Table 4). Even when the data were submitted to the regression study, no significant difference between IBA concentrations was observed either in the quick or in the slow immersion method. In *Sapium glandulatum,* Cunha et al. (2004) found no significant effect of IBA concentration on sprout height in rooted cuttings.

The quick immersion method, in comparison with the slow one, promoted the highest results of rooting percentage and sprouts height. The immersion methods did not differ with regards to cuttings survival percentage, total root length per cutting, number of roots per cutting, new plants survival percentage, and percentage of plants showing sprouts.

Plants which easily root may justify the non use of using auxins. Auxins are more efficient in promoting rooting when the species is of low to difficult rooting. Although auxins are useful for plant propagation, and may increase the time and production efficiency, the final size and vigor of such plants are not larger than when no auxin is applied (Hartmann et al., 2002).

Conclusion

K. senegalensis may be propagated by means of leafy steam cuttings taken from seedling branches without the use of IBA.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENT

This study was sponsored by Brazilian National Council for Research (CNPq).

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academicJournals

Vol. 11(23), pp. 2058-2063, 9 June, 2016
DOI: 10.5897/AJAR2014.9475
Article Number: C78E82858908
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Effect of *Vitex agnus-castus* on *in vitro* digestibility in ruminant

Sibel Soycan-Önenç

Department of Animal Science, Faculty of Agriculture, Namık Kemal University, Tekirdağ, Turkey.

Received 25 December, 2015; Accepted 17 February, 2016

The objective of this study was to evaluate the effects of different levels of *Fructus agni-casti* (FAC) on *in vitro* gas production (GP), organic matter digestibility (OMD) and net energy lactation (NEL) using an *in vitro* gas-production method. Two rumen-fistulated sheep were used in the experiment. The sheep were fed 60% alfalfa hay and 40% concentrate feed twice daily. Five different levels of FAC were added to the concentrate (CON). The volume of gas produced was recorded at 2, 4, 8, 12 and 24 h after incubation. In this study, the GP-improving effect occurred at 2 h of incubation in FAC_3 and FAC_4 groups. Degradation of the water-soluble carbohydrates promoted the concentrate feed with FAC (3 and 4% level) additions. However, the GP-reducing effect occurred from 8 to 12 h of incubation in all the treatments. It was shown that, the concentrate feed with FAC addition reduced degradation of storage polymers such as starch. In addition, GP (at the 24 h), OMD and NEL were not affected by supplementation with FAC.

Key words: *Vitex agnus-castus*, essential oil, feed digestibility, *in vitro* gas production

INTRODUCTION

The aim of manipulating rumen fermentation is to improve the feed efficiency for the production of meat, milk or wool from ruminant animals. The fermentation pattern can be selectively modified to maximize microbial protein synthesis and produce end-products of digestion. Antibiotic feed additives, such as ionophore and antibiotic monensin, have been widely used for this purpose (Nagaraja, 1995). However, the use of antibiotics as feed additives in intensive production systems was banned in the European Union because of the presence of residues in milk in meat (EC, 2003, Regulation 1831/2003/EC). This ban has created the need for suitable alternatives for these antibiotics.

One of these alternatives is the use of herbal extracts and their active substances such as essential oils (Greathead, 2003). The essential oils are drawing interest both in the industry and the scientific research due to their antibacterial and antifungal properties, which make them more useful as natural additives in feeds. Previous *in vitro* studies on different plant extracts and plant metabolites showed the potentials of some extracts, including saponins, anise oil, capsicum extract, eugenol

E-mail: ssonenc@nku.edu.tr.

and cinnamaldehyde, to modify ruminal microbial fermentation. Calsamiglia et al. (2005) indicated that the combination of additives with different mechanisms of action might result in synergistic effects that may enhance ruminal fermentation. Active components or mixtures of EO have the potentials to modify rumen N metabolism by reducing degradation of proteins and ammonia production in the rumen at low concentrations (McIntosh et al., 2003; Molero et al., 2004; Newbold et al., 2004; Busguet et al., 2005 a, b; Chaves et al., 2008).

Vitex agnus-castus L. (Verbenaceae) is a small tree or shrub, widely distributed along the Anatolian coastal lane (Davis, 1982). The fruits were formerly used as a substitute for pepper from Italy to Eastern Georgia (Stojkoviç et al., 2011). In Anatolian folk medicine, *V. agnus-castus* is used as diuretic, digestive, antifungal, anti-anxiety, early-birth and stomach-ache drugs (Baytop, 1999; Honda et al., 1996).

