African Journal of Agricultural Research

Volume 10 Number 46 12 November 2015

ISSN 1991-637X

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African Journal of Agricultural Research

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Vol. 10(46), pp. 4204-4208, 12 November, 2015
DOI: 10.5897/AJAR2015.10274
Article Number: ED9282256148
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Wind in the production of lettuce in Brazil (*Lactuca sativa* L.)

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Received 11 August, 2015; Accepted 6 October, 2015

Lettuce (*Lactuca sativa* L.) is a vegetable of higher consumption and economic value in Brazil. Due of the sensitivity of the leaves, the wind can bring losses to the perfect harmony of growth and physiological development the plant. Wind can also cause irreversible mechanical damage, such as senescence, burning, breaking, fall leaves and tear. The city of Cascavel in western Paraná is at an average altitude of 760 m enabling the continuous occurrence of strong winds. The aim of this study was to evaluate the effect of this meteorological variable in the production of lettuce and water evapotranspiration phenomenon with plants. Wind were made ducts with fans who had average wind speeds of 0, 2, 4, 6, 8 and 10 km/h, positioned at 2 m of plants in greenhouses. The evapotranspiration was verified by a evaporimeter installed in each wind duct for three sunny days, rainy and cloudy. After 49 days, they were taken and analyzed the height of plants and roots, number of leaves, fresh and dry mass of leaves and roots. It was found that the increase in wind speed also increases the evaporation, however, other environmental factors influence this parameter and the production decreases, as we increase the wind speed.

Key words: Greenhouse, evapotranspiration, *Lactuca sativa* L., wind.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a herbaceous plant belonging to the family of Asteraceae and is a typical salad vegetable. Considered as a tranquilizing properties plant and due to being consumed raw, retains all of its nutritional properties. It is a big source of vitamin A, and fewer vitamins: B1, B2, B5 and C, besides the minerals: Ca, Fe, Mg, P, K and Na. It has on average 96% water and it has low calorie (100 g of lettuce have an average of 15 calories). It is the leafy vegetable of higher consumption and economic importance in Brazil (Lisbão et al., 1990; Sonnenberg, 1985; Maroto-Borrego, 1983; Campbell-Clause, 1994; CEAGESP, 2013). Lettuce is grown in all regions of Brazil and has an area of 35,000 ha and the optimum temperature for development of lettuce is around 23°C during the day and 7°C at night. When is grown in regions with very high temperatures, burning occurs at the edges of the sheets (Resende et al., 2007; Jackson et al., 2013). American cultivars are

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characterized by leaves together to the center, forming a cabbage head and compact and can be curly or flat sheets. The roots are of the pivoting type having thin and short branches, exploiting only the first 25 cm of soil (Feltrim et al., 2005; Filgueira, 1982). The main purposes of the cultivation of lettuce protected structures are nullify the negative effects of low temperatures, frost, wind, excessive rain and hail; shorten the production cycle; increase productivity and get better quality products (Sganzerla, 1990).

The amount of evapotranspiration is of great importance in assessing the severity, frequency and distribution of water deficits, project design and management of irrigation and drainage. Evapotranspiration can be defined as a combined process of soil water transfer into the atmosphere, including the process of transpiration through the plant tissue and is an important factor in water balance, it helps to quantify water demand in a particular region (Junior and Resende, 2011; Vescove and Turco, 2005; Silva et al., 2007).

The wind is air movement in relation to the ground. The gradient of the atmospheric pressure is responsible for their training, being modified by the earth's rotation, the friction with the earth's surface and centrifugal force to its movement. The wind with height below 500 m going to suffer the effect of the frictional force, which acts in the same direction, but with the opposite direction to the wind speed. In this way, winds which occur continuously and excessively show up as a major problem for the development of agricultural activities, with a need for alternatives such as windbreaks to protect crops (Tubelis and Birth, 1980; Pereira et al., 2007).

As the wind increases, the shoot tends to send efforts to support, through the deepening of the root system and greater induration the stem. Mechanical stresses induced by the wind affects the root activity since there is increased growth and diameter. The same goes for the shoot, the wind action induces changes in the development, resulting in a more compact plant (Coutts et al., 1990; Fayle, 1976; Telewski, 1993). Assuming the report, the aim of this study is to evaluate the consequences of the wind, with different rates applied on plants of lettuce.

MATERIALS AND METHODS

The survey was conducted in Cascavel, Paraná, Brazil, at latitude 24°53'47 "S and longitude 53°32'09" W, on the campus of the State University of Western Paraná, in plastic greenhouse. The average annual rainfall is 1971 mm and the average temperature is 19.6°C, with the region's climate and temperate mesothermal and super humid (Iapar, 2011).

The arrangement used was experimental entirely randomized design with 4 repetitions for each treatment, in plastic greenhouse, planted on August, 15, 2013, in pots of 20 L in humus, with 5 cm seedlings and two leaves, with one plant each, installed in wind ducts separated by fences. The pots were set at 2 m away from the fans of 40 cm diameter and an angle between the main beam and wind plants of approximately 20°. In ducts, average wind speeds over the plants were applied: 0 (T1), 2 (T2), 4 (T3), 6 (T4), 8 (T5) e 10 (T6) km/h, whereas the value of 0 km/h as witness and daily leaving the fans connected between 9:00 and 18:00 pm, and used an anemometer to measure the measurements. Every day, they collected data of the minimum and maximum temperature inside the greenhouse, as well as the minimum and maximum relative humidity.

Irrigation was 180 daily ml in each pot on sunny days, and on cloudy and rainy days, was flooded 180 ml every 2 days, amount that based on the average evapotranspiration index in the city of 7 liters per square meter per day about (Silva et al., 2007). After 49 days, samples were collected and analyzed: the number of sheets (NF), plant height (AP) and root (AR) fresh mass of the leaves (MFF) and roots (MFR) and dry mass of the leaves (MSF) and roots (MSR). These results were submitted to analysis of variance (ANOVA) and their means compared by Tukey test, adopting the level 1-5% significance using the statistical Assistat® version 7.7 beta package (Silva, 2014).

RESULTS AND DISCUSSION

Figure 1 shown the contents of average daily evapotranspiration, on sunny days, rain and cloudy for each wind speed used in the ducts. It was found that the evaporation is larger as the wind speed increases. The typical of evapotranspiration values in Western Rio Grande do Sul region is 6 to 7 mm/day in sunny days and 4 to 5 mm/day in days rainy, differing from those obtained that were between 2.123 and 11.818 mm/day on sunny days and 0.557 mm and 1.337 mm/day on rainy days (Tabbal et al., 2002).

Figure 2 shows the values of minimum and maximum temperature and the maximum and minimum relative humidity collected. It was observed that the average temperature during the experiment was 20.37°C, however, there is a large variation in temperature of the region, and days 35.4°C with maximum temperature only 0.9°C minimum temperature, testifying to the measurements made by Iapar (2011).

The average relative humidity during the trial period was 65.51%, but with wide variations, with a minimum of 28% and a maximum of 94%, confirming Amorim et al. (2001) who obtained the average relative humidity 75 to 81%.

It showed that rainy days have a higher relative humidity and consequently, low evaporation. In contrast, the sunny days have a high evapotranspiration and lower relative humidity. The microclimatic behavior of rainy and cloudy days were similar, however, the sunny days showed high amplitude variation in temperature, relative humidity and evaporation.

The results of the samples taken are shown in Table 1. It was found that strong winds cause a deficit in the production of lettuce, having damage and reducing the amount of fresh dough, so the leaves, the roots.

Thus, it is seen that strong winds cause a deficit in the

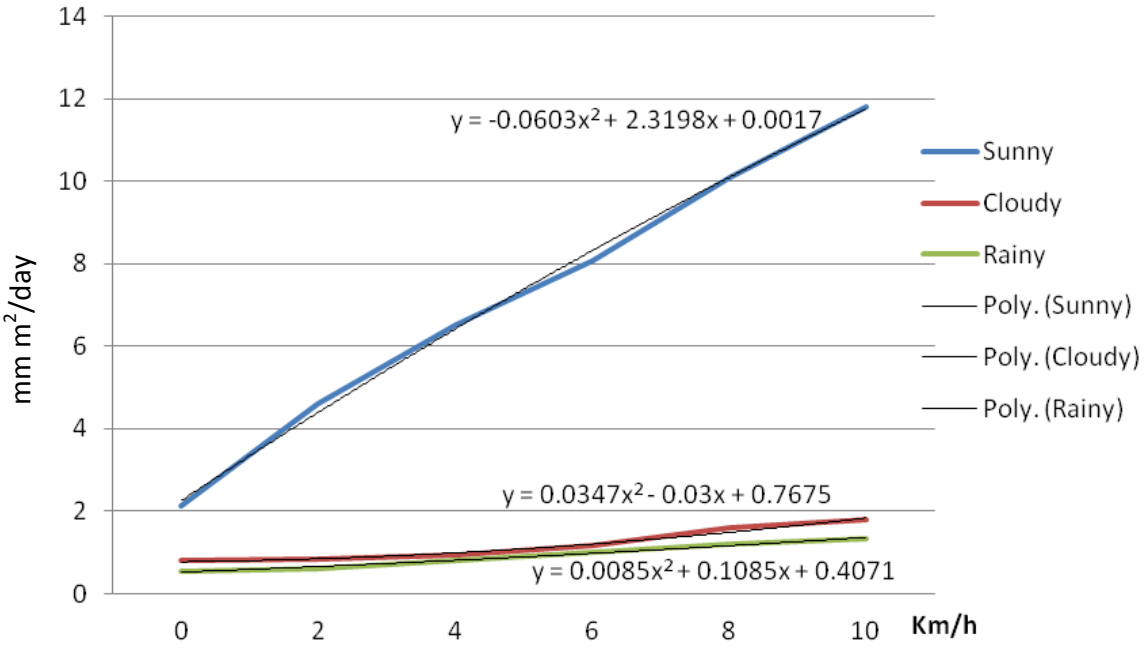

Figure 1. Evaporation average (mm day -1) in the wind ducts in sunny, cloudy and rainy days.

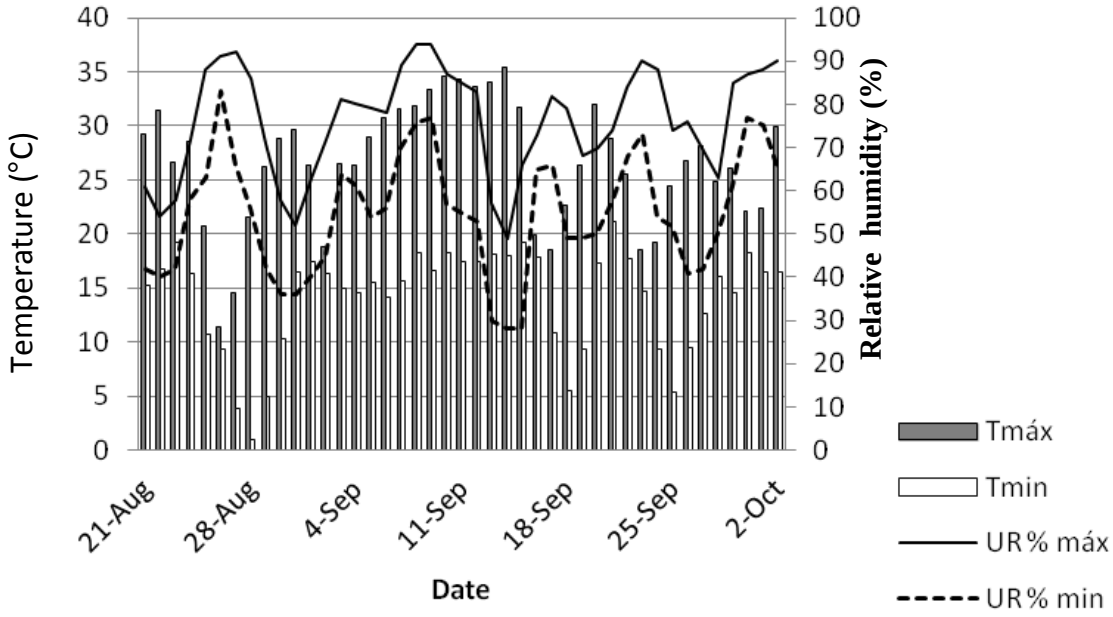

Figure 2. Temperature (T °C) Minimum (Tmin) and maximum (Tmax) and relative humidity (%) Minimum (URmin) and maximum (URmáx).

production of lettuce crop, or increased as the wind speed, the plants showed damage to leaves by reducing the amount of fresh mass, so the leaves, the roots. In winds of 10 km/h only remaining 2 plants, and fresh mass of leaves average was only 2.46 g. Campbell-Clause (1994) evaluated the effect of the winds in grape cultivars Rubi and Italy, using windbreaks, with permeability of 40% and height around 4 m, arranged perpendicular to

Table 1. Results obtained in the experiment.

Wind	AP (cm)	NF	MFF (g)	MSF (g)	AR(cm)	MFR (g)	MSR(g)
T1	24.125[a]	18.500[a]	159.292[a]	5.807[a]	9.750[ab]	5.965[a]	1.237[ab]
T2	26.625[a]	19.750[a]	126.622[ab]	5.282[ab]	12.000[a]	4.945[a]	1.437[a]
T3	29.625[a]	18.500[a]	104.150[bc]	4.182[bc]	9.500[ab]	3.460[ab]	0.910[abc]
T4	24.125[a]	16.250[a]	72.125[cd]	3.104[c]	7.625[b]	2.032[bc]	0.297[c]
T5	23.375[a]	14.500[a]	67.105[d]	3.075[c]	7.625[b]	2.210[bc]	0.500[bc]
T6	5.475[b]	3.000[b]	1.232[e]	0.170[d]	2.807[c]	0.222[c]	0.042[c]
F	17.437[**]	28.191[**]	44.587[**]	38.814[**]	14.698[**]	13.738[**]	7.919[**]
CV%	18.37	15.51	18.59	17.96	19.72	36.01	52.83
GA	22.222	15.083	88.421	3.604	8.218	3.199	0.737

[a] Means followed by the same letter within each analyzed parameter (column), do not differ by Tukey test at 5% error probability. (**) = Significant at 1% probability (*) = significant at the 5% probability (NS) = not significant. CV%: coefficient of variation. GA: General Average.

the direction of the winds, in Western Australia and obtained a productivity and fresh pasta grape of up to 23% compared to the control that did not use the windbreak, corroborating the data obtained in the experiment and noting that, for different cultures, the winds are harmful.

Conclusion

Winds applied to the crop of lettuce significantly increase evapotranspiration on sunny days, but on cloudy and rainy days, this occurs less. Wind action result in mechanical damage and disorderly growth of leaves, which reduces the fresh mass of aerial part of the plant. At speeds of 10 km/h, the culture is practically lost.

Conflict of Interests

The authors have not declared any conflict of interest.

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Vol. 10(46), pp. 4209-4214, 12 November, 2015
DOI: 10.5897/AJAR2015.10391
Article Number: EE9957756151
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Productivity of *Citrus latifolia* plants subjected to branch girdling

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Received 15 September, 2015; Accepted 16 October, 2015

Abscission of flowers and fruit in *Citrus latifolia* cultivars is more intense than in seeded *Citrus* ones. The purpose of this study was to evaluate the effect of different levels of branch girdling in *C. latifolia* fruit fixation. The work was carried out in an orchard of *C. latifolia* grafted onto three-year-old *Citrus limonia* spaced 3x1, located in the Goiás State University experimental field. The experiment was set up according to the randomized block design with four treatments (girdling of 0, 25, 50 and 100% of the branches existing in the plant), five replications, parcels of four usable plants and total of 20 plants. Girdling was accomplished on 29/08/2014 and full flowering occurred on 13/09/2014. The plants that had 100% of their branches girdled presented high productivity of fruit with low juice volume. Those with 50% of branches girdled showed higher productivity and good juice yield per fruit. Results indicate that total blocking of transportation of assimilation from the canopy to the root system has negative impact on the volume of fruit juice.

Key words: Tahiti lime, carbohydrates, citrus culture, photoassimilates.

INTRODUCTION

The name 'lime' is used to refer to citrus fruit with highly acidic juice. Available statistics do not make a distinction between lemon and acid lime. However, estimates are that 70% of the world's total production consists of lemons and 30% of acid limes. The world's most produced and consumed type of acid lime is *Citrus latifolia* (Embrapa, 2015). Brazil is the world's greatest producer of sweet oranges and the fourth major producer of lemons, ranking only after Mexico, India and Argentina. Citrus culture holds key importance in Brazil's economy as it generates an annual contribution of about 5.2 million dollars to GNP. In the country, São Paulo state is the main producer of *C. latifolia* (Lopes et al., 2011; Soares et al., 2015).

The *C. latifolia* species is original from tropical regions, but the exact place of origin is still unknown. It is accepted, though, that is comes from citrus fruit seeds imported from Tahiti (Coelho, 1993). *C. latifolia* belongs

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in the Rutaceae family, with size ranging from medium to large, reaching up to 4 meters high green lanceolate mature leaves, and thin-peeled oblong oval fruit with smooth greenish to yellowish surface and maturing time (from anthesis up to harvesting) of around 170 days. It is a precocious culture with nearly year-round flower blossoming, though with higher concentration of flowers during the months of September and October (Rocha, 2008).

Long blossoming period coupled with high number of flowers and intense flower and fruit abscission are the main barriers to achieving great productivity of *C. latifolia* plants. Flower and fruit abscission in this species is higher than in seeded *Citrus* cultivars. Final fixation is barely around 1.85% in the September blossoming (Spósito and Mourão Filho, 2003). The factors leading to flower fall are still unknown, though they are assumed to be mostly physiologic and related to plant phenology as a result of competition for metabolites (Junqueira, 2013; Santos et al., 2014). According to Rivas et al. (2007), intensive blooming disturbs the partitioning of assimilates and a hormonal signal activates the abscission process to balance out the availability of carbohydrates and the fruit load to come. However, even after part of the flowers have fallen, significant fruit abscission occurs in the initial growth stage.

Adoption of management practices is required in order to minimize the abscission of reproduction structures and increase the productivity of *C. latifolia*. Among such practices branch girdling stands out, which consists in cutting off and removing a strip of bark around the trunk circumference (Pereira et al., 2010). Girdling prevents assimilation from being transported to the root system, increasing temporary retention of carbohydrates in the tree canopy and thus promoting greater fruit fixation (Santos et al., 2014). According to Pereira et al. (2014), girdling increases the number and size of *C. latifolia* fruit. Despite the benefits of girdling, the incision into the stem may cause accumulation of phytoregulators and other metabolites, bringing undesired effects such as reduced plant size, lower photosynthesis rate, yellowing leaves and reduced root growth (Pereira et al., 2010, 2011).

Inconsistent results obtained with girdling of *C. latifolia* and the effects of the incision on the plant's vegetative growth point to the need for research, in order to better understand the physiological aspects involved and thus justify the adoption of this agricultural practice. Therefore, this study was aimed at evaluating the effect of girdling of different amounts of branches on the fixation of *C. latifolia* fruit.

MATERIALS AND METHODS

The work was carried out in an orchard of *C. latifolia* grafted onto three-year-old *Citrus limonia* spaced 3x1 located in Goiás State University experimental field, Ipameri Campus (17°43'19''S, 48°09'35''W, Alt. 773 m). According to Köppen classification, the region has tropical climate with dry winter and rainy summer (Aw). There are two well-defined seasons: rain from October to April and drought from May to September. The soil of the experimental area is red-yellow Oxisol.

The experiment was set up following the randomized block design with four treatments (girdling of 0, 25, 50 and 100% of the number of branches existing in the), five replications, parcels of four usable plants and total of 20 plants. The girdling was accomplished by cutting into the phloem all around the stem circumference without damaging the xylem. The procedure was done with a jackknife that made it possible to remove a 2 mm-thick ring from the bark. Girdling was carried out on 29/08/2014 and full blooming occurred on 13/09/2014. Evaluations regarding the vegetative growth (plant height and stem diameter) were made on the day of girdling and 150 days thereafter (two times). Total foliar concentrations of chlorophylls and carotenoids were measured 60 days after girdling, and productivity variables (fruit diameter and weight, juice volume, total acidity, juice pH and productivity) were recorded during harvest.

Growth and reproduction variables

Plant height and stem, canopy and fruit diameter were measured using a graded ruler and a digital pachymeter. The number of fruits was determined by counting, and the mass was weighed with digital scales. We use the minimum diameter of 40 mm as a reference for the harvesting of fruits.

Determining juice total acidity, volume and pH

The juice volume was determined with a manual extractor and a beaker for precision measurement of four fruits per plant. After the juice was extracted, 10 ml of it were pipetted into an Erlenmeyer flask and distilled water was added to bring the volume to 60 ml. Subsequently, four drops of 1% phenolftalene solution were added and titrated with 0.1 M sodium hydroxide solution until pink color was reached. Calculation of total acidity was made through the equations proposed by Adolfo Lutz Institute (2008). Juice pH was determined with a previously Tri-Meter calibrated digital pH meter.

Photosynthetic pigments

In order to determine the concentration of chlorophylls were removed with two discs 1.2 cm in diameter each on fully expanded leaves located between the 3rd and 4th leaf pair stems and placed in glasses containing dimethyl sulfoxide (DMSO). Next, extraction was performed in water bath at 65°C for four hours. Aliquots were extracted for spectrophotometric reading at 480, 649.1 and 665.1 nm. The chlorophyll a (Chl *a*) and chlorophyll b (Chl *b*) contents were determined through the equation proposed by Wellburn (1994).

Statistical procedures

The experiment was set up according to the randomized blocks design with four treatments (girdling of 0, 25, 50 and 100% of the branches existing in the plant), five replications and parcels of two usable plants. The variables were submitted to one-way ANOVA and Newman-Keuls test at 5% probability, using statistical software SISVAR (Ferreira, 2011).

Table 1. Minimum and maximum values, average, standard deviation, coefficient of variation (CV) and Shapiro-Wilks normality test (SK) of variables plant height and diameter (mm/month), number of fruits per plant, productivity (t ha^{-1}), fruit diameter (mm), total acidity (%), pH, juice volume (ml), total carotenoids (Car) and total chlorophylls (total Chl) in *C. latifolia* plants with different percentages of branch girdling, Ipameri, GO.

Variable	Minimum	Maximum	Average	DV	CV (%)	SW
Height	4.5	23.25	13.8	4.83	43.05	0.96^{ns}
Diameter	0.75	2.67	1.56	0.53	34.65	0.98^{ns}
No. of fruit	274.0	1192.0	692.9	249.1	35.9	0.94^{ns}
Productivity	2.46	8.38	5.12	1.60	31.37	0.91^{ns}
Fruit diameter	40.6	50.25	46.5	2.65	5.70	0.97^{ns}
Total acidity	0.50	0.95	3.78	0.03	18.65	0.94^{ns}
pH	3.72	3.86	3.78	26.23	0.86	0.94^{ns}
Juice volume	35.0	134.0	77.0	26.23	33.73	0.95^{ns}
Car.	0.18	0.65	0.44	0.12	29.10	0.90^{ns}
Total Chl.	0.81	2.89	1.62	0.52	32.51	0.85^{ns}

Table 2. Variance analysis and means comparison of plant height, diameter (mm/month), number of fruits per plant, productivity (t ha^{-1}), and fruit diameter (mm) in *C. latifolia* plants with different percentages of branch girdling, Ipameri, GO.

Source of variation	GL	Mean squares				
		Height (cm/month)	Stem diameter (mm/month)	N° fruits	Prod. (t ha^{-1})	Fruit diam. (mm)
Treatment	3	179.11^{*}	0.68^{ns}	889818.4^{**}	32.87^{**}	8.84^{ns}
Residue	12	77.68	1.61	250748	18.30	148.46
CV (%)		18.72	26.13	18.49	21.05	6.54
Treatment				**Averages**		
0		16.25^{b}	2.02^{a}	638.6^{b}	3.43^{c}	46.64^{a}
25%		21.00^{a}	1.25^{a}	382.2^{c}	4.72^{bc}	45.69^{a}
50%		14.37^{b}	1.45^{a}	734.2^{b}	6.69^{a}	47.67^{a}
100%		6.25^{c}	1.86^{a}	971.4^{a}	6.12^{ab}	46.44^{a}

*significant at 5% probability; **significant at 1% probability; ns = non-significant by F test. Averages followed by the same letter within the column do not differ by Newman-Keuls test.

RESULTS

Analysis of the results presented significant data variability, as shown in Table 1. The treatments promoted considerable changes in vegetative growth for plant height and stem diameter, which increased by 81 and 71% when comparing the lowest and highest values obtained, respectively. The same comparison of number of fruits, productivity, juice volume and total chlorophylls showed variations of 77, 71, 74 and 72%, respectively.

Variance analysis and means comparison of plant height, stem diameter, number of fruits per plant, fruit productivity and diameter are shown in Table 2. Variables of stem diameter and fruit diameter did not present significant variations at 5% probability. Plant height increased significantly among the different treatments. Monthly increase in height was 70% lower in plants with 100% of branches girdled compared to plants with 25% of branches girdled. The number of fruits per plant was on average 60% higher in plants with 100% of branches girdled compared to plants with 25% of branches girdled, whereas the control treatment had intermediate value. Productivity was greater in girdled plants compared to the control. The average productivity of girdled plants was 41% greater than that of the non-girdled control.

Variance analysis and means comparison of total acidity, pH, juice volume per fruit, total carotenoids and chlorophylls are shown in Table 3. Variables of total acidity, pH, and total carotenoids and chlorophylls did not present significant variations at 5% probability. Juice volume per fruit was on average 40% greater in plants with 25% of branches girdled compared to plants with 100% of branches girdled. The control treatment presented intermediate values.

Table 3. Variance analysis and means comparison of total acidity (%), pH, juice volume (ml), total carotenoids (Car), and total chlorophylls (Chl) in *C. latifolia* plants with different percentages of branch girdling, Ipameri, GO.