Nutritional quality of feeds are usually evaluated with *in vitro* techniques due to their convenciences, adaptability and efficiency (Getachew et al., 2005). The close association between rumen fermentation and gas production has long been recognized. Menke et al. (1979) developed *in vitro* gas measuring technique for feed evaluation. Feed fermentation is conducted in a 100 ml calibrated glass syringes containing the feedstuff and buffered rumen fluid. The gas measuring technique was reported had a high correlation between gas production *in vitro* and *in vivo* apparent digestibility (Menke et al., 1979). The gas produced in the gas technique is the direct gas produced as a result of fermentation (CO_2 and CH_4) and the indirect gas produced from the buffering of short chain fatty acids (CO_2 released from the bicarbonate buffer) (Getachew et al.,1998).

The objective of this study was to evaluate the effects of increasing doses of *V. agnus-castus* on *in vitro* gas production, organic matter digestibility and net energy lactation contents by using *in vitro* gas production method.

MATERIALS AND METHODS

Animal and experimental diets

Two rumen-fistulated rams (Tahirova breed and East Friesian 75% x Kivircik 25 %) were used. The rams were fed 60% alfalfa hay and 40% concentrate feed twice daily, as described by Steingass and Menke (1986). The feed material consists of fattening concentrate (CON) and *F. agni-casti* (FAC). Five different levels of FAC (6 replications per treatment) were added to the concentrate (CON) to produce 200 mg DM (CON- without FAC; 1 - 2 mg FAC + 198 mg CON; 2 - 4 mg FAC + 196 mg CON; 3 - 6 mg FAC + 194 mg CON; 4 - 8 mg FAC + 19 2 mg CON; 5 - 10 mg FAC + 190 mg CON). The results of the crude nutrient analysis of CON and FAC are presented in Table 1.

Chemical analysis

The concentrate feed and the aromatic plants were ground on a 1 mm screen in preparation for chemical analysis. The dry matter (DM), crude protein (CP), ether extract (EE), crude ash (CA) and crude fibre (CF) were analysed according to Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten, VDLUFA (Naumann and Bassler, 1993). The metabolisable energy (ME) was calculated based on the chemical composition (Anonymous, 1991).

The rumen fluid was collected from two fistulated ruminal rams before the morning-feeding. The estimates of gas production were obtained using the method of Menke and Steingass (1988). A buffer solution (macro and micro-minerals) was prepared on the day prior to the analysis, and incubated in a water bath at 39°C under a continuous CO_2 stream (DLG, 1981). Incubations were terminated after 24 h for the organic matter digestibility (OMD) and for net energy lactation (NEL) estimations of the concentrate and for the aromatic plant mixtures. The volume of gas produced was recorded at 2, 4, 8, 12 and 24 h after inoculation; the gas production (GP) results were applied in order to calculate OMD and NEL, using the following equations:

OMD (%) = 0.889 x GP + 0.448 x CP* + 0.651 x CA* + 14.88 (Menke and Huss, 1987). *in % DM.

NEL (MJ/kg DM) = 3.95 + 0.3305 x GP-0.0023 x GP^2 + 0.0535 x CP + 0.0132 x EE^2-0.0336 x CF -0.1073 x CA (Aiple, 1993).

GP: 24-h cumulative gas production in DM.

The essential oil from 10 g of dry fructus was extracted by hydro-distillation for 3 h using a Clavenger-type apparatus, according to the European Pharmacopoeia (1975), with three replications. The GC analyses were performed at the Medicinal Plants, Drugs and Scientific Research Center of Antolian University using a Shimadzu GC-9A gas chromatograph.

Statistical analysis

The data obtained were evaluated using the GLM procedure of SPSS V10 software. Duncan's test was employed for the comparison of the differences between the group averages (Efe et al., 2000).

RESULTS AND DISCUSSION

As shown in Figure 1A and Table 2, the highest and the lowest GP values were found after 2 h of incubation respectively, for FAC_3, FAC_4 and CON. The highest and the lowest GP values were found after 4 h of incubation, respectively, for CON and FAC_5 (Figure 1B).

Gas production mainly reflected carbohydrate degradation. Cone et al. (1997) and Cone and Van Gelder (1999) showed that initially, gas is produced from fermentation of the water-soluble components, such as sugars and protein. In this study, the GP-improving effect occurred at 2 h of incubation in FAC_3 and FAC_4 groups. Degradation of the water-soluble carbohydrates increased the concentrate feed with FAC (3 and 4% level) additions. However, gas produced at 4 h incubation was reduced by FAC_4 and FAC_5. The GP values determined after 8 h of incubation (Figure 1C) showed a significant difference between CON and all the treatments ($P<0.01$). After 12 h, the highest GP was found in CON (48.49±0.55), whereas

Table 1. The nutrient composition of CON and FAC, and essential oil ratio of VAC and the chemical content of essential oil.

Sample name	DM (%)	CA (%)	CP (%)	EE (%)	CF (%)	NFE (%)	ME (Kcal/kg)
CON	89.38	4. 8	15.15	4.39	6.27	58.03	2725
FAC	91.12	3.72	6.49	6.26	64.63	10.01	490

	EOR (% DM)	1,8-cineole	Sabinen	α-Terpenyl acetate	α-Pinene	Unknown	Total compounds
FAC	0.27	32.10	20.70	7.4	7.00	32.8	100

EOR: Essential oil ration, DM: dry matter, CA: crude ash, CP: crude protein, EE: ether extract, CF: crude fiber, NFE: nitrogen free extract, ME: metabolic energy.

Table 2. GP, OMD and NEL contents of CON and FAC.

Samples	Gas production (ml/200 mg DM) incubation period					OMD (%)	NEL (MJ/kg DM)
	2-h	4-h	8-h	12-h	24-h		
CON	13.12^{b}±0.18	22.44^{a}±0.31	39.81^{a}±0.44	48.49^{a}±0.55	60.13±0.52	79.47±0.47	8.01±0.03
FAC1	13.43^{b}±0.11	22.27^{a}±0.01	37.47^{bc}±0.01	46.84^{b}±0.01	58.59±0.17	78.11±0.16	7.92±0.01
FAC2	13.43^{b}±0.22	22.44^{a}±0.29	37.46^{bc}±0.39	46.91^{b}±0.45	59.46±0.56	78.88±0.50	7.97±0.03
FAC3	14.36^{a}±0.17	22.38^{a}±0.16	37.90^{b}±0.21	46.19^{b}±0.27	60.28±0.40	79.61±0.34	8.02±0.02
FAC4	14.27^{a}±0.18	22.11^{b}±0.29	37.19^{bc}±0.45	46.10^{b}±0.57	60.10±0.78	79.45±0.69	8.00±0.04
FAC5	13.46^{b}±0.09	21.31^{b}±0.17	36.29^{c}±0.25	44.49^{c}±0.25	58.67±0.40	78.18±0.35	7.93±0.02
P	0.001	0.014	0.001	0.001	0.077	0.077	0.084

[abc:] Means with different letters in the same column are statistically significant (P<0.01). CON: Concentrate, FAC: *Fructus agni-casti*, 1, 2, 3, 4, 5: FAC dose in concentrate (200 mg DM).

the lowest value was found in FAC_5 (44.49±0.25). Gas produced at 6 h incubation was a good estimate of the extent of fermentation of non structural carbohydrate (estimated primarily as sugars, pectin and starches). Gas production level from 6 to 24 h of incubation was a good estimate of the amount of fermentation of structural carbohydrate that occur in cows at high feed intake levels (Orskov and McDonald 1979). The soluble carbohydrates are more rapidly digested by ruminal microorganism than the structural polysaccharides such as cellulose or the storage polymers such as starch (Stefanon at al., 1996). In this study, the GP-reducing effect occurred from 8 to 12 h of incubation in all the treatments (Figure 1C and D). It was shown that, the concentrate feed with FAC addition reduced degradation of storage polymers such as starch.