Source of variation	GL	Mean squares				
		Total acidity (%)	pH	Juice volume (ml fruit^{-1})	Car (mg g^{-1})	Total Chl (mg g^{-1})
Treatment	3	0.107^{ns}	0.06^{ns}	3052.4*	0.06^{ns}	1.07^{ns}
Residue	12	0.191	0.014	6427.5^{ns}	0.07^{ns}	2.40^{ns}
CV (%)		16.49	0.82	28.31	24.68	32.23
Treatment				**Averages**		
0		0.66^{a}	3.76^{a}	20.75^{a}	0.37^{a}	1.98^{a}
25%		0.80^{a}	3.81^{a}	21.95^{a}	0.34^{a}	1.52^{a}
50%		0.59^{a}	3.79^{a}	19.00^{a}	0.29^{a}	1.21^{a}
100%		0.72^{a}	3.79^{a}	13.18^{b}	0.50^{a}	1.98^{a}

* = significant at 5% probability; ** = significant at 1% probability; ns = non-significant by F test. Averages followed by the same letter within the column do not differ by Newman-Keuls test.

Table 4. Pearson correlation coefficients for stem diameter, productivity, fruit diameter, number of fruits, total acidity, pH, juice volume per fruit, total carotenoids, and total chlorophylls in *C. latifolia* plants with different percentages of branch girdling, Ipameri, GO.

Variables	Stem diam.	Prod.	Fruit diam.	No. of fruits	Total acidity	pH	Juice volume	Total car.	Total chl.
Height	-0.93^{ns}	-0.25^{ns}	0.89^{ns}	-0.93^{ns}	0.76^{ns}	-0.58^{ns}	0.94*	-0.83^{ns}	-0.77^{ns}
Stem diameter		0.02^{ns}	-0.85^{ns}	0.90^{ns}	-0.72^{ns}	0.34^{ns}	-0.90^{ns}	0.83^{ns}	0.70^{ns}
Product.			0.12^{ns}	0.44^{ns}	-0.67^{ns}	-0.09^{ns}	-0.44^{ns}	0.52^{ns}	0.23^{ns}
Fruit diameter				-0.69^{ns}	0.40^{ns}	-0.74^{ns}	0.70^{ns}	-0.52^{ns}	-0.96*
No. of fruits					-0.93^{ns}	0.31^{ns}	-0.99**	0.97*	0.50^{ns}
Total acidity						-0.08^{ns}	0.93^{ns}	-0.98**	-0.18^{ns}
pH							0.33^{ns}	0.11^{ns}	0.83^{ns}
Juice volume								-0.97*	-0.51^{ns}
Car									0.30^{ns}

ns = non-significant; * = significant at 5%; ** = significant at 1%.

The Pearson correlation for all variables analyzed is shown in Table 4. High and positive correlations were found between the number of fruits per plant and total carotenoids, plant height and juice volume, total acidity and carotenoids, and juice volume and carotenoids. Significant high and negative correlations were observed between fruit diameter and total chlorophylls and number of fruits per plant and juice volume per fruit.

DISCUSSION

The adoption of practices to reduce the abscission of fruit and increase productivity represents an important alternative to raise profitability for *C. latifólia* producers. Overall, branch girdling caused significant changes to vegetative growth and fruit yield, as discussed below.

Lower height increase in plants with 100% of branches girdled combined with high number of fruits therein indicates possible competition for assimilation between vegetative and reproducible growth, for while girdling prevents partitioning of assimilates into the root system, it makes more carbohydrates available to the canopy, but does not inhibit competition among the shoot organs. The results corroborate those found by Pereira et al. (2010) when studying fruit fixation in *C. latifólia* girdled plants.

Despite the absence of statistical difference, a trend towards increased foliar concentration of carotenoids in plants with 100% girdling is evident. Accumulation of carbohydrates in the canopy may cause retroinhibition of photosynthesis, hinder CO_2 assimilation and cause oxidative stress (Pereira et al., 2014; Rivas et al., 2007). Under such circumstances, carotenoids take on photoprotective importance, as they remove the reactive

species that cause oxidative stress. High correlation between carotenoids and the number of fruits is associated to high accumulation of carbohydrates in the canopy and a consequent higher number of fruits in girdled plants. Although the accumulation of carbohydrates and decrease in leaf concentration of carotenoids is leaf senescence clue in girdled plants (Parrott et al., 2010; Tang et al., 2015), there was no visual symptoms of senescence and carotenoid concentrations remained high and we attribute this phenomenon to the photoprotective role of these pigments.

Regardless of the amount of girdled branches, treated plants presented greater fruit productivity to the detriment of vegetative growth. Plants with 50% of girdled branches, however, presented greater productivity (41% superior to the control) and vegetative growth similar to those of the controls. The 50% branch girdling treatment may possibly have provided more assimilates for the development of fruit, while not fully hindering the transportation of assimilates required to support the absorption of mineral nutrients in the root system. According to Pereira et al. (2010), insufficient mineral nutrients in the canopy are a negative effect of branch girdling in *C. latifólia*.

The high negative correlation between juice volume and number of fruits indicates that greater fruit fixation may reduce juice yield and possibly hamper the product's commercial value, since both the number of fruits and the juice volume depend on and compete for assimilates. The plants with 100% of branches girdled presented higher productivity and lower juice volume per fruit. It suggested that inhibited transportation of assimilates to the root system may have hindered nutrient absorption and played a critical role in the plants' vegetative growth and juice yield. Thus, it is evident that such barrier to the transportation of assimilates from the canopy to the root system may hamper commercial quality of the product.

Plants with 50% of branches girdled presented higher productivity, vigorous vegetative growth, considerable juice yield and quality similar to those of the controls. Notwithstanding, further research is required, with analyses of the vegetative and reproducible growth and nutritional status along several years, for validation and recommendation of the girdling practice.

Conclusions

Plants with 50% of branches girdled presented the highest productivity and greatest juice yield per fruit. Plants with 100% of branches girdled presented high productivity and low juice yield per fruit. Fully blocking the transportation of assimilates from the canopy to the root system has negative impact on the fruit juice volume.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

To Goiás State University (UEG), Coordination for the Improvement of Higher Education Personnel (CAPES) and Research Support Foundation of the State of Goiás (FAPEG) for financing project AUXPE 2370/2014.

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academicJournals

Vol. 10(46), pp. 4215-4221, 12 November, 2015
DOI: 10.5897/AJAR2015.10413
Article Number: D8D7F6F56153
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Influence of *Uroclhoa brizantha* cv. Marandu phytomass in the control of *Bidens subalternans* under dystrophic yellow latossol

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Received 20 September, 2015; Accepted 16 October, 2015

The objective of this work was to evaluate the interference resulting from the use of different phytomass quantities of *Uroclhoa brizantha* cv. Marandu and the management of straw to control beggar-ticks (*Bidens subalternans*). The experiment was performed under greenhouse from May to August, 2014, using a randomized block experimental design with four replicates, in a factorial scheme (3 × 4) +1, with Factor A constituted by three management methods (incorporated, incorporated+surface and surface), and Factor B constituted by five straw levels (3; 6; 9 and 12 t ha^{-1}), plus one treatment without the usage of any cover plant (control treatment). The variables evaluated were the total number of plants emerged, the index of emergence velocity, dry phytomass of the aerial plant portion, leaf area, dry phytomass of roots and the volume of roots. Different management methods at different straw levels were efficient, with an emphasis to the surface management in the suppression of beggar-ticks (*B. subalternans*).

Key words: Allelopathy, beggar-tick, *Brachiaria brizantha,* weed.

INTRODUCTION

Bidens subalternans, commonly known as beggar-ticks, occurs in several agricultural regions in Brazil (Gazziero et al., 1998). Until recently, its existence was little mentioned, probably due to the fact of the species being particularly similar to *Bidens pilosa*, being basically differentiated by the number of beards in the achenes (Kissmann and Groth, 1993). However, in numerous areas its occurrence has significantly increased and, in many farm properties within the Brazilian States of Mato Grosso and the northern region of Mato Grosso do Sul, the infestation is greater than the one observed for *B. pilosa* (Sanches and Zandonade, 1997). Furthermore, its resistance to ALS inhibitors was reported by Monqueiro et al. (2000).

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Among the species infesting corn plantations, the genus *Bidens* sp. is to be highlighted. Species that fit within this genus influence plant growth, development and productivity of crops, due to the competition for light, nutrients and water, as well as increasing the operational expenses for harvesting, drying and processing of grains (Fleck et al., 2002). Moreover, in hot regions, the species *B. subalternans* may produce three to four generations within the period of one year. In addition, this species is a plant native to South America, with an annual cycle and very similar to *Bidens pilosa*. In soybean plantations where the species is found in high level of infestation, the production may have an average reduction of 30% (Kissmann and Groth, 1993).

Non-tillage system (DPS), as a sustainable alternative of agricultural production (Pacheco et al., 2008), offers organic matter to the soil. However, in the conditions of the Cerrado's region, with an area under DPS exceeding twenty-six millions of hectares (FEBRAPD, 2010), the system has exhibited difficulties to achieve two basic requirements: crop rotation and straw's formation/conservation (Ceretta et al., 2002). Moreover, the use of cover plants leads to a reduction in expenses and the use of fertilizers, being able to return considerable amounts of nutrients to the crops, once these plants assimilate nutrients from sub-superficial layers and release them later to the surface layer by the decomposition of their residues (Torres et al., 2008). Allelopathy is characterized primarily by a negative interaction between plants through chemical signaling (El-khawas and Shehata, 2005).

According to Golisz et al. (2008), allelopathy consists in the plant ability to synthetize metabolites that inhibit or stimulate the growth and development of other neighboring plants, with a competition for limited resources as light, water and nutrients. The allelochemicals found in soil cover plants are soluble in water, and are released in the environment by volatilization, radicular exudation, lixiviation and plant tissue breakdown. These released substances cause physiological and/or morphological modifications, influencing processes such as germination, growth, flowering, fructification, senescence and abscission in sensitive species (Correia et al., 2005).

The interference of one plant on another may occur in direct or indirect way. When direct, the allelochemical molecules links to the membranes of the receptor plant or penetrates into cells, directly interfering in metabolism, and due to the transformation of the allelochemicals in the soil and/or by the activity of microorganisms. Allelopathic substances may induce the rise of abnormal plantlets, with the most common symptom being radicular necrosis (Ferreira and Borghetti, 2004).

With a concern about agricultural practices, the need for the utilization of sustainable techniques that will preserve the environment avoiding contamination becomes evident, thus adequate production models that will reduce costs come to be significant. Therefore, the present study had the objective to evaluate *Uroclhoa brizantha* cv. Marandu used under different phytomass' quantities and management methods to control beggar-ticks (*B. subalternans*).

MATERIALS AND METHODS

The experiment was performed in greenhouse from August to October 2014, at the campus of the Federal University of Piauí (UFPI/CPCE), located within the county of Bom Jesus (Latitude 9° 16' 78"S, Longitude 44° 44' 25"W and Altitude of 300 m), in the State of Piauí, Brazil.

The experimental was designed under random blocks, with four replicates in a factorial scheme (3 × 4) + 1, with Factor A constituted by three methods of management (incorporated, incorporated+surface and surface) and Factor B constituted by five straw levels (3; 6; 9 and 12 t ha^{-1}), plus one treatment without the use of any cover plant (control treatment), with a total of 52 experimental units.

The composition of each experimental unit was distributed in pots with 8 dm^3 soil capacity, and 35 cm diameter. The substrate used was constituted by soil samples obtained from 40 to 60 cm layers of a dystrophic yellow latossol (oxissol). This sampling depth was chosen in order to avoid seeds from weed pants existing predominantly at soil superficial layers.

Seeds of *B. subalternans* used in the experiment were collected in August 2014, from a central pivot area at the Agricultural School from Bom Jesus (CABJ). Fifteen seeds were randomly sowed and covered with an approximately 1.0 cm soil layer. The fresh vegetal cover was incorporated, preserved in the surface and part ($^1/_2$) incorporated + other part ($^1/_2$) preserved in the soil surface, in quantities equivalent to the different treatments (3, 6, 9 and 12 t ha^{-1}) in dry mass. The vegetal material was collected and fractionated the same day of the experiment installation, with the aim of avoid potential allelochemical losses.

In order to obtain enough phytomass, seeds of *Urochloa brizantha* were sowed manually, cultivated in beds of 5 m^2 and the aerial parts were collected 60 days after sowing. Vegetal residues were segmented in sections of approximately 2 to 3 cm, weighted and corrected by the reference of a dry base, after that, plant samples remained in an oven at 65°C for 72 h and/or until reaching constant weight. The humid material was set according to the desired dry mass per hectare, where latterly was homogenized and preserved in the soil surface, incorporated to the soil and one part ($^1/_2$) incorporated + another part ($^1/_2$) in the surface, according with the treatments. Irrigation was performed daily according to the plants requirements.

The variables evaluated were: total number of emerged plants (NEP), emergence velocity index (EVI), leaf area (LA), dry phytomas of the aerial plant portion (DPAPP), volume of roots (VR) and dry phytomas of roots (DPR). The EVI was calculated by the formula described by Maguire (1962): EVI=[N1/1+(N2-N1)/2+(N3-N2)/3+....(Nn-Nn-1)/n], where N1, N2, N3...Nn, correspond to the number of emerged plantlets and 1, 2, 3...n, are the number of days after sowing (DAS).

Leaf area (LF) was determined when the weeds in average, reached the pre-flowering stage, with the aid of a LI-3100 Area meter equipment (LI-COR, Inc. Lincoln, NE, EUA). Leaves were detached from the plant stalk to perform the evaluation, expressed as cm^2 pot^{-1}. Besides that, roots were separated from the plant, washed with tap water and removed from the soil, and then the volume of roots (VR) was determined and expressed in terms of cm^3 pot^{-1} utilizing the test-tube method (Basso, 1999). The aerial part as much as the radicular portion were dried in an oven at a

Table 1. Summary of the variance analysis (F values) of the total number of emerged plants (NEP), emergence velocity index (EVI), leaf area (LA), dry phytomass of the aerial plant portion (DPAPP), dry phytomass of roots (DPR) and volume of roots (VR) of *Bidens subaltern.*

Source/ variation	Mean square					
	NEP	EVI	LA	DPAPP	DPR	VR
Management method (MM)	121.43**	1.82**	1241794.55**	36.19**	344.32**	337.22**
Straw levels (SL)	156.03**	2.69**	1331834.88**	40.07**	276.69**	267.90**
MM x SL	9.17**	0.21**	93056.54**	3.47**	27.39**	35.80**
Residue	2.09	0.04	35982.91	0.60	4.91	9.27
CV (%)	26.23	29.42	34.21	24.22	26.12	34.19

**significant at 1%; CV – coefficient of variation.

Table 2. Number of plants emerged and index of emergence velocity of *Bidens subalterna* as a result of management method and straw levels of *Urochloa brizantha*.

Management method	Straw levels (t ha^{-1})				
	0	3	6	9	12
	Number of plants emerged (units pot^{-1})				
Incorporated	11.50^{a}	9.66^{a}	6.66^{a}	7.00^{a}	6.66^{a}
Incorporated + Surface	11.50^{a}	5.00^{b}	4.66^{a}	2.50^{b}	0.66^{b}
Surface	11.50^{a}	3.00^{b}	2.00^{b}	0.66^{b}	0.00^{b}
CV (%)			29.23		
	Index of emergence velocity				
Incorporated	1.42^{a}	1.19^{a}	0.87^{a}	0.68^{a}	0.65^{a}
Incorporated + Surface	1.42^{a}	0.64^{b}	0.58^{b}	0.15^{b}	0.07^{b}
Surface	1.42^{a}	0.15^{c}	0.21^{c}	0.06^{b}	0.00^{b}
CV (%)			29.42		

Means followed by the same letter within the column do not differ statistically by Tukey's test at 5% probability; CV - coefficient of variation.

temperature of 65°C until reached constant weight, in order to obtain their dry phytomass.

Data was submitted to variance analysis (ANOVA) and when relevant for qualitative data, Tukey's test 5% was used to compare mean values, with the aid of the software SISVAR 4.2. Quantitative data was set in equations, with the aid of the software SIGMA PLOT 10.1.

RESULTS AND DISCUSSION

For variables of total number of emerged plants (NEP), emergence velocity index (EVI), leaf area (LF), dry phytomass of the aerial plant portion (DPAPP), volume of roots (VR) and dry phytomass of roots (DPR), a positive interaction was observed (P<0.01) between the factors: management method (MM) and straw level (SL) of *Urochloa brizantha* (Table 1).

All management methods of *U. brizantha* tested promoted a reduction in NEP and EVI, with an emphasis for surface and surface+incorporated management, which showed the greatest reduction within all straw levels studied (Table 2). These results may be explained by the physical control promoted by the soil cover (Severino and Christofoletti, 2001), by diminishing the luminosity required for germination of *Bidens subalterna* (Theisen et al., 2000), as well as by constituting a physical obstacle able to produce the exhaustion of the reserve material from plantlets during the early developmental stage (Pacheco et al., 2013). These results also confirm the observations mentioned by Ferreira et al. (2007), when extracts of *Eucalyptus citriodora* significantly reduced the germination velocity index (GVI) in beggar-ticks.

Management methods of *U. brizantha* showed exponential decreasing behavior for variables NEP and EVI as a function of straw levels (Figure 1). These results demonstrated that treatments with soil surface management, had the most significant effects in the reduction of these variables at the initial straw levels, with 3 t ha^{-1} of straw reducing NEP and EVI in 73.91 and 89.21%, respectively, when compared to the control (0 t ha^{-1}). In a previous study performed by Pacheco et al.

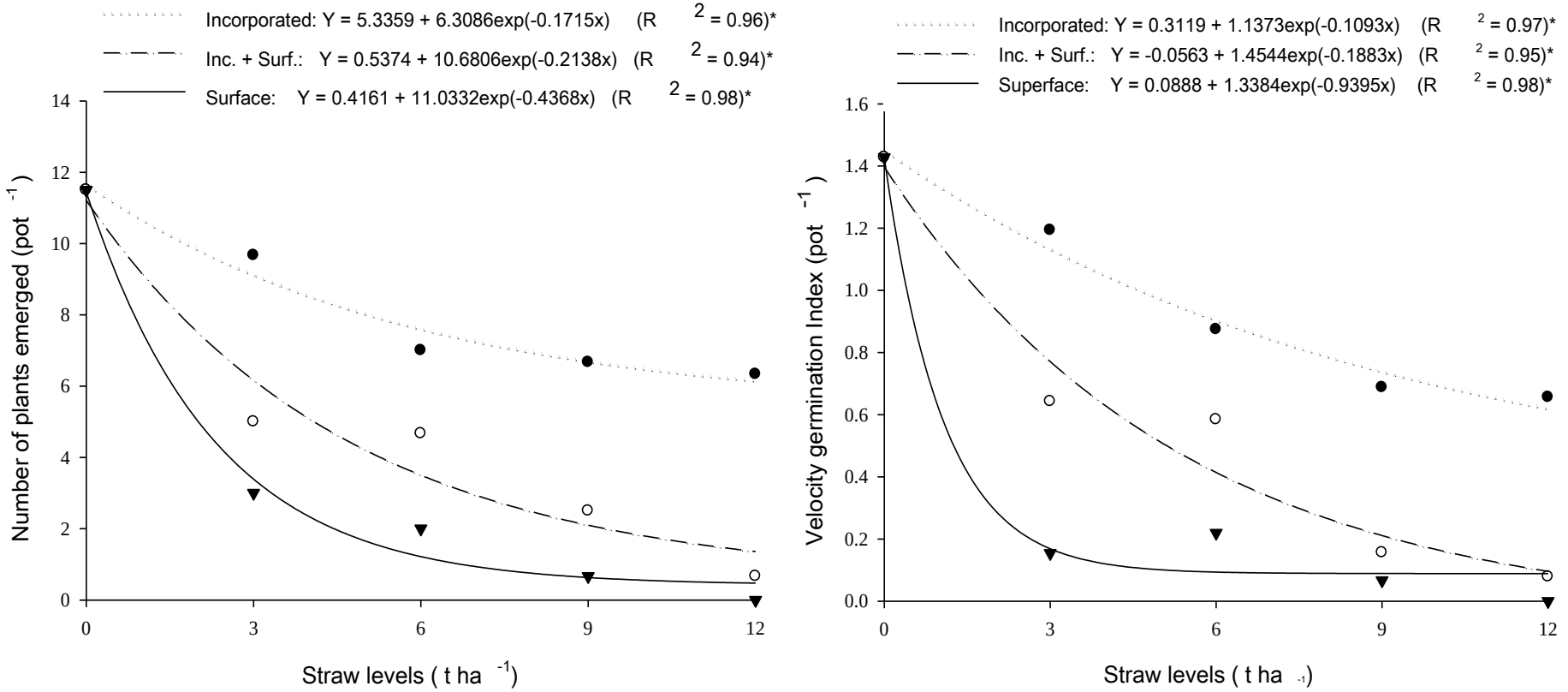

Figure 1. Number of plants emerged and index of germination velocity of *Bidens subaltern* as a function of straw levels of *Urochloa brizantha*. ** and * significant at 1 and 5%, respectively.

(2013) with cover plants, the authors reported that 4 t ha^{-1} of *Urochloa ruziziensis* in soil surface reduced NEP and GVI of *Bidens pilosa* in 61.63 and 75.91%, respectively.

The management of *U. Brizantha* by the method incorporated + surface, even when it resulted in lower means for variables NEP and GVI and compared to the surface management method, still promoted a reduction of such variables in 56.52 and 59.09%, respectively, when using 3 t ha^{-1} of straw, compared to the control treatment (0 t ha^{-1}) (Figure 1). The incorporated management method showed an expressive reduction of such variables at levels higher than 3 t ha^{-1} of straw.

The lowest mean values for variables LF and DPAPP of *Bidens subaltern,* were observed when sowed in pots with surface management and incorporated + surface management treatments (Table 3). These results may be explained by the exponential reduction in the number of emerged plants (Figure 1), as well as by the delay of plantlets' emergence. Promissory results were observed by Moraes et al. (2011), with a significant reduction of LF and DPAPP of *Bidens pilosa,* when using a phytomass of 4 t ha^{-1} of Italian ryegrass (*Lolium multiflorium*), preserved in the soil surface.

Concerning variables LF and DPAPP, management methods of *U. brizantha* showed an exponential decreasing behavior (Figure 2).

Table 3. Leaf area and dry phytomass of the aerial plant portion of *Bidens subaltern,* as a function of the management method and straw levels of *Urochloa brizantha*.

Management method	Straw levels (t ha^{-1})				
	0	3	6	9	12
	Leaf area (cm^2 pot^{-1})				
Incorporated	1110.35^a	933.34^a	708.52^a	680.90^a	632.32^a
Incorporated + Surface	1110.35^a	545.66^b	534.71^a	338.84^b	141.46^b
Surface	1110.35^a	241.46^b	189.90^b	38.10^b	0.00^b
CV (%)			34.21		
	Dry phytomass of the aerial portion (g pot^{-1})				
Incorporated	6.28^a	4.28^a	4.13^a	3.99^a	3.96^a
Incorporated + Surface	6.28^a	3.84^a	3.20^a	2.41^b	0.74^b
Surface	6.28^a	1.60^b	1.21^b	0.12^c	0.00^b
CV (%)			24.22		

Means followed by the same letter within the column did not differ statistically by the Tukey's test at 5% probability; CV – coefficient of variation.

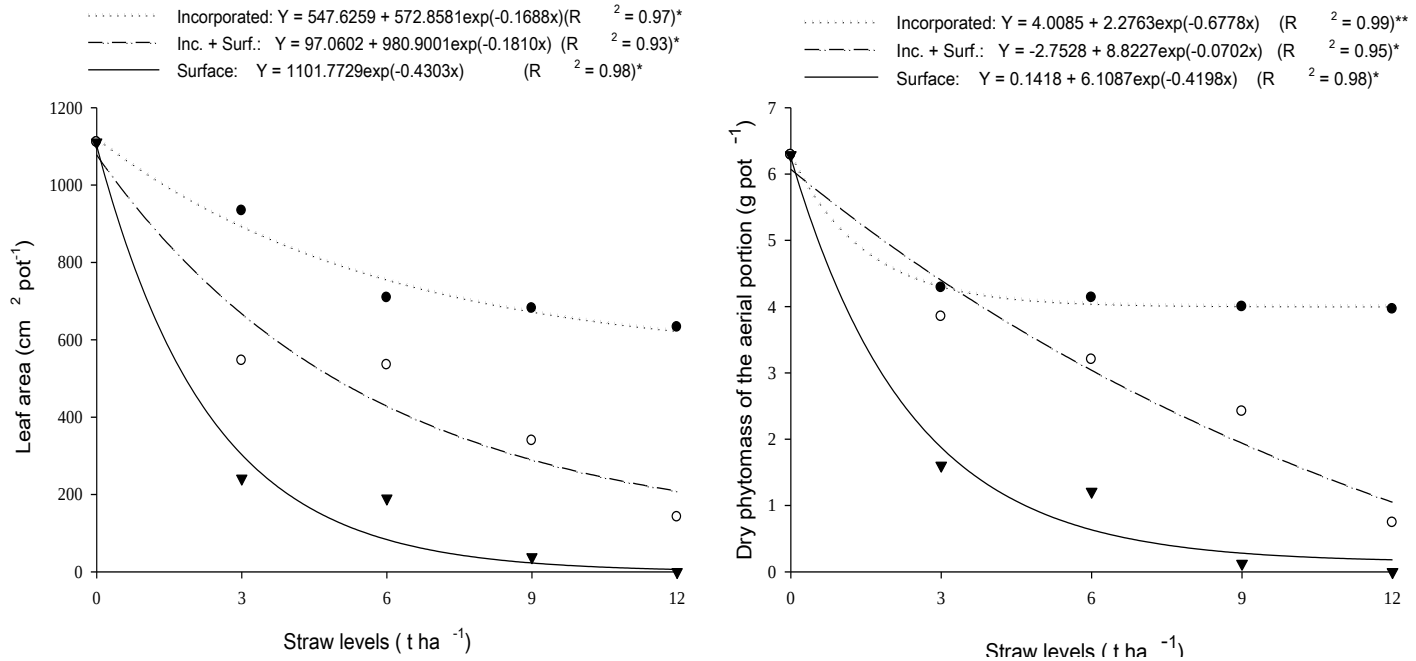

Figure 2. Leaf area and dry phytomass of the aerial portion of *Bidens subalterna* as a function of management method and straw levels of *Urochloa brizantha*. ** and * significant at 1 and 5%, respectively.