The highest GP (at the 24 h) value was found in the FAC_3 group at 60.28±0.40 mL/200 mg DM. However, FAC addition had slightly changed GP values as compared to CON group. Similarly, there was no significant difference found between OMD and NEL (P>0.01). Busquet et al. (2005b) found that cinnamaldehyde and garlic oil had no effect on DM, OM, NDF and ADF digestibility or on the total VFA concentration, and suggested that these additives cannot modify the overall diet fermentability. Castillejos et al. (2005) determined that 1.5 mg/L BEO (Crina ruminants) supplemented with high concentrate and forage rations (100 forage and 900 concentrate versus 600 forage and 400 concentrate) cannot affect DM, OM, NDF, ADF and CP digestion, though BEO increased the total VFA concentration (122.8 versus 116.2 mM). In another study, Newbold et al. (2004) observed a reduction in the *in situ* DM degradation of soya-bean meal after 8 and 16 h of incubation when 110 mg/d essential oil (a mixture of thymol, guaiacol and limonene) was added to the diet of sheep. However, the mixture had no effect on DM degradability of rapeseed meal and hay. Similarly, the digestibility of NDF and ADF was not affected in lactating dairy cows supplemented with a mixture of EO compounds according to Benchaar et al. (2003). Garcia et al. (2007) reported that the addition of carvacrol reduced *in vitro* DM, CP and neutral-detergent fibre (NDF) digestion. The effects induced by 250 mg/L carvacrol on DM digestion after 72 h of incubation were comparable to those of monensin, whereas a greater decrease was noticeable when carvacrol was added at a concentration of 500 mg/L. The researchers explained that the reduced CP potential degradability by addition was caused by the reduction of the slowly degradable fraction. Azar et al. (2011) evaluated the effects of three doses of *Zataria multiflora* water extract (0, 0.15 and 0.3 ml/30 ml buffered rumen fluid) on the short chain fatty acid, net energy, metobolizable energy and organic matter digestibility of canola meal using *in vitro* gas production technique and

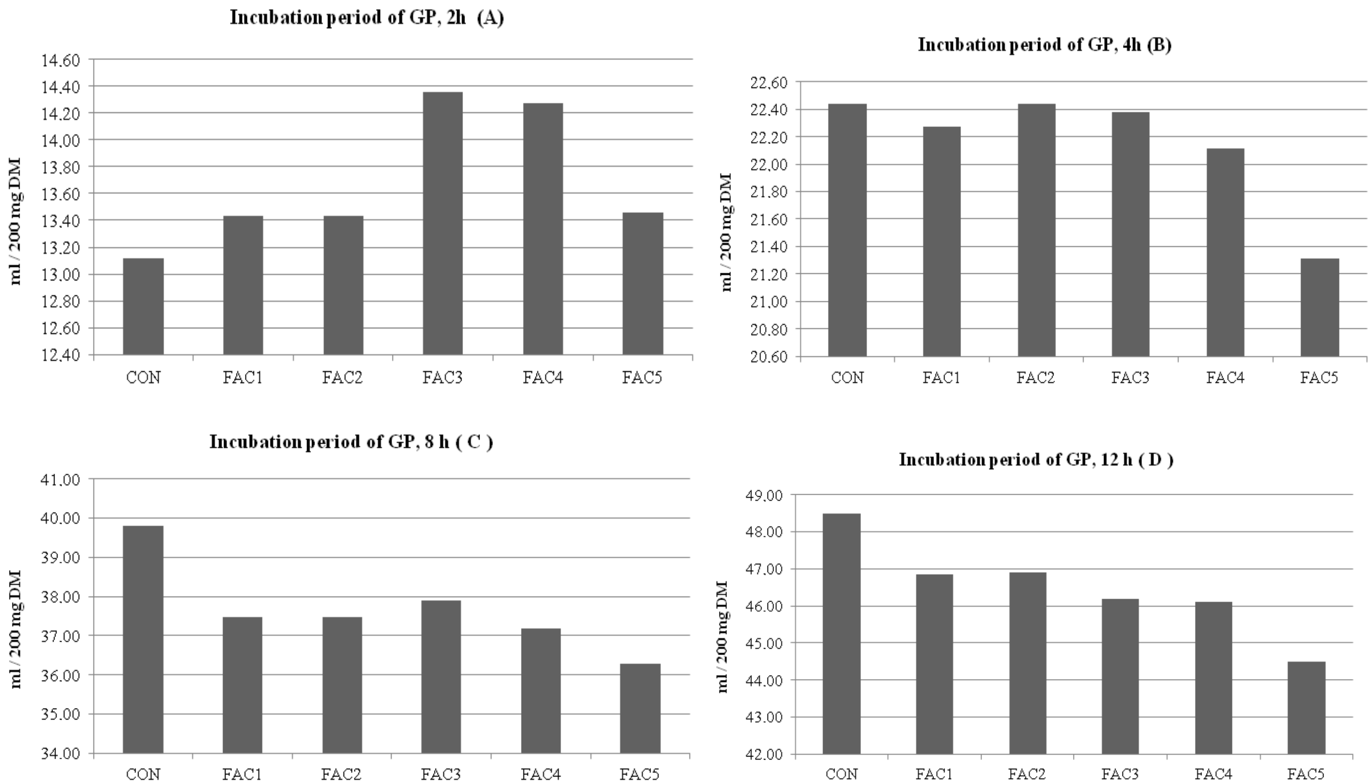

Figure 1. Incubation periods of GP at 2 h (A), 4 h (B), 8 h (C), 12 h (D).