Surface management expressively reduced variables of LF and DPAPP even at the initial quantities of straw, with 3 t ha^{-1} of straw being enough to reduce these variables in 78.25 and 74.49%, respectively, compared with the control treatment (0 t ha^{-1}) (Figure 2). Gimenes et al. (2011) demonstrated that 10 t ha^{-1} of phytomass of *B. decumbens* after 60 days of emergence, were enough to reduce more than 80% of the leaf area in *Digitaria horizontalis* and *C. echinatus.* Pacheco et al. (2013) also reported efficient results with 4 t ha^{-1} of *U. ruziziensis* in soil surface, resulting in a reduction of LF and DPAPP of *B. pilosa* in 71.24 and 76.66%, respectively.

Management methods of *U. brizantha* also promoted reductions in variables of DPR and VR, with emphasis on surface management with the higher reduction in the radicular system of *B. subalterna* (Table 4). These results may be explained by the decrease in the number of germinated plants, as well as by the delay in plantlets'

Table 4. Dry phytomass of roots and root's volume of *Bidens subalterna* as a function of management and straw levels of *Urochloa brizantha*.

Management method	Straw levels (t ha^{-1})				
	0	3	6	9	12
	Dry phytomass of roots (g pot^{-1})				
Incorporated	16.38^{a}	13.11^{a}	12.39^{a}	11.73^{a}	11.08^{a}
Incorporated + Surface	16.38^{a}	9.36^{a}	7.84^{b}	3.66^{b}	1.66^{b}
Surface	16.38^{a}	4.39^{b}	2.76^{b}	0.12^{b}	0.00^{b}
CV (%)			26.12		
	Volume of roots (cm^{3} pot^{-1})				
Incorporated	50.05^{a}	36.11^{a}	35.33^{a}	33.22^{a}	32.38^{a}
Incorporated + Surface	50.05^{a}	20.00^{b}	18.33^{b}	14.33^{b}	2.66^{b}
Surface	50.05^{a}	13.33^{b}	5.66^{c}	1.33^{c}	0.00^{b}
CV (%)			34.19		

Means followed by the same letter within the column did not differ statistically by the Tukey's test at 5% probability; CV – coefficient of variation.

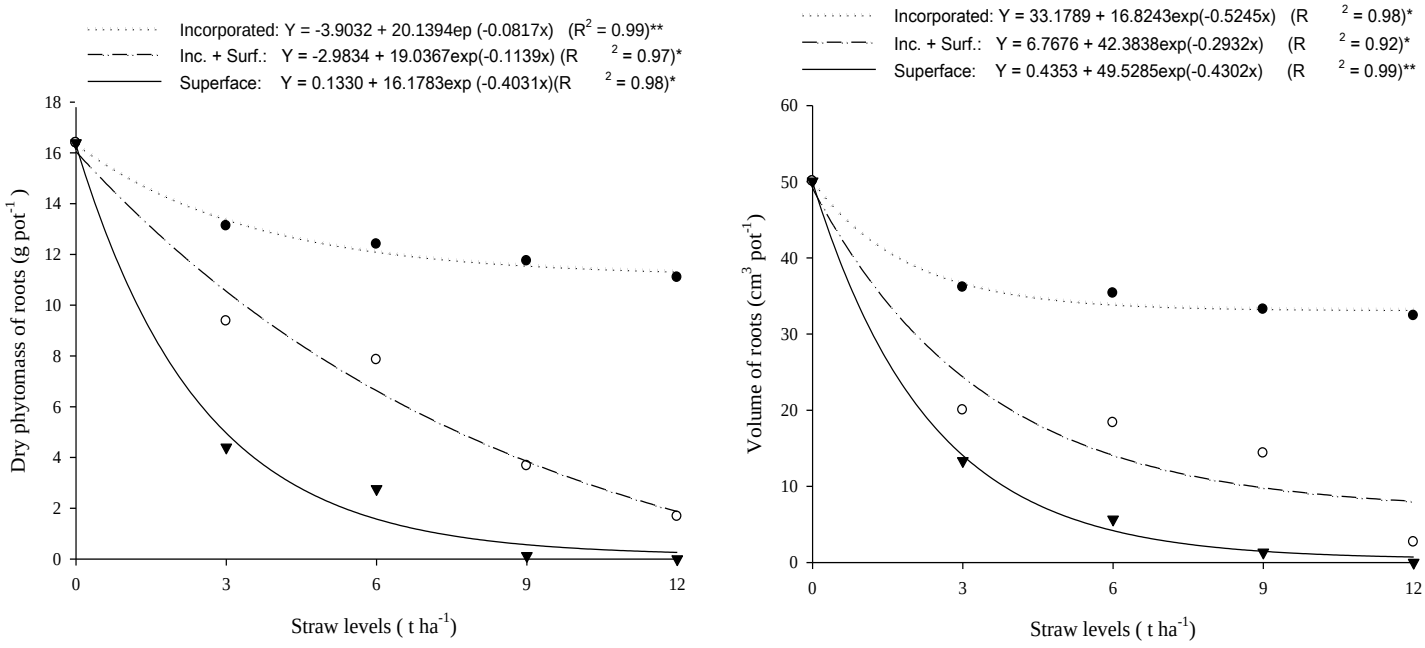

Figure 3. Dry phytomass of roots and volume of roots of *Bidens subaltern* as a function of management method and straw levels of *Urochloa brizantha*. ** and * significant at 1 and 5%, respectively.

emergence. In such way, the lower development of the radicular system may result in reduction of the competitive capacity of the infesting plants, by the reduction of the nutrient and water absorption capability, especially under conditions of water stress (Pacheco et al., 2013).

Management methods of *U. Brizantha* showed a decreasing exponential behavior for variables of DPR and VR, with the exception for the method of Incorporated + Surface, with a linear reduction (Figure 3). These results reveal that when using 3 t ha^{-1} of *U. Brizantha* straw in the soils surface, a reduction of 83.14% and 88.68% in DPR and VR respectively, is expected, when compared to the control treatment (0 t ha^{-1}). In a similar study performed by Moraes et al. 2011), utilizing rapeseed (*Brassica napus*), radish (*Raphanus sativus*), arrowleaf clover (*Trifolium vesiculosum*) and Italian ryegrass (*Lolium multiflorum*), (the authors

demonstrated that the radicular system of *B. pilosa* plants was reduced with the increment of phytomass levels of these species in the soil surface.

From the results, it is possible to see that the surface management method of *U. Brizantha* shows the highest efficiency to control *B. Subalterna*. A quantity of 3 t ha^{-1} of *U. Brizantha* in surface management is enough to promote a significant reduction in the emergence and growth of *B. Subalterna*.

The incorporated + surface management method of *U. Brizantha* results in a reduction of more than 50% of the germination and growth, when applied in quantities of more than 6 t ha^{-1} of straw. Incorporated management of *U. Brizantha* shows efficiency reducing 50% of *B. Subalterna* emergence when used at only 6 t ha^{-1} of straw.

Conflict of Interests

The authors have not declared any conflict of interest.

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academicJournals

Vol. 10(46), pp. 4222-4230, 12 November, 2015
DOI: 10.5897/AJAR2015. 10329
Article Number: F44548A56155
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Genetic parameters in *Stylosanthes* using different statistical methods

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Received 21 August, 2015; Accepted 25 September, 2015

Genetic parameters in *Stylosanthes* accessions were estimated through (ANOVA) and REML/BLUP (Restricted Maximum Likelihood /Best Linear Unbiased Prediction), to compare them for the genetic values in order to select superior accessions. Twenty five genotypes were evaluated in two environments in a randomized blocks experimental design with four replications. The genetic parameters were estimated for 12 descriptors by the two methods. Both methods indicating that the accessions presented genetic variability for the descriptors, but, the ANOVA and the REML/BLUP presented divergent values for the unbalanced data and for descriptors with high environmental influence, as it happens with mass descriptors, what, in turn, indicates that the method REML/BLUP leads to a more accurate predictions and allows for the selection of *Stylosanthes* accessions. It allows the inclusion of characters, even if they present heterogeneity of the residual variance. The indirect selection may be used, because the primary stem length descriptor revealed itself as a good option due to the correlation with the total dry mass. The accessions BGF-016 and BGF-015 are the most promising ones to be taken up by a Stylosanthes plant breeding program for fodder in the Brazilian Semiarid region.

Key words: Semiarid, forages, breeding.

INTRODUCTION

The *Stylosanthes* genus calls our attention due to its number of species with extraordinary fodder potential with excellent nutritional quality and their easy adaptation to different environmental conditions. Even though Brazil is the centre of diversity of these species, Australia, African and Asian countries have released cultivars with higher impact on the production system than the releases made in Brazil (Resende et al., 2006). In Brazil the most used cultivar is the Estilosantes Campo Grande, which belongs to the species *Stylosanthes capitata* (Embrapa, 2007), however this variety was not developed for the Semiarid. A survey done in the herbariums from the universities of Bahia and Embrapa Genetic Resources and Biotechnology indicated the existence of different species in the State of Bahia, revealing the great diversity for the genus (Costa, 2006), and thus demonstrating that there is a great potential for the study of this germplasm, so far quite neglected, in order to develop cultivars for the

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Brazilian Semiarid.

The procedures normally used for the selection of fodder plants is done by the evaluation of accessions and the selection of the superior ones (Chakraborty, 2004). However, this selection is more efficient when it is based on the genetic values estimate and in association with variables of economic importance (Assis et al., 2010). Due to the lack of *Stylosanthes* cultivars developed for the region, it is desirable to establish a plant breeding program for this fodder plant, and thus the genetic parameters estimation is needed to identify the best strategies to be used in the selection of *Stylosanthes* individuals which are tolerant to droughts, has high fodder productivity and high nutritional value.

For a long time, the variance analysis (ANOVA) method has been the most used in plant breeding to estimate variance components, but, in situations where there are unbalanced data, environmental variance, variance heterogeneity among experiments, competition between genotypes due to the difference in aggressively and sensibility of different genetic materials (Resende, 2007) its use is limited.

This study had the objective to estimate the genetic parameters through the statistic models (ANOVA and REML/BLUP), to select the best method of identifying the most prominent accessions in the selection for superior materials for starting a breeding program of this fodder in the Semiarid of Bahia, which is also very important for in the Brazilian semiarid.

MATERIALS AND METHODS

Two field experiments were carried out between July 2012 and January 2013. The first one was done at the experimental station Horto Florestal (12° 16'087''S; 38° 56'346"W; 243 m) which belongs to the State University of Feira de Santana - UEFS in Feira de Santana, BA (A_1) and the other one at the experimental field of the Technology and Social Sciences Department (09° 24' 50"S; 40°30' 10"W; 368 m) of the University of Bahia - UNEB in Juazeiro, BA (A_2).

For the experimental set up, 25 accessions of *Stylosanthes* spp., from collections acquired between the years of 2008-2011 in three semiarid regions in the state of Bahia (Sisaleira, the Middle Lower São Francisco River Basin and the micro region of Feira de Santana) were used along with a commercial variety, Estilosantes Campo Grande, as a control (Table 1).The fruits of each accession obtained in the collecting expeditions were sent to the Evolutive Ecology Laboratory - LEE from the Estate University of Feira de Santana, Feira de Santana, BA for manual processing, consisting of the removal of loment by friction using rubber devices of four mm of thickness. The seeds from each accession were put in labelled envelopes, and kept in air tight bottles with silica gel as a humidity indicator and were kept in Lab conditions (temperature of 25 °C). Between the months of April and May 2012, 54 seeds of each accession were mechanically scarified with wood sandpaper n° 150 and sown in polyethylene tubes with dimensions of 6 x 20 cm filled with the commercial substrate. They were kept under greenhouse conditions, and watered twice daily. The seedlings in both experiments were produced in green house of the State University of Feira de Santana (UEFS) with the purpose of standardizing the procedures for obtaining the seedlings and after three months they were transplanted to the field.

The soil, in both experiments, was prepared by disk harrow and the marking of the plots were marked and, then pitting was performed with the aid of a manual digger. A complete randomized block design with four replications and four plants per useful area of each plot was used, with a total of 16 plants per treatment and the spacing of 3.0 m between rows and 0.8 m between plants was adopted. There was a basal fertilization, in both trials, with the application of 30 kg ha^{-1} P, 30 kg ha^{-1} K and 20 kg N, and after around 35 to 40 days of the transplanting, topdressing fertilization with 30 kg ha^{-1} K and 20 kg ha^{-1}N was used. Both experiments were irrigated using the drip irrigation system whenever necessary.

The evaluations were performed between four and five months after transplanting. For morphological descriptors the measurements were made on all the plants of useful area from each treatment. They were: PD - Plant Diameter (mm) measured at the base of the plant; PH – Plant Height (cm), measured from the ground level to the highest leaf on the stem; PBL - Primary branch length (cm), its measurement was taken from the insertion of the primary branch at the bottom of the stem up to the last leaf; PAL-Length of the central axis of the plants (cm), measured from the ground level to the highest leaf of the main stem; NS - Number of stems (units), counting of the number of stems from ground level to the last stem inserted in the main axis; CLL - central leaflet length (mm) and CLW - width of the central leaflet (mm), measured in the longitudinal part of the central leaflet of the third definitive leaf of the plant, inserted in the first ten centimeters of the central axis; SLL - Side leaflet length (mm) and LLW- Lateral width of leaves (mm), measured in the longitudinal and latitudinal part, respectively, of the right of the third leaf stage of plant, inserted in the first ten centimeters from the central axis lateral leaflet. The TDM descriptors (total dry mass - g), DSM (dry stalk mass - g) and DLM (dry leaf mass - g) were evaluated in one plant per plot.

Initially the individual variance analysis was done for each place to check if the accessions differed significantly among themselves. Then, the homogeneity of variance was tested (F maximum - ratio between the largest and smallest mean squared residual for each descriptor) following the test of Hartley (1950) recommended by Cruz et al. (2004), excluding descriptors that presented F maximum superior to 7.

In this study, each accession was formed by a group of individuals which represented a given population, and therefore, the genotypes effects were regarded as random and the environment effects as fixed using the following model (Cruz et al., 2004): Yijk = μ + (B / A)j*k* + G*i* + A*j* + GA*ij* + E*ijk* where: Y*ijk* = observation in the *k*-the block, reported in the *i*-the genotype and *j*-the environment; μ is the general mean of the test; (B/A) the block effect *k* within the environment *j*; Gi = effect of genotype *i*; Aj = effect of environment *j*; GAij = effect of the interaction between genotype *i* and environment *j*; and Eijk = random error associated with the *ijk* observation.

From the joint analysis, the genetic parameters were estimated (Cruz et al., 2004), thus the decomposition of the mean squares estimate of the interaction in simple and complex parts was measured (Cruz and Castoldi, 1991).

Genetic parameters were also estimated using the methodology of mixed models REML/BLUP (Restricted Maximum Likelihood /Best Linear Unbiased Prediction) to observe possible differences from ANOVA (Resende, 2007). In this analysis each individual has an individual genotypic value allowing for a more accurate estimate of the genetic value and more adequate ordering of superior individuals, leading to a better selection (Martinez et al., 2011).

In the analysis of mixed models with unbalanced data such as the present one, the effects of the model were tested via LRT (likelihood ratio test) over the F test which is used in the analysis of variance and in replacement of the framework of ANOVA a similar framework called analysis of *deviance* (ANADEV) was performed. All these effects along with the estimates of the variance components and genetic parameters were obtained by the

Table 1. Origin and description of *Stylosanthes* accessions stored in BGF/UEFS.

Acession	City	Geographic coordinates	Year	Species
BGF 08-001	Araci	11°36'20"S e 39°09'52.1''O	2008	*S. viscosa* (L.) Sw.
BGF 08-002	Araci	11°27'24.5''S e 39°26'43.6''O	2008	*S. scabra* Vogel
BGF 08-003	Ichu	11°42'24.2''S e 39°09'59.4''O	2008	*S. scabra* Vogel
BGF 08-004	Serrinha	11°40'29.9''S e 39°04'38.1''O	2008	*S. scabra* Vogel
BGF 08-005	Serrinha	11°47'46.8''S e 38°53'24.5''O	2008	*S. scabra* Vogel
BGF 08-006	Serrinha	11°26'36.4''S e 39°12'00.8''O	2008	*S. scabra* Vogel
BGF 08-007	Valente	11°22'13.0''S e 39°17'28.1''O	2008	*S. scabra* Vogel
BGF 08-010	Nova Soure	10°29'36.7''S e 39°20'44.0''O	2008	*S. scabra* Vogel
BGF 08-011	Valente	11°27'12.6''S e 39°25'24''O	2008	*S. scabra* Vogel
BGF 08-012	S. Domingos	11°27'27.1''S e 39°32'46.4''O	2008	*S. scabra* Vogel
BGF 08-014	Tucano	11°01'59''S e 38°48'17''O	2008	*S. scabra* Vogel
BGF 08-015	Queimadas	10°54'40''S e 39°12'17''O	2008	*S. scabra* Vogel
BGF 08-016	Queimadas	10°54'40''S e 39°12'17''O	2008	*S. scabra* Vogel
BGF 08-017	Queimadas	11°19'26''S e 39°49'13''O	2008	*S. scabra* Vogel
BGF 08-018	Candeal	11°49'49.8''S e 39°07'08.5''O	2008	*S. scabra* Vogel
BGF 08-019	Cansanção	09°50'78.7'' e 39°28'05.1''O	2008	*S. scabra* Vogel
BGF 08-020	Candeal	11°49'49.8''S e 39°07'08.5''O	2008	*S. scabra* Vogel
BGF 08-021	Casa Nova	09°16'50.5''S e 41°29'15.5''O	2008	*S. humilis* Kunth
BGF 08-023	Casa Nova	09°21'36''S e 41°47'17.5''O	2008	*S. humilis* Kunth
BGF 08-024	C. A. Lourdes	09°35'15.1''S e 42°54'02.1''O	2008	*S. capitata* Vogel
BGF 08-026	Casa Nova	09°10'33.3''S e 40°50'17.1''O	2008	*S. viscosa* (L.) Sw.
BGF 08-029	Canudos	09°54'29.9''S e 39°03'17.2''O	2008	*S. viscosa* (L.) Sw.
BGF 08-032	Sento Sé	10°09'11.3''S e 41°39'01.1''O	2008	*S. scabra* Vogel
BGF 08-033	Sento Sé	10°10'22.6''S e 41°58'24.0''O	2008	*S. humilis* Kunth
BGF 08-034	F. Santana	12°09'719''S e 38°57'696''O	2011	*S. scabra* Vogel

BGF - Number in the Bank of Germplasm of Forage.

SELEGEN-REML/BLUP software (model 23 - selection based on various replications, taking into consideration one or more plants per plot, evaluation in more than one location and experiments in completely randomized blocks) .

According to Resende (2007) using the following statistical model $y = Xr + Zg + Wi + e$, where: y= data vector; r is the vector of the effects assumed to be fixed (repetition + overall mean); g is the vector of genotypic effects, i is the effects vector of the genotype x environment interaction, where: e is the vector of errors or residues (random), where: X, Z and W are the incidence matrices for the referred effects. Based on the genetic parameters it was possible to estimate the selection genetic gain calculated by the formulae $G_{si(j)} = h^2_{g_i} * ds_{i(j)}$ where $G_{si(j)}$ is the gain in environment *i*, with selection based on environment *j*; *dsi* (*j*) is the selection differential in the environment *i* in which the individuals selected have the best performance in the environment *j*; and h^2_g is the heritability of the character in the environment *i*, adopting a selection intensity of 20% for each analysis. To estimate the gain selection percentage it was used the following formulae: $G_s\% = (G_s / \bar{x}) * 100$.

RESULTS AND DISCUSSION

The joint analysis, using ANOVA, showed that the mean squares of the effects of genotypes and environments presented high (P≤0.01) for almost all variables except lateral leaflet width (LLW), for environment (P≤0.05). The effects of G x E interaction were also great (P≤0.01), except plant diameter (PD) and lateral leaflet width (LLW) (Table 2). When considering the values between the two environments, most of the descriptors presented F maximum below seven (Table 2), except the total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM) descriptors and the genetic variances (V_g) were higher in comparison to variances of the interaction GxE (V_{gxe}) and environmental variance (V_e) for almost all descriptors except for side leaflet length (SLL) (Table 3) indicating genetic diversity among the accessions evaluated and also demonstrated that the genetic variability among the accessions is partially due to differences among individuals within each accession and, also, due to characteristics of different species, since the 25 treatments comprised four species (*Stylosanthes scabra, Stylosanthes viscosa, Stylosanthes capitata* and *Stylosanthes humilis*).

The relative coefficient of variation (CV_r) was almost equal to 1.0 side leaflet length (SLL) and higher than 1.0 for the remaining variables. As for broad-sense

Table 2. Joint variance analysis in genotypes of *Stylosanthes.*

SV	DF	Means square								
		PD	PH	PBL	PAL	NS	CLL	CLW	SLL	LLW
G	25	56.20**	994.66**	2124.10**	883.27**	108.36**	127.72**	14.86**	45.01**	6.99**
E	1	13.51**	790.35**	4230.96**	1378.72**	58.55**	272.69**	9.85**	157.36**	3.76*
GxE	25	2.92^{NS}	151.39**	128.58**	156.36**	14.05**	11.81**	0.99 ^{NS}	15.44**	2.25**
Res.	150	2.28	36.75	51.97	42.32	4.48	4.85	0.69	3.81	0.62
CV	-	12.95	13.60	11.58	17.72	12.98	15.6	12.54	17.55	16.48
M	-	11.66	44.57	62.24	36.72	16.31	14.11	6.61	11.11	4.79
Fmax	-	1.71	1.53	1.09	1.87	1.02	2.94	1.69	1.45	1.13
Excluded descriptors										
Fmax		**TDM**		**DSM**			**DLM**			
		8.64		16.00			8.17			

G - Genotype; E - Environment; GxE - interaction Genotype x Environment; CV - Coefficient of Variation; M - Mean; Fmax - relation between the biggest and smallest square mean of the residue. SV - Source of variation; DF - Degree of freedom; PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central Leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. **, * = significant at 1 and 5% of probability respectively; ^{NS} = not significant.

Table 3. Estimates (Est) of genetic parameters using ANOVA in accessions of *Stylosanthes.*

Est	PD	PH	PBL	PAL	NS	CLL	CLW	SLL	LLW
V_g	6.66	105.41	249.44	90.86	11.79	14.49	1.74	3.70	0.59
V_e	0.25	31.35	61.30	32.11	0.55	7.71	0.31	3.81	0.12
V_{gxa}	0.08	14.33	9.58	14.26	1.20	0.87	0.04	1.45	0.20
V_f	6.99	151.09	320.32	137.23	13.54	23.07	2.09	8.96	0.91
h^2_g	0.95	0.70	0.78	0.66	0.87	0.63	0.83	0.41	0.64
r_{12}	0.91	0.70	0.84	0.63	0.56	0.83	0.75	0.55	0.45
r_g	0.99	0.88	0.96	0.86	0.91	0.94	0.98	0.72	0.74
CV_g	22.13	23.04	25.38	25.96	21.05	26.98	19.93	17.30	16.07
CV_e	4.26	12.56	12.58	15.43	4.56	19.68	8.46	17.57	7.34
CV_r	5.19	1.83	2.02	1.68	4.62	1.37	2.36	0.98	2.19
%C	-	17.31	1.55	7.41	45.91	7.28	-	12.89	15.56
%S	-	82.69	98.45	92.59	54.09	92.72	-	87.11	84.44

V_g - Genotypic Variance; V_e - Residual Variance; V_{gxa} - genotype x environment interaction variance; V_f - individual phenotypic variance; h^2_g - heritability of the total genotypic effects; r_{12} - genetic correlation he genotypes in both environments; r_g - genetic correlation between both environments; CV_g - genotypic variation coefficient ; CV_e - residual variation coefficient; CV_r - relative variation coefficient; *%C* - Part of the complex interaction; *%S* - part of the simple interaction. PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. (-) - Descriptor not significant at 5% of probability by F test.

heritability (h^2_g), the values ranged from 0.41 to 0.95. The results also showed that all characters expressed interaction of the simple type, except for central leaflet width (CLW) and plant diameter (PD) that were not significant and, therefore, the correlation between environments (r_{12}) were relatively high (Table 3).

On the other hand, the values of ANADEV (Analysis of *Deviance*) show that seven descriptors plant diameter (PD), plant height (PH), primary branch length (PBL), length of the central axis of the plants (PAL), number of stems (NS), central leaflet length (CLL) and central leaflet width (CLW) presented effects of genetic variances (V_g) and broad-sense heritability (h^2_g) high (P≤0.01) and the heritability ranged from 0.59 to 0.83. Regarding the descriptors side leaflet length (SLL), lateral leaflet width (LLW), total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM), the effects of genotypes were not significant via the LRT test. For the interaction effects of GxE and for the coefficient of determination (r^2int) the values of the LRT test for all descriptors were high (P≤0.01), except for plant diameter (PD) and central leaflet width (CLW) (Tables 4 and 5).

Table 4. Analysis of Deviance (ANADEV) evaluated in *Stylosanthes.*

	Descriptors					
Effects	**PD**	**PH**	**PBL**	**PAL**	**NS**	**CLL**
Genotype	35.46**	15.58**	29.80**	13.89**	19.39**	25.62**
G x E	1.61^{NS}	25.56**	9.68**	21.60**	17.15**	9.57**
	Descriptors					
Effects	**CLW**	**SLL**	**LLW**	**TDM**	**DSM**	**DLM**
Genotype	28.94**	3.28^{NS}	2.58^{NS}	2.24^{NS}	1.419^{NS}	2.08^{NS}
G x E	1.36^{NS}	28.99**	23.10**	47.23**	46.21**	26.93**

PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. Table chi-square - 3.84 (*) and 6.63 (**) for the levels of significance of 5 and 1%, respectively; ^{NS} = not significant at the level of 5% probability.

Table 5. Estimates (Est) of genetic parameters by REML/BLUP in accessions of S*tylosanthes*

Est	**PD**	**PH**	**PBL**	**PAL**	**NS**	**CLL**	**CLW**	**SLL**	**LLW**	**TDM**	**DSM**	**DLM**
V_g	10.43	126.26	314.59	107.73	14.48	22.41	2.93	4.48	0.69	26513.08	7232.43	2489.26
V_e	0.26	31.45	21.02	31.14	2.68	2.01	0.09	4.87	0.71	46962.39	17515.12	4332.75
V_{gxa}	1.99	36.99	51.51	42.52	4.30	4.90	0.71	3.83	0.63	33216.22	12664.96	5195.29
V_f	12.68	194.70	387.13	181.40	21.47	29.32	3.72	13.18	2.03	106691.70	37412.51	12017.29
h^2_g	0,83	0.65	0.81	0.59	0.68	0.76	0.79	0.34	0.34	0.25	0.19	0.21
h^2_{mg}	0.96	0.86	0.95	0.83	0.88	0.93	0.96	0.60	0.61	0.47	0.40	0.45
r^2_{int}	0.02	0.16	0.05	0.17	0.13	0.07	0.02	0.37	0.40	0.44	0.47	0.36
r_{gg}	0.98	0.92	0.97	0.91	0.94	0.97	0.98	0.78	0.78	0.69	0.63	0.67
r_g	0.98	0.80	0.94	0.78	0.84	0.92	0.97	0.48	0.49	0.36	0.29	0.37
CV_g	28.96	25.40	29.35	28.46	23.70	33.99	25.95	19.21	17.38	38.30	44.90	33.97
CV_e	12.65	13.75	11.88	17.88	12.91	15.90	12.77	17.78	16.62	42.87	59.41	49.07
CV_r	2.29	1.85	2.47	1.59	1.84	2.14	2.03	1.08	1.05	0.89	0.76	0.69
m_g	11.15	44.24	60.43	36.47	16.06	13.93	6.59	11.02	4.78	425.11	189.42	146.88

V_g - Genotypic Variance; V_e - Residual Variance; V_{gxa} - genotype x environment interaction variance; V_f - individual phenotypic variance; h^2_g - coefficient of heritability in the broad sense; h^2_{mg} -mean genotype heritability; r^2int - coefficient of the determination of interaction G x E; r_{gg} - genetic correlation he genotypes in both environments; r_g - genetic correlation between both environments; CV_g - genotypic variation coefficient ; CV_e - residual variation coefficient; CV_r - relative variation coefficient; m_g - overall mean; m_1 - overall mean of environment A_1; m_2 -overall mean of environment A_2; PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass; m_g - mean of both environments.

When comparing values for the variance components obtained by ANOVA with the ones acquired by REML/BLUP, there was divergence in value, once for the ANOVA method the central leaflet length (CLL) and lateral leaflet width (LLW) were high (P≤0.01), whereas for the REML/BLUP these descriptors showed no significant differences. The mean values of the descriptors that were possible to assess through ANOVA were higher when compared to those obtained by REML/BLUP, thus indicating elevation of means by ANOVA in the joint analysis. As such, this may induce a wrong selection of the best individuals.

It is noteworthy that the descriptors mass total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM), the most important ones from the forage point of view, had to be excluded from the joint analysis by ANOVA due to this method`s limitations on heterogeneity of residual variances (Cruz et al., 2004), thus preventing the evaluation of their genetic parameters, the potential in relation to the genetic gains and consequently the indication of possible accessions for the selection of superior materials for the forage production.

Therefore, the methodology REML/BLUP, despite being a method little used for annual plants, can be used to detect variability among accessions in different species of *Stylosanthes* particularly for descriptors that exhibit heterogeneity of variance and unbalanced data, thus showing, that these factors are not limiting to estimate genetic predictions for descriptors that have a strong environmental influence. This reassures this methodology as essential for breeding programs of forages that need to be grown in different environments, as it is the case for *Stylosanthes* in the Semiarid of Bahia. The methodology of mixed models was also used efficiently to quantify the variation and estimate genetic predictions in some annual species (Borges et al., 2010; Neto et al., 2013; Resende et al., 2006).

It is also worth mentioning that apart from mass descriptors, other relevant traits in *Stylosanthes* need to be considered since in a breeding program the accessions selected by genetic merits of mass production should also be assessed in the association with grasses, produce enough seeds and present tolerance to diseases such as anthracnose – *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. (Resende et al., 2006), although not yet reported in the state of Bahia. On the other hand, the mass descriptors were not significant among the accessions evaluated using REML/BLUP, but the broad-sense heritability and mean of dry matter found in this study was similar to the values found for *Stylosanthes* (Resende et al., 2006; Santana et al., 2013).

The CV_g% ranged from 17.38 to 44.90%, with the lowest value observed for the lateral leaflet width (LLW) descriptor, while the highest variation was for dry stalk mass (DSM); the environment coefficient of variation (CV_e) were superior for all descriptors, except for the mass ones; the relative variation coefficient (CV_r), were above a unit for all descriptors, except for mass descriptors (Table 5). Regarding the mean heritability of the genotype (h^2_{mg}), the values obtained were high for all the descriptors, although lower for mass descriptors. A very high precision, the selective accuracy (r_{gg}, Resende and Duarte, 2007), was observed for most descriptors of the plant and only two of them (side leaflet length -SLL and lateral leaflet width -LLW) presented only high accuracy, however, the mass descriptors showed moderate accuracy.

The genetic correlations (r_g) between the accessions and the environments showed that the plant diameter (PD), plant height (PH), primary branch length (PBL), length of the central axis of the plants (PAL), number of stems (NS), central leaflet length (CLL) and central leaflet width (CLW) descriptors showed higher coefficients than 0.78 and plant diameter (PD), central leaflet width (CLW) and primary branch length (PBL) were highlighted. On the other hand, the characters side leaflet length (SLL), lateral leaflet width (LLW), total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM) displayed genetic correlation between both environments(r_g) values below 0.50 but the lowest levels were observed for total dry mass (TDM), dry stalk mass (DSM) and dry leaf mass (DLM) (Table 5).

From the values of genetic variances, heritability and relative coefficient of variation obtained in this work for mass descriptors, it can be inferred that the genotypes demonstrated potential for obtaining genetic progress with the selection in the environments of study. By comparing the results found here with other annual forages commonly used in the Brazilian Semiarid region, the potential of the genus *Stylosanthes* is confirmed (Assis et al., 2008, 2010; Cunha and Lima, 2010).

Moreover, the values of heritability in the broad sense were also overestimated by the method ANOVA, because nearly all descriptors were superior to the REML/BLUP values, except for the central leaflet length (CLL), confirming that the method of ANOVA for more complex situations leads to inaccurate estimates of the variance components and consequently inaccurate predictions of breeding values (Resende, 2007).

The behavior of the variance components when decomposed to G x E interaction by the two methods (ANOVA and REML/BLUP) were similar to some descriptors except side leaflet length (SLL) and lateral leaflet width (LLW) that showed simple interaction type by ANOVA and complex interaction by REML/BLUP. Then, the choice of the best accessions should happen through the REML/BLUP, because it was more viable for descriptors that exhibit different behavior in different environments (side leaflet length - SLL), lateral leaflet width - LLW, total dry mass - TDM, dry stalk mass - DSM and dry leaf mass - DLM). This method facilitates the evaluation and selection in this situation, similar to what happens with other forages (Cargnin et al., 2006; Luz et al., 2010; Pereira et al., 2010) and studies using this

Table 6. Estimate of simple correlations in *Stylosanthes* accessions.

Desc.	PH	PBL	PAL	NS	CLL	CLW	SLL	LLW	TDM	DSM	DLM
DP	0.53**	0.59**	0.49**	0.55**	0.25**	0.21**	0.22**	0.16*	0.33**	0.29**	0.30**
PH		0.60**	0.96**	0.84**	0.55**	0.07^{NS}	0.11^{NS}	0.01^{NS}	0.14^{NS}	0.13^{NS}	0.11^{NS}
PBL			0.56**	0.63**	0.55**	0.36**	0.48**	0.29**	0.68**	0.68**	0.57**
PAL				0.83**	0.14^{NS}	0.04^{NS}	0.06^{NS}	-0.01^{NS}	0.10^{NS}	0.09^{NS}	0.09^{NS}
NS					0.38**	0.22**	0.30**	0.17*	0.27**	0.25**	0.24**
CLL						0.84**	0.93**	0.75**	0.46**	0.45**	0.42**
CLW							0.81**	0.89**	0.26**	0.25**	0.25**
SLL								0.85**	0.43**	0.42**	0.40**
LLW									0.22**	0.22**	0.22**
TDM										0.99**	0.95**
DSM											0.90**

PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. **, * = significant at 1% and 5% of probability respectively; ^{NS} =not significant.

methodology in selecting genotypes (Arantes et al., 2013; Bastos et al., 2007; Zeni-Neto et al., 2008; Verardi et al., 2009).

The estimate of the simple correlations revealed that the majority of descriptors showed significant correlations (P≤0.01), but of moderate to low magnitude from one character to the other, except for plant height (PH) and length of the central axis of the plants (PAL) that showed no significance with the majority of the characters. For the primary branch length (PBL) it was observed a positive association from moderate to low intensity for most descriptors, however, there was a significant and moderate correlation between PBL with the mass descriptors (Table 6).

Therefore, a possibility to improve the selection of genotypes with efficiency for higher dry matter would be through indirect selection. Moreover, indirect selection can lead to superior gains over direct selection, when the auxiliary character displays heritability above the main one, a positive correlation and of high magnitude (Falconer and Mackay, 1996). Therefore, the indirect selection can be used, once the primary branch length (PBL) is a good option for selection due to the correlation with total dry mass. This is a feature of great significance to the breeding of *Stylosanthes* in the Brazilian Semiarid region with the purpose of increasing the total dry mass, since there is a great variation in the environmental conditions.

The selection of the best five accessions by ANOVA generated total gains ranging from the minimum and the maximum equal to 20.81%, and total dry mass (TDM) presented the smallest increase, while the largest was observed in central leaflet length (CLL). By the REML/BLUP method the amplitude of variation was approximately 38.02%, where the central leaflet length (CLL) had the highest and the lowest value was for dry leaf mass (DLM). As for primary branch length (PBL), it was observed that the two methods presented similar high values to gain selection and the estimation of genetic gains by the two methods showed that the BGF-016 and BGF-015 accessions were the top among the materials evaluated (Table 7).

Thus, the REML/BLUP method allowed the elimination of accessions that had negative genotypic effects (values below the overall mean of the experiment), and this increased the probability of selecting superior individuals; it provided the estimate for the number of individuals to be selected by accession for each variable and also enabled the identification of the plots of the best individuals and the number to be selected in each one (Martinez et al., 2011; Resende et al., 2006).

Besides all these advantages presented here, it is known that the REML/BLUP method is little used for annual species in the area of plant

Table 7. Overall genetic gain (Gt %) and gain of selection (G_S %) by genotype in *Stylosanthes.*

PD				PH				PBL				PAL			
Anova		Blup		Anova		Blup		Anova		Blup		Anova		Blup	
G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*
002	31.94	002	36.73	016	26.89	016	32.59	024	44.74	016	45.80	016	31.00	016	33.36
010	24.77	010	29.42	026	24.39	026	29.64	016	36.24	024	44.50	026	26.13	026	28.20
026	24.77	026	29.42	029	20.12	024	25.56	015	26.68	015	34.80	015	23.08	024	26.13
012	18.90	012	23.40	015	19.76	029	24.56	010	19.93	010	26.70	029	21.07	015	24.97
016	17.68	016	22.19	014	16.98	015	24.13	014	19.31	014	26.00	024	19.41	029	22.83
Gt=23.61		Gt = 4.41		Gt=21.63		Gt =18.48		Gt=29.38		Gt=30.08		Gt =24.14		Gt= 9.26	

NS				CLL				CLW				SLL			
Anova		Blup		Anova		Blup		Anova		Blup		Anova		Blup	
G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*
016	43.85	16	41.10	T	79.56	T	105.00	T	69.82	T	71.41	T	41.92	T	30.49
015	42.73	15	40.05	024	59.20	024	65.20	024	68.56	024	65.06	024	34.98	024	18.62
026	28.59	26	27.18	015	21.43	015	31.00	034	16.07	034	17.50	015	12.40	015	11.38
T	16.80	14	15.45	026	15.45	026	22.60	004	6.15	002	4.94	026	5.72	026	5.39
014	15.68	24	15.45	016	14.20	016	20.90	002	4.39	006	4.37	016	5.72	016	5.39
Gt=29.53		Gt=21.52		Gt=37.97		Gt=39.97		Gt =33.00		Gt =27.74		Gt=20.15		Gt=8.08	

LLW				TDM				DSM				DLM			
Anova		Blup		Anova		Blup		Anova		Blup		Anova		Blup	
G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*	G	*Gs%*
024	57.32	T	23.90	016	25.05	016	31.08	016	27.65	016	28.79	15	29.19	015	28.62
T	51.31	024	22.50	015	24.88	015	30.88	015	26.54	015	27.68	16	25.77	016	25.43
034	12.83	034	7.66	024	19.15	011	12.90	024	26.03	024	11.00	11	14.90	011	15.26
006	10.96	006	5.74	011	9.57	014	10.08	011	7.55	011	8.86	24	12.85	014	7.79
011	8.42	011	4.08	014	7.17	024	9.04	020	7.54	020	8.85	14	6.91	020	5.74
Gt=28.17		Gt=7.11		Gt=17.16		Gt=10.00		Gt=19.06		Gt= 8.09		Gt=17.93		Gt=1.94	

PD - Plant Diameter; PH - Plant Height; PBL - Primary Branch Length; PAL - Length of the Central Axis of the Plants; NS - Number of Stems; CLL - Central leaflet length; CLW - Central leaflet Width; SLL - Side Leaflet length; LLW - Lateral Leaflet Width; TDM - Total Dry Mass; DSM - Dry Stalk Mass; DLM - Dry Leaf Mass. G - genotype; (-) absence of value; T - Control.

genetic resources, but the results presented here show that this methodology can be used routinely in the selection of accessions since it is easily handled, it estimates the genetic values with greater accuracy, increases the efficiency of selection, and consequently decreases the cost of

breeding programs to increase mass descriptors for the species of *Stylosanthes*.

Conclusions

The methods (ANOVA and REML/BLUP) presented divergent values, indicating that the REML/BLUP method estimated the genetic values with greater accuracy, increases the efficiency of selection and decreases the cost of breeding programs. The indirect selection may be used, because the primary stem length descriptor revealed itself as a good option due to the correlation with the total dry mass. The accessions BGF-016 and BGF-015 are the most promising ones to be taken up by a *Stylosanthes* plant breeding program for fodder in the Brazilian Semiarid region.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The Coordination of Improvement of Higher Education Personnel (CAPES) for the scholarship grant; the State University of Feira de Santana (UEFS) and the Department of Technology and Social Sciences (DTCS/UNEB), for the support in carrying out the field experiments.

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Vol. 10(46), pp. 4231-4240, 12 November, 2015
DOI: 10.5897/AJAR2013.8111
Article Number: B01F5A756157
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Impact of conservation agriculture on weed dynamics and maize grain yield in eastern Zambia

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Received 21 October, 2013; Accepted 9 October, 2015

Improved fallows of *Sesbania sesban* (Sesbania) have been known to improve soil physical and chemical properties and increase crop yield compared to traditional fallows. However, the effects of soil tillage practices after improved fallows on soil properties, weeds, labour and subsequent maize crop has not been assessed in Southern Africa. This study aimed to evaluate how tillage practices affect yield of maize and affect soil properties after two years of fallow and subsequent cropping phase. In this study, done at sites in eastern Zambia, maize yield from a two-year planted Sesbania, natural fallow, continuously fertilized and unfertilized maize were compared under conventional, flat till and zero tillage practices. A split plot experiment, with improved fallow systems in the main plot and the tillage practice in the subplot, was established at the sites. The results showed that the increases in grain yield under conventional tillage over zero tillage practice were 17.8 and 28.2% during 2000/2001 and 2001/2002 seasons, respectively, at Msekera. At Chadiza, the increases in grain yield under conventional tillage over zero tillage were 66.3 and 327.4% during 2000/2001 and 2001/2002 seasons, respectively. Greater maize yields were achieved under Sesbania planted fallows compared to the natural fallow and maize monoculture without fertilizer. Overall, zero tillage practice resulted in lower maize grain yield, higher bulk density, reduced water intake, higher weed infestation and high labour demand during weeding compared to conventional tillage.

Key words: Conventional tillage, flat till, grain yield, water intake, weeds, zero tillage.

INTRODUCTION

In traditional shifting and semi-permanent hand-hoe tillage systems, zero or minimum tillage operations are common among small-scale farmers. This is due to labour constraints and lack of draught power. Farmers in eastern Zambia are not exceptional as they are faced with problems of shortage of labour during the growing season. For this reason, maize, a staple crop, is planted on flat land after the vegetation or crop residues are gathered and burned. Most resource poor farmers practice this system, traditionally known as “Galauza”. In other cases, farmers leave fields fallow to natural vegetation for up to 5 years to restore soil fertility (Mafongoya and Bationo, 2006). After this period, farmers gather the natural or crop residues and make ridges or

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mounds using hand hoes by covering the mulch on which crops are planted. Labour shortage, especially at planting, make farmers to opt to plant on the flat.

At Msekera, maize in improved fallow trials is planted on the flat after the soil has been tilled and later ridges are made during weeding when the maize is 50 cm high (Mafongoya et al., 1999). In Zambia, there has been an increased interest in conservation farming because of its benefits in soil erosion control, soil moisture conservation, soil structure improvement and increased net return to farmers. There is little quantitative data, however, that is known about the effect of this tillage system on yield of maize in the farming system of eastern Zambia.

There is need, therefore, to come up with a practice that is both economical and practical under resource poor farmers' depleted soils where such fallows have a potential. Improved fallows of Sesbania have also been known to improve the chemical and physical conditions of the soil (Kwesiga et al., 2005) as well as suppressing weeds during fallow phase. Therefore, the objectives of this study were to (i) evaluate how tillage practices affect yield of maize, (ii) determine the effects of tillage practices on soil properties after two years of fallow and subsequent cropping phase.

MATERIALS AND METHODS

Site description

The study was conducted at two sites in eastern Zambia, at Msekera Research Station (32°34' E, 13°38' S, altitude 1030 m asl.) in Chipata and at Farmers' Training Center in Chadiza (32°30' E 14°03' S, altitude 1061 m asl.) between 1997 and 2001. The soils at Msekera in 0 to 20 cm top soil layer have 1% carbon content, 25 and 58% clay and sand, respectively, and a pH ($CaCl_2$) of 4.8. They are classified as Typic Haplustalfs (USDA, 1975) or Ferric luvisols (FAO, 1988). Msekera receives an average rainfall of 1092 mm per annum (unimodal, November to April). At Chadiza the soils (0 to 20 cm layer) have 0.4% carbon content, and a pH ($CaCl_2$) of 4.2, 6 and 71% clay and sand, respectively. They are classified as Typic Haplustults (USDA, 1975) or Ferric acrisol (FAO, 1988). Chadiza receives an average rainfall of 900 mm per annum (unimodal, November to April).

Experimental design

The experiment was carried out using a split plot design with three replications. The main plots were: (1) Maize (*Zea mays* L.*)* after 2 years *Sesbania sesban* (prov. Chipata dam) fallow, (2) Maize after 2 years natural fallow, (3) Maize monoculture with recommended fertilizer (112 kg N, 18 kg P, and 17 kg K ha^{-1}), and (4) Maize monoculture without fertilizer. The sub-plots consisted of three practices of tillage: (1) Conventional tillage (farmers planting maize on ridges), (2) Flat tilled and (3) Zero tillage. The sub-plots measured 10 by 10 m.

Land preparation and crop management

Sesbania was planted in the field from nursery raised bare rooted seedlings at the age of 5 weeks. The spacing between plants was 1.0 by 1.0 m (10 000 plants ha^{-1}). Trees were felled to ground level after two years of growth in October 1999. Stumps and root systems were left in the soil. The above ground biomass of trees was measured at fallow clearing by separating the biomass components into foliage (leaves and twigs), branches and stems. These components were then weighed as green after which samples of each component were collected on plot basis and oven dried at 70°C to constant moisture.

The plots with conventional tillage practice were prepared by covering the natural vegetation or crop residues with soil by making ridges as a common practice in eastern Zambia using hand hoes. The plots with flat tilled practice were ploughed by digging and burying the natural vegetation or crop residues on the surface to 20 cm depth with a hand hoe. On the zero tillage practice, a 3 cm diameter bamboo stick was used to open a fallow to a depth of 5 cm, where maize seed was placed at planting. Biomass production of natural fallow at the end of two years was assessed using four quadrants of 0.50 by 0.50 m (0.25 m^2) each (Klingman, 1971). Weeds during the cropping phase were only estimated at Msekera before each weeding by the procedure mentioned above. Predominant weeds were *Acanthospermum hispidium* DC, A. *conyzoides, Bidens pilosa* L. and *Cassia obtusifolia* L. (Fabaceae). Weeds were controlled in the conventional tillage practice by re-ridging. In the flat tilled plots, the weeds were controlled by hand hoeing, and in the zero tillage plots by cutting the weeds at ground level with hand hoe. All plots were weeded twice during the crop season. Hybrid maize (*Zea mays* L. var. MM 604) was sown by hand in all tillage practices at 25 cm within the rows and 100 cm between the rows (44 444 plant ha^{-1}). Fertilizer to the 'fertilized maize control plots' was applied at the rate of 20, 18, and 17 kg N, P and K ha^{-1}, respectively, using Compound D at sowing and 92 kg N ha^{-1} using urea, four weeks after sowing. The experiment was done over 2 seasons, in the 2000/2001 and 2001/2002 seasons.

Sample collection and analyses

Six replicate samples were taken from 0 to 20 cm soil depth in all plots for determination of total inorganic N. The first sampling was taken at fallow clearing (post-fallow preseason sampling, October 2000) and the second sampling was done in February 2001 (wet season sampling). Ammonium N was determined by colorimetric method (Anderson and Ingram, 1993). Nitrate concentrations were determined by cadmium reduction (Dorich and Nelson, 1984). The sum of NH_4^+-N and NO_3^--N constituted the total inorganic N.

Soil samples for determination of bulk density from all plots were collected using standard core rings (100 cm^3) from 0 to 20 cm soil layer at fallow clearing (October 2000) and start of the second crop season (October, 2001) and oven-dried to constant weight at 105^oC and weighed. Infiltration was monitored only at Msekera at fallow clearing towards the end of the dry season (October, 2000) and before start of the second cropping season (October, 2001) using the double ring infiltrometer (Bouwer, 1986). Measurements were recorded from 3 double rings inserted diagonally in a systematic design in the net plot for three hours at 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150 and 180 min. The average readings were used to calculate infiltration rate per plot using Kostiakov (1932) model.

The data were subjected to analysis of variance (ANOVA) using the generalized linear model (Proc GLM) of the Statistical Analysis System, SAS (1996). The least significant difference (LSD) method was used at 5% to separate treatment means in case of a significant F-test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Above ground tree biomass

At both sites, no significant difference was recorded in

Table 1. Above ground biomass (tha^{-1}) at fallow clearance at Chadiza and Msekera as affected by fallow system and tillage practice in October 2001.

Fallow system (FS)	Tillage practice		
	Conventional tillage	Flat till	Zero tillage
Msekera			
Two years Sesbania fallow			
Leaf and twig	0.4	0.4	0.5
Stem	6.6	7.4	6.2
Total above ground biomass	7.0	7.8	6.7
Two years natural fallow			
Total above ground biomass	7.1	7.9	8.5
$LSD_{(0.05\ level)}$: FS = NS, Tillage = NS, F x Tillage = NS			
Chadiza			
Two years Sesbania fallow			
Leaf and twig	0.4	1.0	0.3
Stem	15.7	12.0	8.3
Total above ground biomass	16.1	13.0	8.6
Two years natural fallow			
Total above ground biomass	6.1	6.4	6.2
	$LSD_{(0.05\ level)}$: FS = NS, Tillage = NS, FS x Tillage = NS		

LSD = least significant difference; FS = Fallow system; NS = not significant.

above ground biomass in relation to tillage practice or land use system (LUS) (Table 1). Despite this, sesbania had the highest standing total above ground biomass of 16.1 tha^{-1} under conventional tillage practice and 7.8 tha^{-1} under flat till practice at Chadiza and Msekera respectively (Table 1). Conventional tillage practice at Chadiza had biomass of 15.7 tha^{-1} and 0.4 tha^{-1} for wood and foliage (leaf + twigs), whereas wood biomass was 7.4 tha^{-1} and foliage (leaf + twigs) was 0.4 tha^{-1} for flat till practice at Msekera.

The above ground biomass reported in this study relates well to that reported by Kwesiga et al. (1995) and Mafongoya et al. (1999) under similar conditions. The high sesbania biomass at Chadiza site was attributed to the good rainfall (1144.4 mm p.a.) of 1997/1998 followed by another good season with a total of 1062 mm p.a. The other reason is that the type of soils at Chadiza has a top 40 cm sand layer followed by a clay subsoil which traps leached nutrients.

Top soil nitrogen dynamics

Pre-season inorganic NO_3^- - N before sowing crop was not significantly affected by LUS or tillage practice at Msekera (Table 2). However, NO_3^- - N and total inorganic N in both October 2000 and February 2001 was highest under conventional tillage compared to zero or flat till practice (Table 2). The interaction between LUS and tillage practice was not significant. This could be attributed to the dry conditions experienced at the time of soil sampling. During the wet season sampling at Msekera, significant differences were observed for LUS and tillage practice. There was also significant interaction between LUS and tillage practices.

At Chadiza site, there was no significant difference at both times of sampling in relation to soil nitrogen (Table 3). Despite this, zero tillage practice had generally lower concentrations of NO_3^- - N in the top 20 cm. This is in contrast to Khant (1971), who proposed greater N concentration in zero tillage plots due to less uptake and movement as a result of the absence of thorough land preparation. The low NO_3^- - N levels under Sesbania at both sites during wet season sampling could be a result of rapid N uptake by growing maize and rapid leaching of NO_3^- - N during high rainfall of 2000 (1342 mm p.a.). Okonkwo et al. (2008) reported similar results of NO_3^- - N being leached beyond rooting depth of maize.