reported that the amounts of organic matter digestibility, metabolizable energy, short chain fatty acid and net energy for lactation (NEL) of canola meal (79.46 g/ kg DM, 10.27 MJ/kg DM, 1.046 mmol and 5.28 MJ/kg DM respectively) were high as compared to *Z. Multiflora* water extract (0.3 ml/30 ml buffered rumen fluid) which were 41.85 g/kg DM, 3.63 MJ/kg DM, 1.047 mmol and 1.22 MJ/kg DM, respectively.

In the present study, it was found that GP (at the 24 h), OMD and NEL were not affected by addition of FAC. These findings corresponded with the findings of Busquet et al. (2005b), Castillejos et al. (2005) and Newbold et al. (2004). Essential oils have antimicrobial activities against Gram-negative and positive bacteria, which were related to a number of small terpenoid and phenolic compounds (Helander et al., 1998; Dorman and Deans, 2000). Pattnaik et al. (1997) stated that 1,8-cineole and camphor exhibit strong antimicrobial effects. Lis-Balchin and Deans (1997) showed that essential oils containing large amounts of 1,8-cineole are better anti-listerial agents than those without 1,8-cineole. Mourey and Canillac (2002) reported that 1,8-cineole is more bacteriocidal than β-pinene and are ineffective bacteriostatic at concentrations less than 0.062%. In another study, Hayouni et al. (2008) reported that the antimicrobial activity of *S. officinalis* essential oil is related to the 1,8-cineole, α/β-thujone and borneol content in the oil. Nagy and Tengerdy (1968) evaluated the sensitivity of ruminal microorganisms to the essential oil of *Artemisia tridentate* (main compound 1,8-cineole) because some evidence indicated that high intake of this plant can cause digestive problems in wild deer. In addition, Soycan-Onenc (2008) reported that varying levels of FAC addition to dry timothy grass and barley decreased the total GP and OMD, which was associated with a decrease or inhibition of the activities of amylolytic and sellulolitic bacteria in the rumen. Santoyo et al. (2005), Pattnaik et al. (1997) and Hayouni et al. (2008) reported that antimicrobial effect is related to borneol and camphor concentrations. In this study, FAC contains 0.8% essential oil (0.27% ground FAC in DM), which comprised of 32.10% 1.8-cineole and 20.70% sabinen (Table 1).

Antimicrobial activity of EO is dependent on ruminal pH and a more pronounced effect at a lower ruminal pH (Calsamiglia et al., 2007), whereas low pH can increase the influence of some active compounds of EO due to conformational changes in their structures and the higher sensibility of rumen bacteria to these compounds (Skandamis and Nychas, 2000). Also, most studies have been carried out on rumen fluids and diets of dairy cattle, but the generalized results that beef cattle consume high levels of concentrate can be misleading. This is because the effects of EO were indicated to be highly dependent on the diet and the ruminal pH (Cardozo et al., 2005;

Castillejos et al., 2005).

Conclusion

In this study, the GP-improving effect occurred at 2 h of incubation in FAC_3 and FAC_4 groups. Degradation of the water-soluble carbohydrates promoted the concentrate feed with FAC (3 and 4% level) additions. However, the GP-reducing effect occurred from 8 to 12 h of incubation in all the treatments. It was shown that, the concentrate feed with FAC addition reduced degradation of storage polymers such as starch. In addition, GP (at the 24 h), OMD and NEL were not affected by supplemented with FAC. The effects of FAC on GP, OMD and ME varied according to the feed used, the pH of the rumen fluid, the EO content of the aromatic plant and the amount supplemented.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The author sincerely thank the Turkish Scientific and Technical Research Council for funding this project within the framework of WHAG-1984 and also thank Mustafa Şenyel and Mine Kürkçüoğlu for the determination of essential oil components.

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