Cumulative water intake

Significant difference ($p<0.05$) was found in all LUS and tillage practices in both October 1999 and October 2000 seasons (Table 4). Cumulative water intake by the LUS was 14.9 and 12.7% higher under conventional tillage than zero tillage practice in both years after three hours (Table 4). Natural fallow under conventional tillage practice had significantly higher cumulative water intake than under flat till or zero tillage practice in 1999 season (Table 4). This could be attributed to less runoff, high root mass, less compaction during the fallow period (Sjogren

Table 2. Inorganic soil NO_3^--N and total inorganic-N (mg N kg^{-1}), at 0 to 20 cm depth, before sowing crop and during the wet season of first post-fallow crop (2000/2001) as affected by cropping system and tillage practice at Msekera.

Cropping system (CS)	Tillage practice					
	Conventional tillage		Flat till		Zero tillage	
	Nitrate-N	Total inorganic-N	Nitrate-N	Total inorganic-N	Nitrate-N	Total inorganic-N
Inorganic nitrogen in October 2000						
Maize with fertilizer	5.12	6.99	1.90	3.22	2.60	4.24
Maize after Sesbania fallow	3.44	4.64	2.98	3.14	3.52	5.22
Maize after natural fallow	1.88	3.18	2.84	4.82	1.40	3.30
Maize without fertilizer	2.52	3.97	3.06	4.01	1.47	2.24
Inorganic nitrogen in February 2001						
Maize with fertilizer	15.16	19.64	1.81	4.99	1.94	6.23
Maize after Sesbania fallow	2.26	7.55	1.63	4.93	1.04	3.64
Maize after natural fallow	2.36	6.96	1.84	5.87	1.76	6.03
Maize without fertilizer	1.97	3.08	1.22	2.94	1.84	4.56
Nitrate-N: $LSD_{(0.05\ level)}$: CS = 1.82, Tillage = 0.72, CS x Tillage = 2.00						
Total-N: $LSD_{(0.05\ level)}$: CS = 2.80, Tillage = 1.23, CS x Tillage = 3.18						

LSD = least significant difference; CS = Cropping system; NS = not significant.

et al., 2010). Natural vegetation regrowth consists of many plant species with different types of root systems, which have the capacity to increase infiltration of water in the soil. Fallowing with various legumes and grass cover crops is known to improve soil infiltration (Chintu, 2004). Low cumulative water intake in maize with or without fertilizer on zero tillage practice could be attributed to deterioration of soil physical properties leading to high bulk density and reduced porosity.

Similarly, Good and Beatty (2011) reported a decline in cumulative water intake in continuous maize with fertilizer, which they attributed to high soil bulk density, and penetrometer resistance under no till treatment. Generally there was a decline in all LUS in cumulative water intake in the second post fallow season (October, 2000) than the first post fallow season (October, 1999). This decline could be attributed to break down of soil physical properties. The benefits accrued during fallowing are easily lost by cultivation (Wilkinson and Aina, 1976). This decline was more pronounced for natural vegetation fallow under conventional tillage practice and the least was for unfertilised, monocultivated maize under zero tillage practice. Continuous cultivation has been reported by several researchers (Liu et al., 2006) as being responsible for structural degradation, decrease in soil organic matter content.

Soil bulk density

The bulk density measured at fallow clearance was lowest under the maize planted after the natural fallow (1.14 gcm^{-3}) and sesbania fallow (1.23 gcm^{-3}) flat till practice compared to maize monoculture with fertilizer (1.54 gcm^{-3}) and maize after natural fallow (1.53 gcm^{-3}) on zero tillage practice (Table 5). The higher bulk density under zero tillage practice could be attributed to the non-incorporation of organic matter which was left on the soil surface. These soils are normally compacted if no tillage is used.

Therefore, where minimum tillage or mixing of soil with organic matter is employed, bulk density is bound to be lowered. This is contrary to other researchers (Diana et al., 2008) who reported that the presence of residue on the soil surface is responsible for maintaining low soil bulk density. Bulk density measured after one year of cropping

Table 3. Inorganic soil NO_3^--N and total inorganic-N (mg N kg^{-1}), at 0 to 20 cm depth, before sowing crop and during the wet season of first post-fallow crop (2000/2001) as affected by cropping system and tillage practice at Chadiza.

Cropping system (CS)	Tillage practice					
	Conventional tillage		Flat till		Zero tillage	
	Nitrate-N	Total inorganic-N	Nitrate-N	Total inorganic-N	Nitrate-N	Total inorganic-N
Inorganic nitrogen in October 2000						
Maize with fertilizer	4.91	7.63	2.20	5.68	2.04	4.12
Maize after Sesbania fallow	4.94	7.51	5.67	8.11	5.77	10.24
Maize after natural fallow	1.35	4.96	1.63	5.47	1.12	5.60
Maize without fertilizer	5.40	8.24	1.88	4.39	1.12	3.84
Inorganic nitrogen in February 2001						
Maize with fertilizer	1.49	7.30	3.1	6.22	2.9	6.58
Maize after Sesbania fallow	2.55	6.48	0.55	4.26	0.59	4.43
Maize after natural fallow	1.21	5.77	0.52	2.93	0.27	3.38
Maize without fertilizer	2.03	4.80	0.19	2.55	0.46	3.85
Nitrate-N: $LSD_{(0.05\ level)}$: CS = NS, Tillage = NS, CS x Tillage = NS						
Total-N: $LSD_{(0.05\ level)}$: CS = NS, Tillage = NS, CS x Tillage = NS						

LSD = least significant difference; CS = Cropping system; NS = not significant.

Table 4. Cumulative water intake (mm) after 3 hours before sowing the first crop (October 2000) and before sowing second crop (October 2001) as affected by cropping system and tillage practice at Msekera.

Cropping system (CS)	Tillage practice		
	Conventional tillage	Flat till	Zero tillage
Water intake in October 2000			
Maize with fertilizer	190.0	154.3	152.3
Maize after Sesbania fallow	255.0	251.7	234.7
Maize after natural fallow	306.0	256.7	262.7
Maize without fertilizer	140.3	130.3	126.0
$LSD_{(0.05\ level)}$: CS = 7.1, Tillage = 4.2, CS x Tillage = 9.2			
Water intake in October 2001			
Maize with fertilizer	108.7	102.0	96.7
Maize after Sesbania fallow	170.7	160.0	111.0
Maize after natural fallow	158.3	179.0	174.3
Maize without fertilizer	95.0	94.7	93.0
$LSD_{(0.05\ level)}$: CS = 17.5, Tillage = 12.6, CS x Tillage = 25.0			

LSD = least significant difference; CS = Cropping system.

(October, 2000) was lowest in maize planted after natural fallow (1.27 gcm^{-3}) and highest under maize monoculture with fertilizer (1.62 gcm^{-3}) under zero tillage practice (Table 5). In this study, bulk density measured after one year of cultivation led to progressive deterioration of the soil structure under all LUS. The results from this experiment confirm the earlier findings by Liu et al.(2006) who reported high bulk density after continuous monocropping. The increased bulk density could be linked to high soil compaction under the zero tillage practice, which could have impended root growth to exploit nutrients hence lower maize grain yields. No tillage, although advantageous through reduction of erosion and soil organic matter maintenance, could eventually lead to soil compaction with shallow rooting crops and insufficient residue return (Juo et al., 1996).

Table 5. Dry bulk density (g cm^{-3}) before sowing the first crop (October 2000) and before sowing second crop (October 2001) as affected by land-use system and tillage practice at Msekera.

Cropping system (CS)	Tillage practice		
	Conventional tillage	Flat till	Zero tillage
Dry bulk density in October 2000			
Maize with fertilizer	1.30	1.36	1.54
Maize after Sesbania fallow	1.33	1.23	1.45
Maize after natural fallow	1.38	1.14	1.53
Maize without fertilizer	1.38	1.31	1.36
$LSD_{(0.05\ level)}$: CS = NS, Tillage = 0.07, CS x Tillage = 0.16			
Dry bulk density in October 2001			
Maize with fertilizer	1.42	1.50	1.62
Maize after Sesbania fallow	1.45	1.37	1.57
Maize after natural fallow	1.48	1.27	1.59
Maize without fertilizer	1.52	1.45	1.47
$LSD_{(0.05\ level)}$: CS = NS, Tillage = 0.06, CS x Tillage = 0.17			

LSD = least significant difference; CS = Cropping system; NS = not significant.

Maize grain yields

At Chadiza site, there was no interaction between land use system and tillage practice with respect to maize grain yield in both crop seasons. However, the maize yields of maize monoculture with fertilizer during 2000 season under conventional tillage practice performed better than the rest of the LUS and tillage practices (Table 6). During 2000 season the maize yields of fertilised monocultivated maize, maize after Sesbania fallow, maize after natural fallow and maize monoculture without fertilizer from conventional tillage practice were 49, 46, 42 and 47% above the zero tillage system respectively. On the other hand, maize yields from the flat till practice were not significantly different ($p>0.05$) from the zero tillage practice. Similarly, maize yields during 2000/2001 season were highest in maize monoculture with fertilizer under conventional tillage compared to zero tillage or flat till practice (Table 6).

The increases in grain yield under conventional tillage over zero tillage respectively were 66.3 and 327.4% during 2000/2001 and 2001/2002 seasons. In both cropping seasons at Chadiza site, zero tillage decreased maize grain yields compared to conventional tillage. This could be due to weed infestation, outbreak of *Cercospora* grey leaf spot disease during grain filling period and deterioration of soil properties under continuous zero tillage practice. Madal et al. (1994) reported higher yields under conventional tillage practice, which they associated with better root growth and higher water use. No till has also been reported to cause significant reductions in maize yield compared with conventional cultivation and deep tillage (Arora et al., 1991; Archarya and Sharma, 1994). At Msekera, in both 2000/2001 and 2001/2002 seasons, maize yields were significantly different ($p<0.05$) among the LUS and tillage practices. No interaction between LUS and tillage practice with respect to maize grain yield in both seasons was recorded at Msekera site. In spite of this, the highest yields, irrespective of tillage practice were from maize monoculture with fertilizer in 2000 and 2001 season. The increases in grain yield under conventional tillage over zero tillage practice respectively were 17.8 and 28.2% during 2000 and 2001 season respectively (Table 6).

Kwesiga et al. (2005) showed that improved fallow of sesbania of one to three year duration has the capacity to increase yield of subsequent maize crops on N-deficient soils. Sesbania leaf biomass is higher in N and decomposes rapidly to supply N to maize crops in the first season. Mafongoya and Bationo (2006) reported similar benefits of sesbania leaf biomass on subsequent maize grain yield. Whereas high maize yields in the control with fertilizer could be ascribed to N from fertilizer. Maize yields under conventional tillage practice surpassed yields from other tillage practices and this could be attributed to the improved soil fertility, concentration of organic matter along the ridge, and reduced weed infestation. On the other hand, low maize yields from zero tillage were a result of high weed infestation and pests or disease outbreaks (Sileshi, personal communication, Chitedze Research Station, Zambia).

Dry weed biomass

Significant differences ($p<0.05$) were observed in weed infestation among the LUS and the tillage practices at fallow clearance and at the two weeding times (Table 7).

Table 6. Maize grain yields (t ha^{-1}) at Chadiza and Msekera as affected by cropping system and tillage practice.

Cropping system (CS)	Tillage practice		
	Conventional tillage	Flat till	Zero tillage
Chadiza 2001			
Maize with fertilizer	4.23	3.13	2.14
Maize after Sesbania fallow	3.76	3.77	2.01
Maize after natural fallow	1.07	0.86	0.60
Maize without fertilizer	1.42	1.03	0.75
$LSD_{(0.05\ level)}$: CS = 0.43, Tillage = 0.37, CS x Tillage = 0.74			
Chadiza 2002			
Maize with fertilizer	1.65	0.50	0.32
Maize after Sesbania fallow	0.99	0.99	0.32
Maize after natural fallow	0.74	0.37	0.26
Maize without fertilizer	0.55	0.21	0.03
$LSD_{(0.05\ level)}$: CS =0.41 Tillage = 0.36, CS x Tillage = NS			
Msekera 2001			
Maize with fertilizer	4.31	3.97	3.65
Maize after Sesbania fallow	3.35	3.28	3.39
Maize after natural fallow	2.12	2.06	1.92
Maize without fertilizer	1.15	0.96	0.86
$LSD_{(0.05\ level)}$: CS =0.50, Tillage = NS, CS x Tillage = NS			
Msekera 2002			
Maize with fertilizer	4.77	4.15	3.98
Maize after Sesbania fallow	1.69	1.70	1.67
Maize after natural fallow	1.10	1.02	0.93
Maize without fertilizer	0.67	0.53	0.38
$LSD_{(0.05\ level)}$: CS =0.28, Tillage = 0.32, CS x Tillage = NS			

LSD = least significant difference; CS = Cropping system; NS = not significant.

Table 7. Total dry weed biomass production (kg ha^{-1}) as affected by cropping system and tillage practice at Msekera, Zambia.

Cropping system (CS)	Tillage practice		
	Conventional tillage	Flat till	Zero tillage
Weed biomass at fallow clearance			
Maize with fertilizer	2440	2200	2667
Maize after Sesbania fallow	0	0	0
Maize after natural fallow	7063	7920	8450
Maize without fertilizer	2577	2713	2703
$LSD_{(0.05\ level)}$: CS = 370, Tillage = 453, CS x Tillage = 412			
Weeds at 2 WAP			
Maize with fertilizer	1920	2033	2200
Maize after Sesbania fallow	223	277	303
Maize after natural fallow	3133	3217	3333
Maize without fertilizer	1917	2030	2117
$LSD_{(0.05\ level)}$: CS = 499,Tillage = 35, CS x Tillage = 500			

Table 7. Contd.

Weeds at 7 WAP			
Maize with fertilizer	193	240	263
Maize after Sesbania fallow	157	240	327
Maize after natural fallow	1970	2060	2563
Maize without fertilizer	603	707	780
$LSD_{(0.05\ level)}$: CS = 153, Tillage = 131, CS x Tillage = NS			
Total weed biomass			
Maize with fertilizer	26.7	47.3	43.3
Maize after Sesbania fallow	29.0	40.0	70.0
Maize after natural fallow	41.0	57.7	87.3
Maize without fertilizer	24.7	44.3	54.3
$LSD_{(0.05\ level)}$: CS = 11.5, Tillage = 6.5, CS x Tillage = 14.4			

LSD = least significant difference; CS = Cropping system; NS = not significant.

Sesbania planted fallow had no weed biomass at fallow clearance compared to natural fallow. In general the highest and lowest weed cover was found in natural fallow and sesbania fallow, respectively. Overall, the zero tillage practice and the natural fallow system had significantly high weed infestation at all times during the 2000/2001 season. The low weed infestation under sesbania LUS at fallow clearance could be attributed to its ability to suppress weeds in relation to other LUS. These results conform to Sileshi and Mafongoya (2003) findings under similar conditions. Significant difference in weed biomass was recorded for LUS and tillage practice at 2 and 7 WAP (Table 7).

Higher weed infestation occurred under natural fallow for zero tillage practice, compared to other LUS during the first and second weedings. Similarly, total weed biomass was also significantly affected by LUS and tillage practice. The zero tillage practice under the natural fallow practice had the highest total weed infestation (Table 7). This could be as a result of weed seeds, which were still in the soil, which came up after the soil was slightly disturbed at weeding. Conventional tillage was able to suppress weeds more than other tillage practices throughout the subsequent weeding times during the crop growth. Whereas crop residues from maize monoculture with or without fertilizer, mostly consisted of stalks which were not able to suppress weeds. Böhringer (1991) reported that mulch morphology plays an important role in controlling weeds and facilitating hand hoe weeding.

Total labour requirement

At land preparation, the zero tillage practice was the easiest to prepare and took less man hours compared to the flat till or conventional tillage practice (Table 8). The low labour for sesbania under zero tillage practice could be attributed to low weed infestation as well as the improved soil structure, which made it easier to make fallows with a wooden peg. On the overall, the maize monoculture with fertilizer on the flat till tillage practice took 115.3 man hours ha^{-1} to prepare compared to 5.3 man hours ha^{-1} for sesbania fallow zero tillage practice (Table 8).

Tillage practice had no significant difference ($p>0.05$) in the time it took to do the planting operation. Despite this, more time was spent in the natural fallow zero tillage practice compared to maize monoculture without fertilizer under conventional tillage practice during planting (Table 8). Under Sesbania zero tillage practice weeds germinated earlier than other LUS because of the improved fertility of the soil. Whereas under natural fallow there were a lot of weed seeds in the soil, which germinated after favourable conditions were met such as good rainfall and soil condition. The high weed infestation consequently led to increasing labour demand at weeding. Addati and Cassirer (2008) reported that farmers in Africa spent about 40% of their work hours weeding. This is because farmers using hand hoes for weeding would like to clean weed their small areas of land in order to get a good yield.

At 2 WAP the weed infestation was higher under the zero tillage practice and as such more time was spent for clean weeding in the maize after natural fallow and sesbania fallow with zero tillage practices compared to maize mono culture with and without fertilizer under conventional tillage practice (Table 8). The reason has already been mentioned above.After 7 WAP (second weeding) the maize after natural fallow and sesbania fallow with zero tillage still had higher weed infestation as such more time was spent for clean weeding compared to the conventional tillage (Table 8). The major reason has also been mentioned before.

The total labour requirement for land preparation,

Table 8. Labour requirements (man hours ha^{-1}) for different operations as affected by cropping system and tillage practice.

Cropping system (CS)	Tillage practice		
	Conventional tillage	Flat till	Zero tillage
Labour at land preparation			
Maize with fertilizer	75.3	115.3	35.3
Maize after Sesbania fallow	103.3	103.7	5.3
Maize after natural fallow	77	112	60.7
Maize without fertilizer	73.0	103.7	46.0
$LSD_{(0.05\ level)}$: CS = NS, Tillage = 22.6, CS x Tillage = NS			
Labour at planting			
Maize with fertilizer	26.0	48.0	54.3
Maize after Sesbania fallow	26.0	46.0	58.0
Maize after natural fallow	26.0	54.3	60.0
Maize without fertilizer	17.3	29.0	47.7
$LSD_{(0.05\ level)}$: CS = NS, Tillage = 12.7, CS x Tillage = NS			
Labour at 1st weeding			
Maize with fertilizer	20.3	41.0	37.7
Maize after Sesbania fallow	23.3	35.7	64.7
Maize after natural fallow	36.7	54.7	89.9
Maize without fertilizer	20.3	38.7	50.3
$LSD_{(0.05\ level)}$: CS = 12.3, Tillage = 6.3, CS x Tillage = 14.7			
Labour at 2nd weeding			
Maize with fertilizer	26.7	47.3	43.3
Maize after Sesbania fallow	29.0	40.0	70.0
Maize after natural fallow	41.0	57.7	87.3
Maize without fertilizer	24.7	44.3	54.3
$LSD_{(0.05\ level)}$: CS = 11.5, Tillage = 6.5, CS x Tillage = 14.4			
Total labour			
Maize with fertilizer	148.3	251.7	170.7
Maize after Sesbania fallow	181.7	224.7	198.0
Maize after natural fallow	181.0	278.7	265.7
Maize without fertilizer	135.3	215.7	198.3
$LSD_{(0.05\ level)}$: CS = 34.4, Tillage = 33.4, CS x Tillage = NS			

LSD = least significant difference; CS = Cropping system; NS = not significant.

planting and two weeding in one season under different LUS and tillage practice was highest under the maize after natural fallow with the flat till and zero tillage practices compared to the maize mono culture with or without fertilizer under conventional tillage (Table 8). Fertilized plots offered good crop stand, which eventually helped to suppress weeds and reduce labour demands. Low fertility under maize without fertilizer contributed to low weed infestation and less labour demand at weeding. Flat till and zero tillage practice required high labour input because traditionally most of the surface area is weeded, even in the case of scattered weed growth as reported by Vogel (1994).

Generally maize monoculture without fertilizer under conventional tillage practice resulted in low labour demand during land preparation, planting, first and second weeding. This could be ascribed to reduced biomass from maize stalks from previous season, which could have interfered with land preparation, planting and weeding operations.

Conclusions

This study illustrates the various tillage practices and their implication on labour in relation to maize production

under smallholder enterprise. Zero tillage practice resulted in lower maize grain yield, higher bulk density, reduced water intake, higher weed infestation and high labour demand during weeding compared to conventional tillage. Despite zero tillage having less labour demand at land preparation, a farmer will need to invest in herbicides in order to control the weeds if this tillage practice is to be adopted. Conventional tillage improved the soil environment and resulted in increased maize yield in all LUS. Flat till practice has higher labour demand at land preparation in relation to other tillage practices and will cause a serious hindrance to households with shortage of labour.

Conflict of Interests

The authors have not declared any conflict of interests.

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academicJournals

Vol. 10(46), pp. 4241-4251, 12 November, 2015
DOI: 10.5897/AJAR2015. 9835
Article Number: 32F186E56159
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Removal of Cr (III) from contaminated water using industrial waste of the cassava as natural adsorbents

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Received 22 April, 2015; Accepted 9 October, 2015

This innovative research aimed to study the potential of the use of solid wastes from cassava root processing industry (*Manihot esculenta* Crantz) (peel, bagasse and the mix peel + bagasse) as natural adsorbents of Cr^{3+} from waters. In a first step, the biosorbents were characterized chemically, structurally and morphologically. This way were performed infrared spectrum analysis, scanning electron microscopy, point of zero charge and chemical composition for all studied natural adsorbents. After that, studies evolving kinetics, equilibrium, thermodynamics and desorption of Cr^{3+} were performed. According to the obtained data the cassava adsorbents proved to be efficient in the removal of Cr^{3+}, being found in Langmuir and D-R models the best fitting, indicating monolayer chemisorption, with endothermic character. The cassava materials presented lower rates of desorption also suggesting a chemisorption of Cr^{3+} molecules. By the obtained results it was concluded that the use of cassava biosorbents are viable for the decontamination of waters containing Cr^{3+}, being the use of cassava wastes for adsorption a complementation of the final steps of cassava productive chain.

Key words: Contamination of waters, chromium toxicity, biosorbents, natural adsorbents, remediation of waters, biosorption, decontamination of water.

INTRODUCTION

In The solid wastes produced by the industrialization of cassava roots, the peel of cassava roots and the fibrous material originated by the starch extraction (bagasse) do not have any other destiny than animal feeding (Menezes et al., 2004).

Only in Brazil, every year are produced approximately 1 million tons of cassava peel. It is estimated that globally this number can reach 11 million tons every year, being all these wastes a cause of major problems by the incorrect disposal frequently in industries and farms. In function of the many problems of chemical pollution which the environment is exposed, among them, the

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hydric pollution by heavy metals (Li and Bai, 2005), is from major importance the research for new technologies for low cost remediation of toxic metals from hydric bodies, because these metals cause severe risks to humans, animals and environment (Montanher, 2005).

One of the alternatives for natural resources decontamination is the process of adsorption, which regulates the mobility and availability of the pollutants in solution, being the molecules presents in a fluid, liquid or gas, which can spontaneously accumulate above the solid surface (Reddy et al., 2010).

The Cr^{3+} contamination is normally treated with minor attention when compared to Cr^{6+}, due to its toxicity degree, however some researches relates that concentrations of Cr^{3+} cause severe perturbations in human erythrocytes (Suwalsky et al., 2008), being in this way the removal of Cr^{3+} from waters extremely important from the environmental and human point of view. The removal of heavy metals, such as Cr^{3+}, presents in contaminated effluents can be performed using organic and inorganic materials, being this a very attractive option, mainly in function of the higher availability of the materials and for low cost (Demirbas, 2008).

Many authors relate the use of alternative biosorbents for the removal of pollutants from waters, as example of: pine bark (Argun and Dursun, 2008), cocoa husks (Meunier et al., 2003), rice brain (Montanheir et al., 2005); dry mass of *Eichhornia crassipes* (Gonçalves Jr. et al., 2009), sugar cane bagasse in natura and modified (Dos santos et al., 2011), however there are not any studies relating the use of cassava solid wastes and its use as natural adsorbents.

In this way, the objective of this study was to evaluate these renewable materials (Peel, bagasse and peel + bagasse) as natural adsorbents, studying its adsorption characteristics in function to Cr^{3+} remediation.

MATERIALS AND METHODS

Obtaining of the natural adsorbents

The raw matter (peel, bagasse and peel + bagasse) was obtained in industry located at Toledo – Paraná. The material was dried at 60°C for 48 h, crushed and sieved, maintaining the final granulometry ranging from 14 to 60 mesh. Non treatment that modified chemically the adsorbent materials was performed, being these only crushed and sieved for standardization of particle size. It was also performed the chemical characterization of biosorbents, by nitropercloric digestion of the materials (AOAC, 2005), for determination of the concentrations of potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), cadmium (Cd), lead (Pb) and chromium (Cr) by flame atomic absorption spectrometry (FAAS) (Welz and Sperling, 1999). For the evaluation of functional groups responsible for the adsorption with Cr^{3+} was performed the infrared characterization of adsorbents, using a Shimadzu Infrared Spectrophotometer FTIR- 8300 Fourier Transform, in the region between 400 and 4000 cm^{-1}. The spectrum was obtained using KBr tablets.

The material surface was also evaluated morphologically by scanning electron microscopy (SEM), with a FEI Quanta 200 microscope, operating in a voltage of 30 kV. The samples were placed in a double-sided tape attached to a carbon sample holder and then were metallized with gold to a thickness of about 30 nm by using a sputter coater Baltec Scutter SCD 050. It was also determined the zero point charge (pH_{PZC}) of the adsorbent, which refers to the pH which the resultant of adsorbent superficial charges is null (Mimura et al., 2010).

Preparation of contaminated water

The monoelementar solution fortified with Cr^{3+} were prepared using chrome nitrate [$Cr(NO_3)_3$ $9H_2O$ P.A. ≥ 99,0% Sigma-Aldrich]

Adsorption studies in function of pH and adsorbent mass

The removal of chromium by the adsorption process is dependent from many factors, between them it can be highlighted the adsorbent quantity, pH of the contaminated water and contact time between adsorbent/adsorbate. This way, in this preliminary study were maintained constant the system stirring (200 rpm), stirring time (1.5 h) temperature (25°C) and chromium concentration (10 mg L^{-1}), varying only the pH of the solutions and the adsorbent mass.

The Cr^{3+} solutions were adjusted in three pH conditions (4.0; 5.0 and 6.0). The pH values were adjusted using HCl solutions (Vetec, 37%) or NaOH (Vetec, 99%), both in the concentration of 0.1 mol L^{-1}. The adsorbent masses were 200, 400, 600, 1000 and 1200 mg, being each adsorbent material evaluated separately.

In erlenmeyers of 125 ml, were added the adsorbent material and 50 ml of Cr^{3+} solution (10 mg L^{-1}), being after 1.5 hours the samples were filtered with qualitative filter (Unifil), being determined the levels of Cr^{3+} by FAAS. (Welz and Sperling, 1999). By the obtained results for equilibrium concentration was calculated the adsorbed quantity in equilibrium Equation (1).

$$Q_{eq} = [(C_0 - C_{eq}).m]\ V \quad (1)$$

Being: Q_{eq} the quantity of Cr^{3+} adsorbed by adsorbent unit (mg g^{-1}), m is the adsorbent mass used (g), C_0 correspond to the initial concentration of Cr^{3+} in solution (mg L^{-1}), C_{eq} is the concentration of Cr^{3+} in solution in the equilibrium (mg L^{-1}) and V is the volume of solution used (L). The percentage of Cr^{3+} removal was calculated according Equation (2):

$$\%R = 100 - (C_{eq}/C_0)\ .100 \quad (2)$$

Being: %R the percentage of Cr^{3+} removal by the adsorbent, C_{eq} the concentration of Cr^{3+} in equilibrium (mg L^{-1}) and C_0 the initial concentration in solution (mg L^{-1}).

Kinetics and equilibrium studies

In order to determine the influence of contact time in the adsorption process, were used the best conditions found in the previous studies of adsorbent mass and pH in different stirring time intervals. This way, 400 mg was the mass used for the three adsorbents and pH of 5.5 of Cr^{3+} solution. The stirring time intervals were 5, 10, 20, 40, 60, 80, 100, 120, 140 and 160 min, being after these intervals the samples were filtered and aliquots sampled for FAAS concentration determination (Welz and Sperling, 1999).

For the evaluation of the kinetic mechanism which controls the adsorption process, the following models were used, according to the literature citations and studies: Pseudo first order (Aksu, 2001), pseudo second order (Ho and Mckay, 1999), Elovich (Ho and Mckay,2004) and intraparticle diffusion (Witek-Krowiak et al., 2011). Adsorption isotherms were also built, using initial concentrations (C_0) ranging from 5 to 200 mg L^{-1}, these results were linearized by

Table 1. Chemical characteristics of the raw materials peel (P), bagasse (B) and peel + bagasse (P + B)

Adsorbent	K	Ca	Mg	Cu	Fe	Mn	Zn	Cd	Pb	Cr
	g kg^{-1}			-mg kg^{-1}						
P	24.10	35.03	6.83	14.33	35.67	123.33	32.00	<0.005	11.00	<0.01
B	5.77	23.23	4.58	5.67	24.50	27.67	18.67	<0.005	14.67	<0.01
P + B	7.77	22.58	5.12	6.00	26.00	34.00	17.00	<0.005	3.33	<0.01

LQ: K = 0.01; Ca = 0.005; Mg = 0.005; Cu = 0.005; Fe = 0.01; Mn = 0.01; Zn = 0.005; Cd = 0.005; Pb = 0.01; Cr = 0.01.

the models of Langmuir, Freundlich and Dubinin-Radushkevich (D-R) ().

Desorption process

For the desorption process, the adsorbents used for the construction of isotherms were separated by filtration process, washed in ultrapure water and dried at 60 ± 2 °C for 24 h. The adsorbent mass recovered was in contact with 50 mL of HCl 0.1 mol L^{-1}, with stirring at 200 rpm for 60 min at 25°C. The final concentration of the desorbed Cr^{3+} was determined by FAAS and calculated by Equation (3):

$$D=[C_{eq(des)}/C_{eq(ads)}] \cdot 100 \quad (3)$$

Being $C_{eq(des)}$ and $C_{eq(ads)}$ (mg L^{-1}) refer to the Cr^{3+} concentration desorbed and quantity adsorbed of Cr^{3+} in equilibrium by the adsorbents.

Comparative studies with active coal (AC)

With the purpose of comparison the obtained results between the cassava adsorbents and one commercial adsorbent, the active coal P.A. (Synth) powder, which is wildly used for the removal of pollutants from water bodies (Oliveira et al., 2008) was used. This way, the same conditions used for the construction of isotherms and desorption tests were used for the comparison with active coal.

Thermodynamics of adsorption

The influence of the temperature in the adsorption process was evaluated in five conditions: 25, 35, 45, 55 and 65°C. For this purpose 50 mL of Cr^{3+} solution at 50 mg L^{-1} concentration. The solution was added in erlenmeyers of 125 ml with 400 mg of adsorbent material. After that period of time was performed the determination of Cr^{3+} concentration in equilibrium by FAAS (Welz and Sperling, 1999).

By the obtained data the thermodynamics parameters were evaluated being also investigated the nature of the adsorption process. For that purpose the Gibbs free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) were calculated (Sari et al., 2007; Gonçalves et al., 2008). All adsorption and desorption processes described in the items above were performed in triplicate.

RESULTS AND DISCUSSION

Biosorbents characterization

Concentrations of lead were found in the adsorbent materials (Table 1), which is an indicative of the presence of this heavy metal in soil where the plants were cultivated. The lead concentration found in the biosorbents is possibly originated from cassava plants from soil contaminated with this heavy metal.

According to Gonçalves Jr. et al. (2011) evaluating the fertilization residual effect of wheat crop with contaminated fertilizers, found soil contaminated with Pb and consequently absorption of this metal by the wheat plants.Peels from vegetal origin are basically constituted of cellulose, hemicelulose and lignin (Pehlivan et al., 2009). Figure 1 show the micrographs of the adsorbents in magnifications until 20.000 times.

The results (Figure 1) show an adsorbent surface of fibrous and spongy aspect, with irregular and heterogeneous structure. Many of these cavities and cracks can be highlighted, showing a material with high superficial area, with favorable characteristics of adsorption. The pH_{PZC}, according to Mimura et al. (2010), is defined as the pH which the surface of the solid possesses neutral charge. In solutions with pH below pH_{PZC}, in adsorbents surface predominate positive charges and, in solutions with pH above pH_{PZC}, the superficial liquid charge is negative, providing better conditions for cations adsorption.

The obtained results of pH_{PZC} (Figure 2) show that the equivalence point of positive and negative charges for the adsorbents is 6.00 for peel, 6.17 for bagasse and 6.24 for peel + bagasse. This way, the adsorption of cations, in this case Cr^{3+} is favored by pH values above than the pH_{PZC} (Tagliaferro et al., 2011).

Adsorption process in function of pH solution and adsorbent mass

The pH is one of the parameters with great importance in the adsorption process, because it interferes in the solid-solution interface, causing influence in the active sites charges of biomass and also in the adsorbate behavior (Gao and Wang 2007; Fávere et al., 2010). As observed in Figure 3, adsorbent masses higher than 400 mg were not found higher removal rates of chromium, in this manner, for the posterior tests were used 400 mg of the adsorbent material with solution Cr^{3+} at pH 5.5.

It is also possible to observe in Figure 3 that for

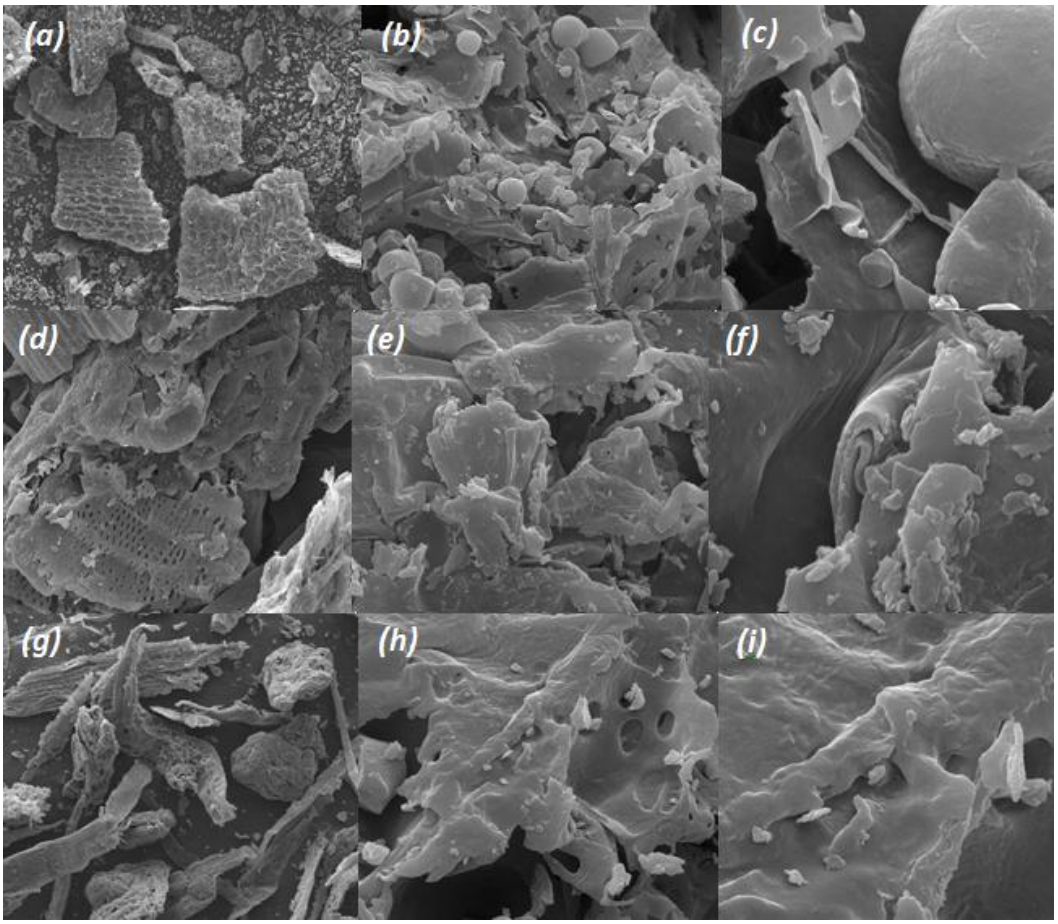

Figure 1. Image of scanning electron microscopy of the adsorbents peel (a, b and c); bagasse (d, e and f) and peel + bagasse (g, h and i) in magnifications of 200 x (left), 3.000 x (middle) and 20.000 x (right).

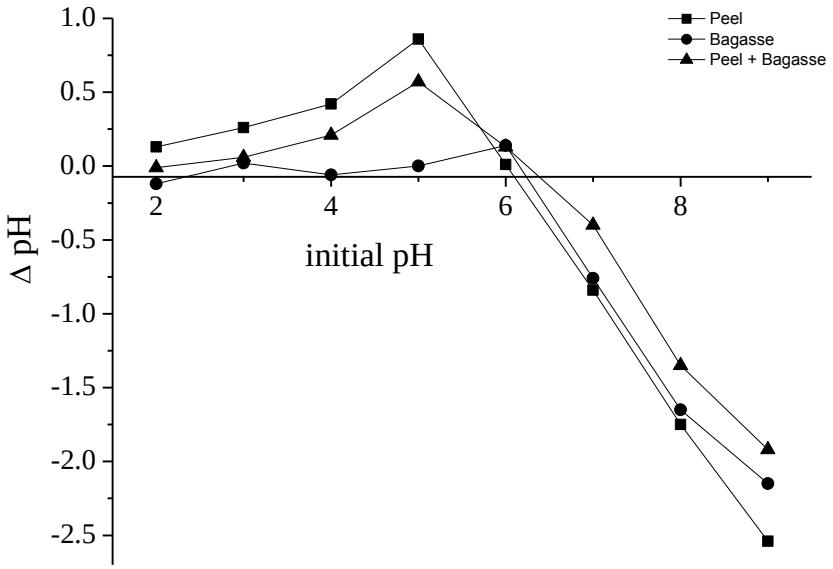

Figure 2. Point of zero charge (pH_{PZC}) in KCl 0.5 mol L^{-1} for peel, bagasse and peel + bagasse.

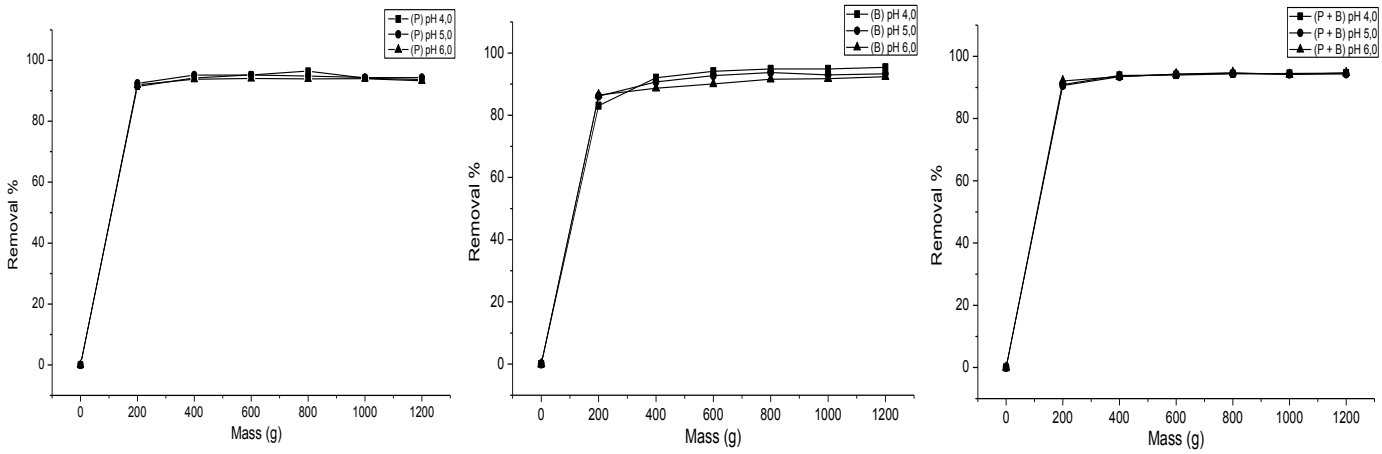

Figure 3. Effect of adsorbent mass and solution pH in the removal % of Cr^{3+} for peel (P), bagasse (B) and peel + bagasse (P + B) in pH 4.0, 5.0 and 6.0.

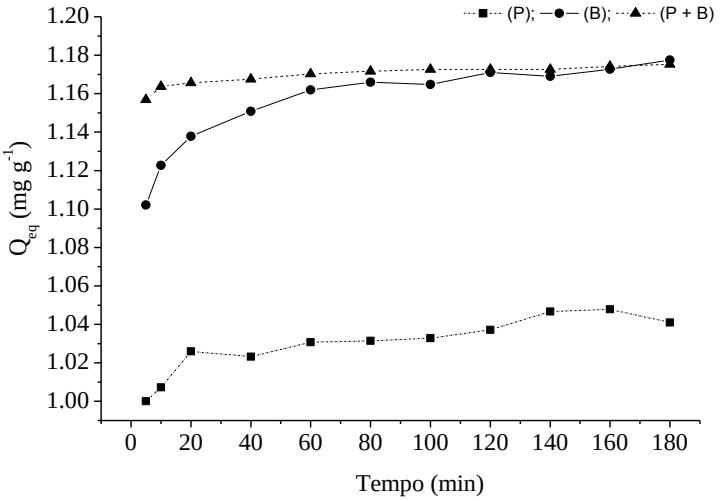

Figure 4. Effect of time (min) in the quantity of Cr^{3+} adsorbed (mg g^{-1}) by peel (P), bagasse (B) and peel + bagasse (P + B).

lower concentrations (10 mg L^{-1}) the materials peel, bagasse and peel + bagasse were capable of removing more than 90% of the Cr^{3+} in solution. It is observed that in the studied pH range, the removal % was superior to 90% in all adsorbent materials, emphasizing the adsorbent peel + bagasse, which removed great quantities of chromium from solution. The value of 8 g of adsorbent per litter of contaminated water is the ideal proportion.

Many materials equally from vegetal origin have the adsorption influenced by pH of contaminant solution, as verified in mesocarp and endocarp macadamia (Vilas Boas et al., 2012), bark of *Pinnus elliottii* (Gonçalves Jr. et al., 2012), rice rusk (Mimura et al., 2010), rice bran (Montanher et al., 2005), peanut husk (Runping et al., 2008), among others.

Influence of contact time between adsorbent/adsorbate

The influence of contact time between adsorbent/adsorbate for the materials peel, bagasse and peel + bagasse is illustrated at Figure 4. It is shown in Figure 4, the occurrence of an increase of Cr^{3+} adsorption by the materials in the course of time, being after 60 min of stirring the dynamic equilibrium is reached, not occurring higher adsorption rates for periods of time higher than 60 min, indicating a fast adsorption.

The obtained results, according to the kinetic models of adsorption are shown in Table 2. For the interpretation of the parameters in Table 2, besides the values of the coefficient of determination *(R^2)* present best fit, the values of Q_{eq} obtained by the model must be very close

Table 2. Kinetic parameters obtained in the adsorption studies of Cr^{3+} for peel (P), bagasse (B) and peel + bagasse (P + B) for the models of pseudo first order, pseudo second order, Elovich and intraparticle diffusion

Models	Mathematical parameter		Adsorbents		
			(P)	(B)	(P + B)
Pseudo first order		$K_{1\ (min^{-1})}$	-0.014	-0.016	-0.015
		$Q_{eq\ (calc)\ (mg\ g^{-1})}$	0.042	0.057	0.014
		R^2	0.599	0.918	0.929
Pseudo second order		$K_{2\ (g\ mg^{-1}\ min^{-1})}$	1.482	1.184	4.556
		$Q_{eq\ (calc)\ (mg\ g^{-1})}$	1.046	1.178	1.175
		R^2	0.999	0.999	1.000
Elovich		$A\ (mg\ g^{-1}\ h^{-1})$	0.982	1.076	1.151
		$B\ (g\ mg^{-1})$	0.012	0.019	0.004
		R^2	0.912	0.976	0.964
Intraparticle diffusion		K_{id} (g mg^{-1} $min^{-1/2}$)	0.012	0.015	0.004
	Line A	C_i (mg g^{-1})	0.972	1.069	1.149
		R^2	0.953	0.931	0.678
		K_{id} (g mg^{-1} $min^{-1/2}$)	0.003	0.006	0.0010
	Line B	C_i (mg g^{-1})	1.010	1.109	1.161
		R^2	0.735	0.974	0.943
		K_{id} (g mg^{-1} $min^{-1/2}$)	-0.004	1.142	-
	Line C	C_i (mg g^{-1})	1.089	0.0025	-
		R^2	0.158	0.757	-
Experimental values	$Q_{eq\ (exp)}$		0.982	1.076	1.151

K_1: constant of velocity from pseudo first order; *Qeq (exp)*: quantity of adsorbate retained per gram of adsorbent in equilibrium (experiment determination); *Qeq (cal)*: quantity of adsorbate retained per gram of adsorbent in equilibrium (estimated by model); K_2: constant of velocity from pseudo second order; *A*: constant which indicates the velocity of initial chemisorption; *B*: number of adequate active sites for adsorption, related to the extension of the surface cover and the activation energy for chemisorption; R^2: coefficient of determination. K_{id}: constant of intraparticle diffusion; C_i: suggests the thickness of the boundary layer effect.

from the obtained experimentally *($Q_{eq\ exp.}$)* (Febrianto et al., 2009). Comparing the values obtained in Table 2, the model of pseudo first order, which suggests physisorption predominance, does not prove convincingly the observed adsorption rates, with unsatisfactory R^2 values.

According to Farooq et al. (2011), the pseudo first order model do not fit in most of the biosorption systems, what explain why this model does not presented satisfactory fitting in the present study. However, the results of R^2 and Q_{eq} calculated by the pseudo second order are satisfactory, suggesting a chemisorption of Cr^{3+} by the materials *'P', 'B'* and *'P + B'*, as observed in Table 2. The model of Elovich dos not explain the behavior observed by the adsorbents (Table 2), in function of the lower values of R^2 obtained. When observed the values of R^2 for the intraparticle diffusion (Table 2), it is verified a multilinear division, this was performed in order to find some step of adsorption which is conducted by the model of intraparticle diffusion. However all obtained R^2 values found were unsatisfactory.

Influence of initial concentrations of Cr^{3+}

The adsorption isotherms describe the relation between the ion adsorbed quantity by adsorbent mass related to the solution concentration in dynamic equilibrium (Witek-krowiak et al., 2011), being these information important for the interpretation of biosorption process. It is observed in Figure 5 high adsorption rates for cassava biosorbents, being these values very close to the adsorption rates obtained for active coal. It is worth pointing out the obtaining of active coal is from abrupt physical-chemical structure modifications, which result in a higher cost of production, while the natural adsorbents such cassava adsorbents (P, B and P + B) present low cost and high availability in most tropical countries.

It was observed that the efficiency in the removal by the biosorbents decrease rapidly with the increase of C_0, very similar behavior found with active coal (AC). It is important to emphasize that AC is an adsorbent obtained by abrupt physical-chemical modifications, that is, it is

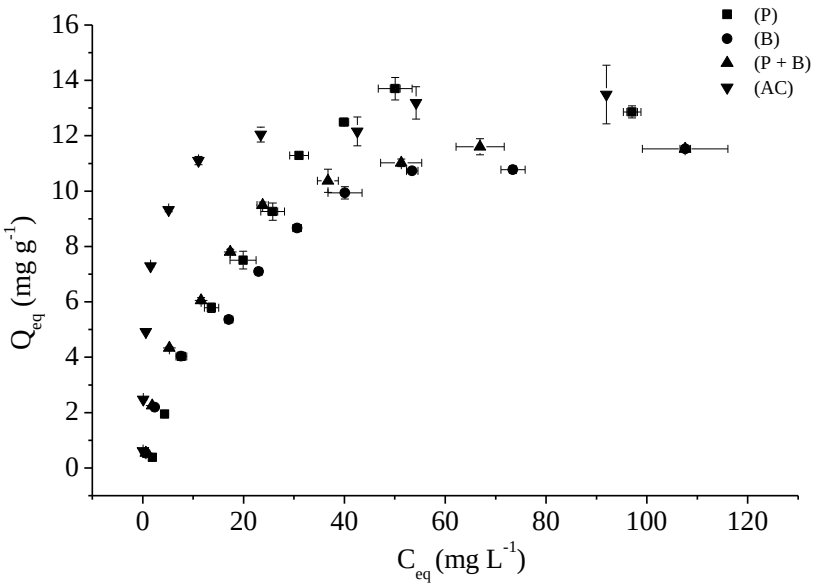

Figure 5. Isotherms of Cr^{3+}adsorption for adsorbents peel (P), bagasse (B), peel + bagasse (P + B) and active coal (AC); (C_0: 5 a 200 mg L^{-1}; 400 mg; pH 5,5, 60 min; 200 rpm; 25°C).

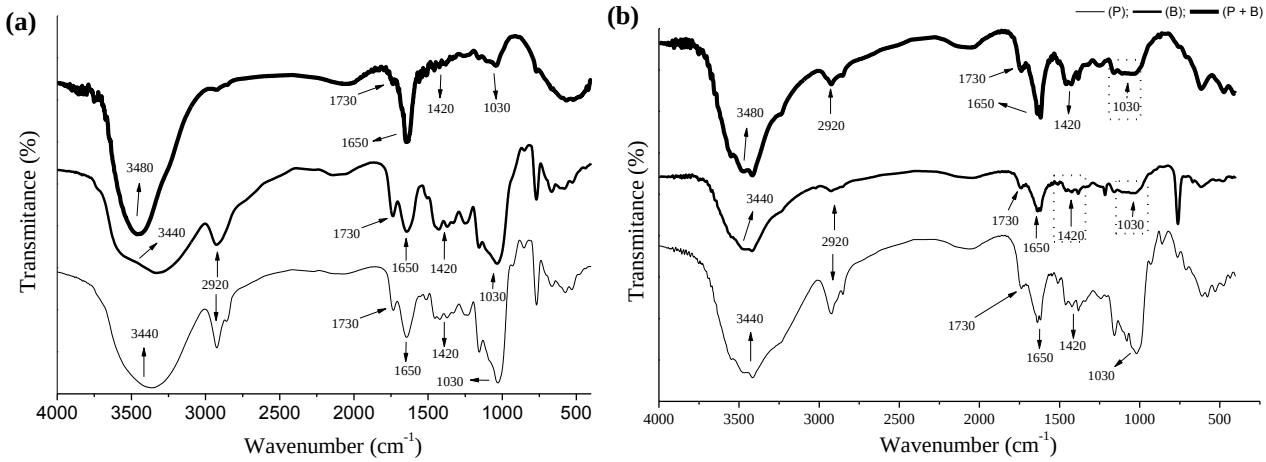

Figure 6. Infrared spectrum after adsorption of Cr3+ for peel (P), bagasse (B) and peel + bagasse (P + B) before adsorption (a) and after adsorption process (b).

more expensive in the monetary point of view (Girods et al., 2009), however presenting lower adsorption rates than cassava biosorbents in higher C_0, such as concentrations of Cr (III) higher than 40 mg L^{-1}. It worth point out that in solutions contaminated with Cr^{3+} in C_0 higher than 140 mg L^{-1}, the natural adsorbents present themselves efficient as active coal (Table 4; Figure 5).

In Figure 5 it is noted a lower efficiency of Cr^{3+} adsorption in lower C_0 by the natural adsorbents when compared to active coal (AC), however in higher C_0 (higher than 140 mg L^{-1}) the natural adsorbents presented high efficiency as active coal (AC). As observed in Figure 5, the obtained isotherms for adsorbents presented typical behavior of isotherms from "L group" (Langmuir type), with initial curvature facing down, due to the decrease of availability of active sites (Giles et al., 1960).

It was also identified in Figure 6 isotherms from "L group" (Langmuir type) and subgroup "2", what indicates the occurrence of surface saturation which the adsorbate has more affinity by the solvent than adsorbed molecules (Giles et al., 1960).

Table 3. Parameters of mathematical models of Langmuir, Freundlich e Dubinin-Radushkevich (D-R) for the process of Cr^{3+} biosorption by peel (P), bagasse (B), peel + bagasse (P + B) and active coal (AC).

Parameter		P	B	(P + B)	AC
Langmuir	Q_m	2.85	13.42	13.01	13.55
	K_L	0.181	0.095	0.064	0.010
	R_L	0.027	0.050	0.073	0.327
	R^2	0.977	0.993	0.997	0.983
Freundlich	K_f	0.714	1.564	2.092	7.224
	n	1.277	2.153	2.391	6.739
	R_2	0.976	0.959	0.925	0.942
D-R	Q_d	0.0029	0.0012	0.0009	0.0004
	E	7.794	9.009	10.174	17.903
	R^2	0.983	0.979	0.992	0.975

Q_m (mg g^{-1}): max adsorption capacity; K_L ou b (L mg^{-1}): constant related to the interaction strength adsorbent/adsorbate; R_L: constant of Langmuir; R^2: determination coefficient; K_f (L mg^{-1}): related to adsorption capacity; n: related to the heterogeneity of solid fraction; Q_d (mol g^{-1}): max capacity of adsorption; E (kj mol^{-1}): average sorption energy.

For C_0 until 80 mg L^{-1} of Cr^{3+}, the active coal (AC) is the best material, adsorbing great quantities of this ion, however, in concentrations superior of this, in function of active sites saturation, this material chemically modified become equally efficient to the cassava natural adsorbents. The parameters obtained for linearization of adsorption isotherms for peel, bagasse, peel + bagasse and active coal in removal of Cr^{3+} are shown in Table 3.

The materials 'B', 'C+B' e 'AC' presented better fitting by Langmuir, suggesting adsorption of Cr^{3+} in monolayer.In relation to Langmuir parameters (Table 3), the max adsorption quantity (Q_m) presented satisfactory results for the cassava adsorbents, with values very close to those obtained for active coal (AC).

Evaluating the values of "R_L" of Langmuir (Table 3), it can be stated that in the experimental conditions the adsorptive process is favorable for all cases, once "R_L" values shown between '0' e '1' (Lin and Juang, 2002).

According Sodré et al. (2001), the "n" parameter from Freundlich indicates the reactivity of active sites, when this variable assume values superior than 1, this is a strong indication of the presence of highly energetic sites, suggesting that these sites are occupied by Cr^{3+} in first place. This behavior of high energetic interaction and high reactivity it is observed in Table 3 for biosorbents "P", because in this case the model of Freundlich present satisfactory fitting.

The model of D-R explains satisfactorily the adsorption of Cr^{3+} by the natural adsorbents (Table 3). According to Wan Ngah and Hanafiah (2008), the average sorption energy (E), related to the mathematical model of D-R, is the free energy evolved in the transfer of 1 mol of solute for surface of adsorbente, this parameter suggests the reaction character as chemical or physical adsorption. Values of "E" between 1 and 8 kj mol^{-1} indicates physical adsorption, whereas values above 8 kj mol^{-1} indicates chemical nature of the adsorption process (Romero-Gonzalez et al., 2005; Fávere et al., 2010). In Table 3, the values of E in most cases assume values above 8 kj mol^{-1}, suggesting predominance of chemisorption for biosorbents, exception only for material "P" in Cr^{3+} adsorption, with values of E inferior to 8 kj mol^{-1}.

Thermodynamics of adsorption

For the comprehension of the temperature effect in the process of Cr^{3+}sorption by the biosorbents and to analyze the nature that rules this process, some thermodynamic parameters were evaluated, as is shown in Table 4. According to Q_{eq} values observed in Table 4 for biosorbents (P, B and P + B), it is noticed the occurrence of a gradual increase in Q_{eq} values, what evidence the influence of temperature in the biosorption process of Cr^{3+}. As shown in Table 4, the values of ΔH are positive, indicating an endothermic system (Wan Ngah and Fatinathan, 2010).

According to Crini and Badot (2008), the laws of thermodynamics indicate that at constant temperature and pressure, the value of ΔG is the main criteria for indication of spontaneity of the system. According to Wan Ngah and Hanafiah (2008), negative values for ΔG indicate the spontaneous nature of the reaction, while positive values for ΔS indicate the increase of the disorder and randomness of the interface solid/solution during the sorption process, as occurred for the evaluated biosorbents (Table 4). By the obtained results, higher temperatures result in higher adsorption of Cr^{3+}, however the adsorption process is not usually operated at higher temperatures, because this would increase the operational costs (Crini and Badot, 2008).

Table 4. Values of Q_{eq} obtained and thermodynamic parameters for Cr^{3+} adsorption for biosorbents peel (P), bagasse (B) and peel bagasse (P + B).

Adsorbents	Temp. (°C)	Thermodynamic parameters				
		Q_{eq}	ΔG	ΔH	ΔS	R^2
		(mg g^{-1})	(kJ mol^{-1})	(J mol^{-1})	(J mol^{-1})	
(C)	25	4.893	2.031	2.832	2.689	0.984
	35	4.895	2.004			
	45	4.930	1.977			
	55	4.955	1.950			
	65	5.003	1.923			
(B)	25	4.631	2.616	20.430	59.782	0.973
	35	4.815	2.018			
	45	5.183	1.420			
	55	5.446	0.822			
	65	5.463	0.224			
(C + B)	25	5.126	1.419	27.175	86.432	0.982
	35	5.345	0.554			
	45	5.607	-0.310			
	55	5.779	-1.174			
	65	5.783	-2.038			

C_0 = 50 mg L^{-1}; Q_{eq}: quantity adsorbed of metallic ion by adsorbente unit of mass; *ΔG*: variation of Gibbs free energy; *ΔH*: variation of enthalpy; *ΔS*: variation of entropy; R^2: coefficient of determination.

Characterization of the adsorbent´s functional groups after adsorption

The infrared spectrum after the adsorption of Cr^{3+} was conducted in order to observe which were the possible functional groups responsible for adsorption process (Figure 6). The bands comprehended at 1730 cm^{-1} still indicate the starch presence and carbonyl groups present in lignin and holocellulose (Horn et al., 2011).

The bands at 1420 to 1650 cm^{-1} are still present in the materials peel (P) and peel + bagasse (P + B), suggesting the presence of amides and carboxylic groups from lignin molecules (Han et al., 2010). However, for the material bagasse (B) the bands at 1420 to 1030 cm^{-1} are missing (dash area at Figure 6 right) or with a decrease of transmittance percentage, suggesting that these adsorption sites were responsible for the binding with Cr^{3+} (Argun and Dursun 2008), probably carboxyl groups (C-O) (Han et al., 2010) and lignin (Guo et al., 2008).

Desorption

According to Mimura et al. (2010), the desorption corresponds to removal of the metal from the binding site from the adsorbent surface and it is expected that H^+ ions can replace the adsorbed cations by the ionic exchange mechanism. The recovery of the adsorbed metal can be performed by many procedures as, for example, the desorption process. This practice is fundamental for the knowledge of characteristics of interaction between adsorbate and adsorbent concerning to its resistance for reuse in new adsorption processes.

Desorption results for biosorbents and activated coal reveal Cr^{3+} low desorption rates, especially for biosorbents. Results may be explained by the fact that Cr^{3+} adsorption by cassava materials appear to be a chemical process.

According to Namasivayam et al. (1998), low desorption rates generally suggest that the adsorbate is strongly linked to the adsorbent´s active sites by chemisorption. It must be underscored that an increase in HCl concentration or the use of other compounds, such as nitric acid (HNO_3), sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH) or even complexant EDTA to promote desorption may provide better results (Sekhar et al., 2004; Bernardo et al., 2009).

Conclusion

By the performed results, it was concluded that the adsorptive process of Cr^{3+} is favorable, presenting best fitting for Langmuir and Dubinin-Radushkevich, what suggests chemisorption in monolayers. The low desorption of Cr^{3+}, the obtained fitting for pseudosecond

order and Dubinin-Radushkevich suggests the occurrence of chemisorption by the cassava adsorbents. With the comparative studies with active coal, it was possible to conclude that cassava materials, which are low cost and high availability biosorbents, presented very similar adsorption rates, demonstrating in some cases higher or equivalent values for adsorption than active coal.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This research was supported by the Brazilian National Counsel of Technological and Scientific Development and by the Brazilian Ministry of Science and Technology (CNPq).

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Vol. 10(46), pp. 4252-4257, 12 November, 2015
DOI: 10.5897/AJAR2015.9838
Article Number: 5D3726656161
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Genetic gain prediction in coffee progenies derived from the cross between 'Híbrido de Timor' and 'Catuaí' cultivars

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Received 22 April, 2015; Accepted 6 October, 2015

The objective of this study was to estimate genetic parameters and predict the genetic gains of coffee plant progenies using characters that are targeted in coffee breeding. The experiment was conducted in an area naturally infested with *Meloidogyne exigua* on Ouro Verde Farm, which is located in the municipality of Campos Altos in the state of Minas Gerais- Brazil. Twenty-three progenies that were potentially resistant to root-knot nematodes were used in the study, and seven commercial cultivars were used as controls. The evaluated progenies are in the fourth generation of a cross between 'Híbrido de Timor' and 'Catuaí', and they were provided by the coffee plant-breeding program conducted in Minas Gerais. The following characters were evaluated in the 2010 to 2011 and 2011 to 2012 crops: Productivity per processed coffee bags per hectare; percentage of grains at the berry stage; percentage of floating grains; grains size; and plant vigor. Furthermore, the number of *M. exigua* eggs per root gram was evaluated in the latter crop. The following genetic parameters were evaluated: Coefficient of environmental variation; phenotypic variance; genotypic variance; broad sense heritability; coefficient of genetic variation; and variation index. Gains by direct and indirect selection and the selection index, based on the sum of ranks of Mulamba and Mock, were used to estimate the genetic gain prediction. The progenies exhibited large genetic variability for the assessed traits. The index based on the sum of ranks presented higher simultaneous gains in relation to direct and indirect selection. The progenies (H493-1-2-2, H514-7-4-5, H518-2-10-1, and 514-5-2-4) were the most promising in the area infested by *M. exigua* and were identified for generational advancement based on the two procedures of analytical gain prediction.

Key words: *Coffea arabica*, selection index, breeding.

INTRODUCTION

The contributions of genetic breeding techniques for coffee cultivation are unquestionable for farmers and especially for the Brazilian economy. Although the selected cultivars had already reached high yield levels,

studies showed that new technologies are still largely demanded by Brazilian farmers and consumers. Those people long for new cultivars, which offer a differentiated product in relation to the drink quality and also an effective reduction in crop losses by rational use of agriculture inputs through resistance to the main pathogens infecting the coffee, especially plant parasitic phytonematodes, which has been responsible for serious damage to coffee crops.

Moreover, the utilization of new cultivars also effective reduction in crop losses, and cultivars that are resistant to major pathogens can be established. Regarding the coffee plant, are one of the main parasites, and they account for severe damage in coffee (Castro et al., 2008; Barbosa et al., 2010). Advances in research are made difficult by the perennial condition of this crop and the period of time required for evaluating the behavior of plants derived from sources of the *Coffea* spp. germplasm in an infested area. In fact, under Brazilian conditions, few research has been conducted under field conditions for genetic selection of plants resistant to nematodes; on the other hand, various studies, among which are Ito et al. (2008) and Boisseau et al. (2009) have been carried out under greenhouse conditions. Nevertheless, evaluation of genetic materials of the coffee plant under the conditions of an infested area in the field allow better knowledge of plant behavior and, according to Alpizar et al. (2007), verify the stability of plant reaction.

Thus, the use of more accurate selection procedures becomes essential. Estimates of genetic parameters are extremely important in breeding programs because they allow genetic effects to be distinguished from environmental effects. This aids the selection of the best breeding strategy by supplying the basis for a posterior selection of higher genotypes, with consequent reductions in the time needed to launch new cultivars (Cruz and Carneiro, 2006).

One of the most important contributions of quantitative genetics in plant breeding is the possibility of gains being obtained in the following generation. Thus, direct and indirect selection arises as the first alternative to providing compensated genetic gains (Bhering et al., 2012; Nick et al., 2013). The selection of higher progenies based on one or a few characters may not be suitable for the breeder. Therefore, the simultaneous selection of many desirable characters aims to increase the probability of program success (Gonçalves et al., 2007; Mendes et al., 2009).

The selection indices are multivariate techniques that allow the combination of various data sources in the experimental unit. This allows the selection of superior materials based on complex of variables that meet attributes of interest to the breeder, which results in best simultaneous gains (Cruz and Carneiro, 2006). The index based on the sum of "ranks" (Mulamba and Mock, 1978) has been shown in the literature by providing better simultaneous gains in various situations (Costa et al., 2004; Santos et al., 2007).Thus, the objective of this study was to predict genetic gains of progenies from the cross between 'Híbrido de Timor' and 'Catuaí' cultivars based on characters targeted by coffee plant breeding programs in an area that is naturally infested with *Meloidogyne exigua*.

MATERIALS AND METHODS

The experiment was conducted in an area infested with *M. exigua* immediately following the uprooting of an old coffee crop (December, 2000), with no soil mobilization, on the Ouro Verde Farm, which is located in the Campos Altos municipality in the Alto Parnaíba region of Minas Gerais State. The materials used in the experiment included 23 progenies that were potentially resistant to root-knot nematodes and seven cultivars that were used as controls (Table 1).

The assessed progenies were from the fourth generation of a cross between the 'Híbrido de Timor' and 'Catuaí' cultivars, and they were provided by the coffee plant-breeding program conducted in Minas Gerais, which is managed by Empresa de Pesquisa Agropecuária de Minas Gerais (EPAMIG) with the participation of Universidade Federal de Viçosa (UFV) and Universidade Federal de Lavras (UFLA).

A random block design with four replicates was used (totaling 120 plots), and each plot contained eight plants. The spacing used in the experiment was 4.0 x 0.8 m between rows and plants, respectively, which corresponded to a total area of 3072 m^2. The experiment was designed and conducted following the technical recommendations for coffee crops in the region. Evaluations of the 2010 to 2011 and 2011 to 2012 harvests were made, including the following characters: productivity per 60 kg bags•ha^{-1}; percentage of grains at the berry stage; percentage of floating grains; grain retained in high sieve (screen 17 or above); plant vigor; and the number of *M. exigua* eggs per root gram.

Yield was assessed in 60 kg bags of hulled coffee per hectare (bags•ha^{-1}). The harvest was carried out in individual plots that were measured in liters of "field coffee" (coffee fruits of mixed maturity) per plot. Subsequently, the volume of coffee harvested was converted to bags•ha^{-1}, considering the mean yield of a 60 kg bag of hulled coffee for each 480 L of "field coffee", and this yield corresponded to the regional average. The percentage of fruit at the cherry stage was determined by counting a 300 ml sample of fruit per plot. The methodology proposed by Antunes Filho and Carvalho (1954) was used to determine the percentage of floating fruits. Based on this method, 100 cherry fruits were placed in water, and the fruits remaining at the surface were considered floating fruits.

Grains size was graded (17 sieve or above) after coffee processing. A 300 g sample was passed through a set of sieve perforations (from 17/64 to 19/64 inch diameter holes). The material retained in each sieve was weighed, determining the percentage of

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Table 1. Relation and genealogy of F_4 generation progenies assessed in Campos Altos – Brazil.

S/N	Progenies	Origin
1	514-5-4-C25	CA IAC 86 x HT UFV 440-10
2	436-1-4-C26	CV IAC 99 x HT UFV 442-42
3	518-7-6-C71	CV IAC 141 x HT UFV 442-34
4	514-7-14-C73	CA IAC 86 x HT UFV 440-10
5	514-5-2-C101	CA IAC 86 x HT UFV 440-10
6	516-8-2-C109	CA IAC 86 x HT UFV 446-08
7	504-5-6-C117	CV IAC 81 x HT UFV 438-01
8	514-5-4-C121	CA IAC 86 x HT UFV 440-10
9	514-7-4-C130	CA IAC 86 x HT UFV 440-10
10	493-1-2-C134	CV IAC 44 x HT UFV 446-08
11	505-9-2-C171	CV IAC 81 x HT UFV 438-52
12	518-2-6-C182	CV IAC 141 x HT UFV 442-34
13	514-7-16-C208	CA IAC 86 x HT UFV 440-10
14	514-7-16-C211	CA IAC 86 x HT UFV 440-10
15	493-1-2-C218	CV IAC 44 x HT UFV 446-08
16	438-7-2-C233	CA IAC 86 x HT UFV 451-41
17	514-7-16-C359	CA IAC 86 x HT UFV 440-10
18	514-7-8-C364	CA IAC 86 x HT UFV 440-10
19	518-2-10-C408	CV IAC 141 x HT UFV 442-34
20	514-5-2-C494	CA IAC 86 x HT UFV 440-10
21	518-2-4-C593	CV IAC 141 x HT UFV 442-34
22	516-8-2-C568	CA IAC 86 x HT UFV 446-08
23	518-2-6-C685	CV IAC 141 x HT UFV 442-34
24	Catuaí Vermelho IAC 99*	-
25	Catuaí Amarelo IAC 62*	-
26	Topázio MG 1190*	-
27	Rubi MG 1192*	-
28	Acaia Cerrado MG 1474*	-
29	Icatu Precoce IAC 3282*	-
30	Icatu Amarelo IAC 2942*	-

CA: Catuaí Amarelo; CV: Catuaí Vermelho; HT: Híbrido de Timor.*Commercial cultivars used as control.

beans graded as screen 17 and above (Brasil, 2003). Plant vigor was evaluated by rank, according to an arbitrary 1 to 10 point scale. As suggest by Carvalho et al. (1979), 1 corresponded to the worst plant with reduced plant vigor and a sharp weakening symptom, and 10 was assigned to plants with excellent vigor, which had more leaves and sharp plant growth of productive branches.

The number of *M. exigua* eggs per root gram was evaluated by collecting the roots at 20 to 40 cm depth on both sides of the plant, perpendicular to the planting line, at an approximated amount of 50 g for all plants in the plot. After being mixed, 100 g of the roots were taken to make a sample composed of specimens from each experimental plot.

This evaluation was conducted during two seasons: the rainy season (January, 2011) and the dry season (July, 2011). Nematodes were extracted in the laboratory following the methodology of Hussey and Barker (1973). After extraction, quantification was performed in an inverted biological microscope using a counting blade to determine the number of eggs per root gram. The mean values from two assessments were considered for the analysis of variance among all traits, and a 1% significance level was adopted for the F test. The Box-Cox methodology (Box and Cox, 1964) was used to transform the data from the number of eggs per root gram with the aid of the statistical software R (R Development Core Team, 2011). The following parameters were estimated from the analyses of variance: coefficient of environmental variance (CV_e), phenotypic variance (σ^2_f), genotypic variance (σ^2_g), broad sense heritability (h^2_a), genetic variance coefficient (CV_g), and variation index (θ).

Regarding the prediction of genetic gains, direct and indirect selection gains were estimated (Cruz and Carneiro, 2006) for all of the assessed characters, and a selection of approximately 20% of the genotypes (that is, the six best progenies) were considered.

In addition to this strategy, the selection process was also conducted based on the index proposed by Mulamba and Mock (1978), in which are added the "ranks" of each genetic material for each of the characters in order to improvement favorable, resulting in selection index. This resulted in the following selection index: $I = r_1 + r_2 + ...+ r_n$, where I is the index value for a given individual or family; r_n is the rank of an individual in relation to the n^{th} trait; and n is the number of traits considered in the index. Moreover, the variables are specified for different weights, so $I = p_1r_1 + p_2r_2 +... + p_nr_n$, where p_n is the economic weight given to the n^{th} trait (Cruz and Carneiro, 2006; Santos et al., 2007).

The following economic weights were adopted in this study: CV_g;

Table 2. Summary of analysis of variance and estimates of genetic parameters for grain yield per bags.ha^{-1} (PROD), percentage of cherry grains (CG), percentage of floating grains (PFG), plant vigor (PV), bean size (S17), and number of eggs/root gram (NERG) of 23 progenies and seven cultivars evaluated in Campos Altos-Brazil.

FV	GL	Mean Square					
		PROD	CG	PFG	PV	S17	NERG[(1)]
Treatment	29	126.66**	336.17**	97.74**	2.09**	428.55**	89.13**
Block	3	65.99	47.64	2.38	0.83	316.18	326.80
Residue	87	50.32	42.24	2.91	0.18	25.29	18.26
Mean	-	41.79	50.80	9.53	6.90	33.80	13.49
CVe (%)	-	16.98	12.79	17.93	6.25	14.88	31.67
σ^2_f	-	31.67	84.04	24.44	0.52	107.14	22.28
σ^2_g	-	19.09	73.48	23.71	0.48	100.81	17.72
h^2_a	-	60.27	87.44	97.02	91.09	94.10	79.51
CVg(%)	-	10.46	16.87	51.12	10.00	29.71	31.19
θ	-	0.62	1.32	2.85	1.60	2.00	0.98

[(1)]Data transformed into (y $^{0.22}$ -1) /0.22.** Significant at 1% of probability by the F test. CVe: coefficient of environmental variation; σ^2_f: phenotypic variance; σ^2_g: genotypic variance; h^2_a: broad sense heritability; CVg: coefficient of genetic variation; θ: variation index (CVg/CVe).

h^2_a; θ; weight 1 for all characters (W1); and values provided by the relative importance (RIP) of magnitudes 100, 40, 40, 60, 60, and 80 for productivity (PROD), percentage of grains at the cherry stage (BS), percentage of floating grains (PFG), plant vigor (PV), screen 17 sieve or above (S17), and number of eggs per root gram (NERG), respectively. Regarding productivity, BS and S17 selection was performed towards addition, and selection for PFG and NERG was done in the opposite direction. All statistical-genetic analyses were performed using the Genes software (Cruz, 2006).

RESULTS AND DISCUSSION

Significant differences were observed for all of the assessed characters, which indicated that progenies exhibited differentiated behavior under the trial conditions (Table 2). Values of CV_e ranged from 6.25% (PV) to 31.67% (NERG), revealing good experimental precision. High CV_e values for the variables related to the nematode population in the roots were also found in other cultures by Moura et al. (2008) and Silva et al. (2011), and they may be explained by the complex methods used to evaluate this pathogen, especially under field conditions.

Broad-sense heritability (h^2_a) reflects the importance of inheritance and the environment in character expression. The values found for heritability were 60.27, 87.44, 97.02, 91.09, 94.10, and 79.51% for PROD, CG, PFG, PV, S17, and NERG, respectively (Table 2). High heritability values were found for all characters when compared to other trials that evaluated *Coffea arabica* progenies (Botelho et al., 2007; Petek et al., 2006). Interestingly, the heritability of the studied trait should be high to achieve successful selection (Miranda et al., 1988). The higher the expression level of genetic variability in relation to the environment, the higher the gains estimated for the next generation.

Thus, such results provide evidence that supports the existence of genetic variability among progenies, with possible selection gains. This indicates the possibility of obtaining high genetic gains with genetics improvement to the characteristics studied, which are of high interest commercial, and that parents with higher PROD, CG, PFG, PV, S17 and NERG can be used in directed crosses genotypes aimed these characteristics.

CV_g expresses the magnitude of genetic variation in relation to the mean of the trait. Estimates of CV_g shown in Table 2 reveal that the analyzed traits generally presented variations ranging from 10.00% (PV) to 51.12% (PFG). Bonomo et al. (2004) and Mistro et al. (2007), when studying progenies derived from the germplasms of 'Híbrido de Timor', in F_3 and F_4 generations, respectively, also found high genetic variability. Moreover, this variability supports the conclusion that the selection of the best progenies will increase expression and the genetic value of the population.

The proportion of CVg to CVe is referred to as the variation index (θ). According to Vencovsky (1987), when this index is near or higher than 1.0, it indicates a situation where selection is favorable. The values found in this study (Table 2) show a satisfactory situation for selection of all characters, with the exception of productivity, which showed a value of 0.62. Values less than 1.0 for productivity were also reported by Bonomo et al. (2004), and can be explained by the fact that productivity is a quantitative inheritance character and also by the coffee biannuality.

Therefore, the genetic variation in productivity depends on allelic variation in a large number of loci, and expression of those loci is highly affected by environmental factors (Guo et al., 2004; Huang et al., 2011). However, productivity is an important variable, and any gain in this character should be taken into account.

Estimates of the direct and indirect gain of selection are

Table 3. Estimates of percentage gains by indirect and direct selection for productivity per bags.ha^{-1} (PROD), percentage of cherry grains (CG), percentage of floating grains (PFG), plant vigor (PV), bean size (S17), and number of eggs/root gram (NERG) from 23 progenies in F_4 generation and seven cultivars evaluated in Campos Altos-Brazil.

Selection on	Expected gain in (%)					
	PROD	CG	PFG*	PV	S17	NERG*
PROD	11.69	8.85	-22.32	6.09	20.36	-13.97
CG	6.38	18.56	-19.78	7.86	5.21	-12.63
PFG*	3.19	-4.08	-48.21	0.16	24.76	-2.81
PV	7.62	16.67	-24.02	12.12	-10.53	-18.69
S17	7.44	13.90	0.59	3.60	39.84	-17.61
NERG*	5.35	2.03	-13.62	6.21	0.07	-35.81

*Variables selected towards decrease (it is desired negative values for those characters).

Table 4. Estimates of percentage gains by simultaneous selection, using the index based on the sum of ranks, based upon five economic weight criteria[(1)], for grain productivity per bags.ha^{-1} (PROD), percentage of cherry (CG), percentage of floating grains (PFG), plant vigor (PV), bean size (S17), and number of eggs/root gram (NERG) from 23 progenies in F_4 generation and seven cultivars assessed in Campos Altos-Brazil.

Trait	Expected gain in (%)				
	CVg	h^2_a	CVg/CVe	W1	WRI
PROD	9.27	9.05	9.27	9.05	10.73
CG	11.21	15.08	11.21	15.08	10.65
PFG[(2)]	-42.27	-30.81	-42.27	-30.81	-29.96
PV	5.80	5.66	5.80	5.66	6.90
S17	24.96	27.61	24.96	27.61	22.30
NERG[(2)]	-19.34	-20.06	-19.34	-20.06	-22.59

[(1)]CVg: coefficient of genetic variation; h^2_a: broad sense heritability; CVg/CVe: variation index; W1: weight 1 for all characters; WRI: values provided by the relative importance (100, 40, 40, 60, 60 and 80).[(2)] Variables selected towards decrease.

presented in Table 3. From this selection, gains were obtained from the main character (upon which selection was performed) with the possible occurrence of favorable and unfavorable responses in the characters of secondary importance (Costa et al., 2004).

Considering that the target was to obtain an increase in the PROD, CG, PV, and S17 characters and a decrease in PFG and NERG, the best result was achieved when selection was performed on the PROD trait, which provided positive gains of 11.69, 8.85, 6.09, and 20.36% for PROD, CG, PV, and S17, and negative values of 22.32 and 13.97% for PFG and NERG, respectively. Thus, based on direct selection on yield, progenies H493-1-2-2, H518-2-10-1, 514-7-4-5, 436-1-4-2, 518-2-6-1, and 514-5-2-4 were selected as the promising ones for the generational advancement. The index based on the sum of ranks (Mulamba and Mock, 1978) was also used to target increasing reliability of the results obtained in this experiment.

The results indicate that it is possible to obtain desirable gains for all assessed characters, considering all the provided economic weights (Table 4). This suggests that it is possible to promote the effective increase of favorable alleles via the stocking of the assessed characters in the population. Among the economic weight options used to obtain selected gains, the results indicated that weight, based on the relative importance (WRI), presented the best results with predicted gains of 10.73, 10.65, 6.90, and 22.30% for PROD, CG, PV, and S17, respectively. This was in addition to the negative gains for PFG (-29.96%) and NERG (-22.59) (Table 4), which were the favorable characters for the selection of higher genotypes, where the number of plants with floating fruits and susceptibility to *M. exigua* tended to be lower.

Although yield gain was smaller than indirect and direct selection, the index of Mulamba and Mock (1978) provided greater magnitudes of predicted gain for all of the other characters, which corroborates the results of Costa et al. (2004) and Santos et al. (2007). Progenies selected by the Mulamba and Mock (1978) procedure, based on WRI, were as follows: H493-1-2-2, H514-7-4-4, H518-2-10-1, H514-7-16-3, H493-1-2-8, and H514-5-2-4. The results show that four of these (H493-1-2-2, 514-7-4-5, 518-2-10-1, and 514-5-2-2) were also selected by the criteria for direct and indirect selection, which confirms the potential of these progenies for generational advancement.

Conclusions

The progenies presented large amounts of genetic variability for the assessed characters, and the index based on the sum of the ranks presented comparable gains relative to direct and indirect selection. The H493-1-2-2, H514-7-4-5, H518-2-10-1, and 514-5-2-4 progenies were the most promising in the area infested by *M. exigua* and pointed to generational advancement based on the two analytical gain prediction procedures.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Consórcio Brasileiro de Pesquisas Cafeeiras and INCT- Café for supporting the Project. We also thanks CNPq for granting the fellowship (RMR and JCR).

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Vol. 10(46), pp. 4258-4267, 12 November, 2015
DOI: 10.5897/AJAR2015.9551
Article Number: 64D668556163
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Characterization of *Pectobacterium* species isolated from vegetable crops in north-west of Iran

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Received 26 January, 2015; Accepted 16 October, 2015

In August 2012, vegetable crops including potato, cabbage, bell pepper and carrot showing symptoms of maceration and water soaked lesions on their tuber, leaf and fruit were collected from major vegetable growing areas in north-west of Iran. Physiological and biochemical assays divided the isolates into two main groups according to their ability to grow at 37°C. In addition, these two groups were differentiated by some biochemical characteristics such as utilization of α –methyl glucoside and maltose, reducing substrates from sucrose and gelatin liquefaction. Partial sequence of polymerase chain reaction (PCR) product from reaction of putative *Pectobacterium* spp. with 16S rRNA confirmed the results obtained from physiological and biochemical assays used for identification of the bacterium. All of the causal organisms isolated from infected tissues were identified as *Pectobacterium* spp. based on biochemical characteristics and PCR amplification of the 16S rRNA gene with specific primers PCCSSF and PCCSSR. Twenty of the isolates were identified as *Pectobacterium carotovorum* subsp. *carotovorum* using a polyphasic approach including physiological, biochemical and molecular characteristics. Only six isolates from infected bell peppers and five isolates from infected potatoes identified as *Pectobacterium wasabiae* and *Pectobacterium atrosepticum* respectively. Our findings based on physiological, biochemical and molecular assays indicated the occurrence of *P. wasabiae* as a novel species in bell pepper in Iran. Moreover, genetic diversity of *Pcc* strains in this study was also performed based on Repetitive extragenic palindromic-PCR (rep-PCR). High genetic variability was revealed among these *Pcc* strains by rep-PCR typing using two primers sets for ERIC-PCR and BOX-PCR.

Key words: 16S rRNA, repetitive extragenic palindromic- polymerase chain reaction (rep-PCR), *Pectobacterium*, soft rot, vegetable.

INTRODUCTION

Bacterial soft-rot caused by *Pectobacterium* spp. has been considered as one of the most recurrent diseases observed in variety of vegetable crops and fruits species worldwide and cause great economic loss of crops (Yahiaoui-Zaidi et al., 2003; Agrios, 2005; Farrar et al., 2000; Toth et al., 2001). Soft-rot symptoms begin as a small water-soaked lesion, which enlarges rapidly in diameter and in depth.

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The affected area becomes soft and mushy while its surface becomes discolored and somewhat depressed. Tissues within the affected region become cream colored and slimy, disintegrating into a mushy mass of disorganized plant cells and bacteria. The outer surface may remain intact while the entire contents have changed to a turbid liquid; alternatively, cracks develop and the slimy mass exudes to the surface and, in air, turns tan, gray, or dark brown. A whole fruit or tuber may be converted into a soft, watery, decayed mass within 3 to 5 days. Infected fruits and tubers of many plants are almost odorless until they collapse, and then secondary bacteria grow on the decomposing tissues and produce a foul odor (Agrios, 2005). These groups of bacteria are not specific to plant hosts and produce pectolytic enzymes which damage fleshy fruits, vegetables, ornamental plants and some other agricultural crops, especially in storage (Fahy and Persely, 1983). Hence, characterization of *Pectobacterium* species and subspecies is very important for effective managing of them (Toth et al., 2001).

The recent studies have clarified the taxonomy of genus and species of Entrobacteriaceae via using biochemical and molecular methods (Gardan et al., 2003). Before these studies, the soft rot causing bacteria of Entrobacteriaceae belonged to *Erwinia* genus, but the results of recent investigations were led to distinction of *Dickeya* and *Pectobacterium* genera (Gardan et al., 2003; Samson et al., 2005). For the first, the *Erwinia* genus was described in 1917 and it included all of Entrobacteriaceae family that caused the disease in different plants (Perombelon, 1990). However, further studying on diversity of these bacteria especially comparison of 16s rDNA sequence, led to identification of new genera including *Dickeya*, *Brenneria*, *Entrobacter*, *Pectobacterium*, *Pantoea* and *Samsonia*.

The genus *Pectobacterium* has been divided into several species and subspecies on the basis of molecular, biochemical and host range differences (Gardan et al., 2003; Duarte et al., 2004; Ma et al., 2007; Van der Merwe et al., 2010). To date, five *Pectobacterium* species have been described: *Pectobacterium atrosepticum, Pectobacterium betavasculorum, Pectobacterium carotovorum, Pectobacterium wasabiae* (Gardan et al., 2003) and the recent one *Pectobacterium brasiliensis* (Daurte et al., 2004; Van der Merwe et al., 2010), which is phylogenetically further apart from the other four *Pectobacterium* spp. (Ma et al., 2007). The species *P. betavasculorum* and *P. atrosepticum* constitute an exception to the broad-host range nature of *Pectobacterium* spp, since they have been reported exclusively on sugar beet and potato respectively (Ma et al., 2007). Whereas the species *P. carotovorum, P. brasiliensis,* and *P. wasabiae* have been reported to cause disease on potato and other plant species (Kim et al., 2009). *P. carotovorum* subsp. *carotovorum* (*Pcc*) has a broad host range in comparison with other subspecies of this bacterium and disperses in the subtropical and moderate zones (Perombelon and Kelman, 1980). *P. c.* subsp. *carotovorum* has been previously isolated from potato producing areas in Iran (Soltani-Nejad et al., 2005; Firoz et al., 2007; Baghaee et al., 2011). *P. atrosepticum* potato soft rot and blackleg causing often disperses in the cold and moderate zones (Perombelon and Van der Wolf 2002). However, there are some reports that *P. atrosepticum* can cause soft rot disease in tomato (Gardan et al., 2003). *P. atrosepticum* also has been reported in south-west and north of Iran (Zohour Paralak et al., 2007; Baghaee et al., 2011). *P. wasabiae* previously had been isolated from radish (Goto and Matsumoto, 1987) and recently it has been reported from potato (Kim et al., 2009; Baghaee et al., 2011). To this date, there is no report of *P. wasabiae* on bell pepper from Iran.

The occurrence of pectolytic enterobacteria causing soft rot have been previously reported in different plants such as radish (Goto and Matsumoto, 1987), aglaonema (Chao et al., 2006), dieffenbachia (MacFadden, 1961; Nieves-Brun et al., 1985), tulip (Boyraz et al., 2006), syngonium (Alippi and Lopez, 2009), potato, tomato, carrot and cabbage (Maisuria and Nerurkar, 2013), artichoke (Loreti et al., 2001), sweet potato, chinese cabbage and eggplant (Golkhandan et al., 2013). The key objective of this research was to identify *Pectobacterium* spp. as the causal organisms of soft rot through physiological, biochemical and molecular techniques in some vegetable crops in north-west of Iran.

MATERIALS AND METHODS

Bacterial strains

The bacterial strains used in this study were isolated from bell pepper (*Capsicum annuum*), potato (*Solanum tuberosum*), carrot (*Daucus carota*) and cabbage (*Brassica oleracea*) collected from different vegetable growing regions in north-west of Iran which were affected by soft rot disease (Table 1). The disease is characterized by dark and small water-soaked lesions or soft rot symptoms on potato tuber, carrot root, cabbage leaf and bell pepper fruit. The type strains of *P. carotovorum* subsp. *carotovorum* (IBSBF-863 = ATCC15713), *P. atrosepticum* (IBSBF-1819 = ATCC33260), *P. betavasculorum* (IBSBF-787 = ATCC43762), *P. carotovorum* subsp. *odoriferum* (IBSBF-1814 = ICMP11533) obtained from the Instituto Biológico Seção de Bacteriologia Fitopatologia (IBSBF) and reference strain of *P. wasabiae* (SCRI 488) obtained from Scottish Crop Research Institute (SCRI) were included for comparison in different tests.

Media and cultural condition

Isolation of bacteria from infected samples was carried out according to Perombelon and Van der Wolf (2002). Briefly, after washing the diseased plants small amounts of root, leaf, fruit and tuber samples from the margin of healthy and diseased tissue were homogenized in 1 to 2 drops of sterile water, allowing 2 to 30 min to stand. The suspensions were placed on nutrient agar sucrose

Table 1. *Pectobacterium* strains isolated from four vegetable crops in some major vegetable growing regions in north-west of Iran.

Source	No. of strain(s)	Geographical origin in Iran	Host species	Plant part
Field	2(CrBo10, CrBo11)	Azarbaijan sharghi- Bostan abad	*Daucus carota*	Root
Field	2(CrTa12, CrTa13)	Azarbaijan sharghi- Tabriz	*Daucus carota*	Root
Field	2(CrMa14, CrMa15)	Azarbaijan sharghi-Marand	*Daucus carota*	Root
Field	2(CrUr17, CrUr18)	Azarbaijan gharghi-Urmia	*Daucus carota*	Root
Field	2(CrKh19, CrKh20)	Azarbaijan gharghi-khoy	*Daucus carota*	Root
Field	3(CbUr21, CbUr22, CbUr24)	Azarbaijan gharghi-Urmia	*Brassica oleracea*	Leaf
Field	5(CbNs25, CbNs30 to CbNs33)	Azarbaijan gharghi- Nushin shahr	*Brassica oleracea*	Leaf
Store	4(PtUr26, PtUr27, PtUr37, PtUr38)	Azarbaijan gharghi- Urmia	*Solanum tuberosum*	Tuber
Store	4(PtBo28, PtBo23 PtBo29, PtBo44)	Azarbaijan sharghi-Bostan abad	*Solanum tuberosum*	Tuber
Store	2(PtMa16, PtMa35)	Azarbaijan sharghi-Marand	*Solanum tuberosum*	Tuber
Store	1(PtBo36)	Azarbaijan sharghi-Bostan abad	*Solanum tuberosum*	Tuber
Store	2(BpMa39, BpMa40)	Azarbaijan sharghi-Marand	*Capsocum annuum*	Fruit
Store	2(BpUr41, BpUr42)	Azarbaijan gharghi-Urmia	*Capsocum annuum*	Fruit
Store	2(BpNs43, BpNs45)	Azarbaijan gharghi-khoy	*Capsocum annuum*	Fruit

(NAS) and eosin methylene blue (EMB) (Schaad et al., 2001). After 24 h of incubation at 25°C, single colonies with white to creamy color and irregular margin on NAS or emerald green on EMB were purified on nutrient agar sucrose medium. All selected isolations were stored in sterile water at 4°C for further investigations.

Physiological and biochemical characterizations

Isolates which were characterized as positive in pectolytic activity were submitted for the biochemical and physiological tests including gram reaction, fermentative metabolism (Hugh and Leifson, 1953), oxidase and catalase activity (Schaad et al., 2001). Hydrolysis of gelatin, casein and lecithin were tested on gelatin agar, skimmed-milk agar and egg-yolk agar respectively (Dickey and Kelman, 1988). They were checked for production of phosphatase, ability to grow at 37°C in nutrient broth, growth in 4 and 5% sodium chloride on nutrient agar at 28°C, urease activity, levan production and gelatin liquefaction (Schaad et al., 2001).

Moreover, production of reducing substances from sucrose, malonate utilization and indole production from tryptophan, anaerobic degradation of arginine and utilization of citrate were examined using previously described methods (Gallois et al., 1992; Gardan et al., 2003).

In addition, the utilization of carbon sources were tested on the basal medium of Ayers et al. (1919) supplemented with 0.1% carbohydrates including lactose, D-fructose, D-Sucrose, D-glucose, D-galactose, trehalose, α-methyl glucoside, D- arabitol, D-melibiose, mannitol, raffinose, sorbitol, maltose, cellubiose and starch. Hypersensitivity reaction (HR) assays were performed with all the isolates (Bauer et al., 1994). Sensitivity assays of the isolates to erythromycin were performed using previously described methods (Schaad et al., 2001; Klement et al., 1990).

Pathogenicity assays

The pathogenicity assay was performed with different vegetable crops including cabbage (*Brassica oleracea*), potato (*Solanum tuberosum*), bell pepper (*Capsicum annuum*), and carrot (*Daucus carota*). The surface of the potato tubers, bell pepper fruits and carrot roots were sanitized in 70% ethyl alcohol for 30 s and were washed with sterilized distilled water. Tubers and fruits then needle punctured with 10 µl (10^8 CFU/ml) overnight culture of each strain grown on nutrient broth (NB) for 24 h at 27°C. Inoculated and non-inoculated (control) tubers and fruits were incubated in a moist chamber with 80 to 90% relative humidity at 27°C. After 72 h, macerated tissue was scooped from the tubers and fruits and weighed to determine the extent of tissue maceration (Yap et al., 2004). Pathogenicity tests were carried out similarly on cabbage leaves. However, in this case, the leaves of each plant were separated before sanitization, injected with 10 µl of a bacterial suspension (10^8 CFU/ml) with a syringe, and then incubated at 28°C for 48 h in a moist chamber with 80 to 90% relative humidity (Kim et al., 2007). Distilled water was used as a negative control. Re-isolations from inoculated plants were confirmed by pectolytic assay, morphology and biochemical tests.

Molecular characterization

Bacterial DNA extraction

Total genomic DNA was extracted from the bacterial isolates according to Cheng and Jiang (2006) protocol. Briefly, 1 ml cell suspension was centrifuged at 8000 g for 2 min. After removing the supernatant, the cells were washed with 400 µl STE Buffer (100 mM NaCl, 10 mM Tris/ HCl, 1 mM EDTA, pH 8.0) twice. Then the cells were centrifuged at 8000 g for 2 min. The pellets were re-suspended in 200 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0). Then 100 µl Tris-saturated phenol (pH 8.0) was added to these tubes, followed by a vortex-mixing step of 60 s to lyse cells. The samples were subsequently centrifuged at 13000 g for 5 min at 4°C to separate the aqueous phase from the organic phase. 160 µl upper aqueous phases was transferred to a clean 1.5 ml tube. 40 µl TE buffer was added to make 200 µl and mixed with 100 µl chloroform and centrifuged for 5 min at 13 000 g at 4°C. Lysate was purified by chloroform extraction until a white interface was no longer present; this procedure might have to be repeated two to three times. 160 µl upper aqueous phase was transferred to a clean 1.5 ml tube. 40 µl TE and 5 µl RNase (at 10 mg/ml) were added and incubated at 37 C for 10 min to digest RNA. Then 100 µl chloroform was added to the tube, mixed well and centrifuged for 5 min at 13 000 g at 4°C. 150 µl upper aqueous phase was transferred to a clean 1.5 ml tube. The aqueous phase contained purified DNA and was directly used for the subsequent experiments or stored at-20°C.

16S rRNA gene sequence analysis

For a rapid and simple identification and differentiation of Iranian isolates, polymerase chain reaction (PCR) assays were applied with primers PCCSSF (5'-ATAACTACTGGAAACGGTA-3') and PCCSSR (5'-TTCTCTTTGTATACGCCATT-3'). The 16S rRNA gene assays for the isolates were carried out using the method described by Yanagi and Yamasato (1993). PCR was performed in 25 µl of a reaction mixture containing 2.5 µl of 10xPCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of deoxynucleoside triphosphates, 1.5 U of Taq polymerase, 10 pmol of each primer and 3 µl of template DNA. PCR amplification was carried out using thermal cycler (Techne-TC-512) with the following thermal regime; initial denaturing for 3 min at 95°C, 35 cycles of denaturing at 94°C for 60 s, followed by annealing at 49°C for 60 s, elongation at 72°C for 60 s and final extension step at 72°C for 10 min. Amplified DNA fragments were detected by electrophoresis in a 1.0% agarose gel stained with ethidium bromide. The PCR products of representative strains were purified and sequenced by Macrogen Inc. (Seoul, South Korea) using an ABI3730 XL automatic DNA sequencer and the primers PCCSSF and PCCSSR. The identification of the isolates was performed using BLAST (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi) in NCBI.

Rep-PCR based DNA finger printing

Two repetitive extragenic palindromic- polymerase chain reaction (rep-PCR), genomic fingerprinting methods were performed using single oligonucleotide primer BOX A1R (5'-CTACGGCAAGGCGACGCTGACG-3') and oligonucleotide primer pair ERIC1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'- AAGTAAGTGACTGGGGTGAGCG-3') based on recommended methods with a little changes (Versalovic et al., 1991; Gillings and Holly, 1997; Weingart and Volksch, 1997). PCR amplifications were performed in 25 µl of a reaction mixture containing 2.5 µl of 10×PCR buffer, 2 mM $MgCl_2$, 0.2 mM of deoxynucleoside triphosphates, 10 pmol of each primer, 1.5 U of Taq polymerase and 3 µl of template DNA. PCR amplification was carried out using thermal cycler (Techne-TC-512) with the following thermal regime; initial denaturing for 5 min at 94°C, 34 cycles of denaturing at 94°C for 40 s (BOX) and 50 s (ERIC), followed by annealing at 50°C for 40 s and 52°C for 90 s for BOX and ERIC primers respectively, elongation at 72°C for 60 s (BOX) and 240 s (ERIC) and final extension step at 72°C for 10 min. After finishing the PCR reactions, 7 µl of amplified products were mixed with 2 µl of loading buffer (Dye) and were electrophoresed through a 1.5% agarose gel in 1x TBE buffer at 80 V for 90 min and stained with ethidium bromide (0.5 µg/ml), and visualized under UV light using gel documentation. The Gene Ruler 1 kb DNA Ladder (Ready to Load, Solis BioDyne) was used to determine fragment size.

Data analysis

Clustering of bacterial isolates in this study was performed with comparison of phenotypic and biochemical characteristics and also based on fingerprints of genomic DNA of *Pcc* isolates in agarose gel. The rep-PCR banding patterns of agarose gel were scored as present (1) or absent (0) for each rep-PCR. Also, each physiological or biochemical characteristic was counted as a unit character; positive (1) or negative (0) test results were scored as binary traits. Cluster analysis and dendrogarm drawing were done by using the NTSYS software (version 2.02; Exeter Software, USA), using Jaccard coefficient and simple matching according to the unweighted pair group method with arithmetic averages (UPGMA) (Sutra et al., 2001). For phylogenetic analysis, 16S rRNA gene sequences were aligned with Geneious software (Java Version 1.7.0_51-b13) and the phylogenetic tree was created using Geneious Tree Builder software according to genetic distance model Tamura Nei, Neighbour-joining (NJ) method, and bootstrap 500.

Nucleotide sequence accession numbers

The 16S rDNA sequences from *Pectobacterium* CbUr23, CbUr22, PtUr26 and BpUr43 strains in this study were deposited in the GenBank database under the following accession numbers: KM371724, KM371725, KM371726 and KM371727 respectively.

RESULTS

Phenotypic and biochemical characteristics

A survey was performed on infected vegetable tissues such as potato tuber, carrot root, bell pepper fruit and cabbage leaf in 2012-2013. In total, 35 isolates from four vegetable crops from north-west of Iran were all identified as *Pectobacterium* spp. by biochemical and phenotypic assays. The physiological and biochemical tests divided the isolates into two main groups according to their ability to grow at 37°C (Table 2). In addition, these two groups were differentiated by utilization of α –methyl glucoside and maltose, reducing substrates from sucrose and gelatin liquefaction. All bacterial cultures isolated from the survey exhibited pectolytic ability on potato slices. The isolates were also negative for indole production, phosphatase activity, acid production from maltose, utilization of malonate and urease activity. All the isolates were positive caseinase activity, methyl red reaction and utilization of trehalose, α-D-melibiose, citrate, L-glutamate and D-glucuronate. The biochemical tests for production of phosphatase and indole, sensitivity to erythromycin, growth in sodium chloride 5% agreed with those expected for *Pectobacterium* spp. (Gallois et al., 1992; Gardan et al., 2003). Therefore, based on biochemical features, all of the isolates of group 2 were determined as *P. carotovoum* subsp. *carotovorum*. Whereas, the isolates of group 1 showed heterogenous biochemical and physiological characteristics. All of the six isolates obtained from bell pepper belonged to group 1 were identified as *P. wasabiae* and they could be differentiated by other biochemical tests such as reducing substances from sucrose, utilization of lactose, and raffinose that were negative for *P. wasabiae*. Only four isolates from infected potatoes belonged to group 1 were identified as *P. atrosepticum*. The rest of group 1 initially described as atypical *P. carotovoum* subsp. *carotovorum* because they did not grow at 37°C. Despite this, there were five *Pectobacterium* spp. strains that their phenotypic and biochemical characteristics were not fitted well with the results obtained from type strains.

Pathogenicity test and hypersensitive response

The pathogenicity of representative strains of potato, bell

Table 2. Comparison of phenotypic features of isolated strains with those of *Pectobacterium* spp. type and reference strains.

Test	Iranian strains		Type and reference strains				
	Group 1 (n=19)	Group 2 (n=16)	Pcc	Pco	Pa	Pb	Pw
Pectolytic ability	+	+	+	+	+	+	+
Grow at 37°C	-	+	+	+	-	+	-
OF	FA[a]	FA	FA	FA	FA	FA	FA
Growth in 5 % NaCl	+	+	+	+	+	+	+
Production of Indole	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Rss [b]	80 [c]	+	-	+	+	+	-
Phosphatase	-	-	-	-	-	-	-
Gelatin liquefaction	+	-	+	+	-	-	+
Lecithinase	-	24	-	-	-	-	-
Caseinase	+	+	+	+	-	-	+
Sensitivity to Erythromycine	-	-	-	-	-	-	-
Methyl Red reaction	+	+	+	+	+	-	+
Utilization of:							
Trehalose	+	+	+	+	+	+	+
Lactose	85	+	+	+	+	+	-
α –Methyl glucoside	74	-	-	+	+	+	-
Maltose	80	-	-	+	+	+	-
D-Sorbitol	-	-	-	+	-	-	-
Raffinose	82	+	+	+	+	+	-
α-D(+)-Melibiose	+	+	+	+	+	-	-
D-(+)-Cellobiose	89	+	+	+	+	-	+
Citrate	+	+	+	-	+	-	+
Malonate	-	-	-	-	-	-	-
L-Glutamate	70	+	+	+	-	+	-
D-Glucuronate	+	+	+	-	+	+	+

+positive reaction; − negative reaction, [a] Facultatively anaerobic, [b] Reducing substrates from sucrose,[c] Percentage of isolates that tested positive.

pepper, carrot and cabbage was quantified using tuber, fruit, root and leaf of these vegetable crops respectively. All inoculated potato tubers, bell pepper fruits, carrot roots and cabbage leaves exhibited soft rot symptoms after 72 h similar to those observed in the fields and stores and the same bacteria were consistently re-isolated. Symptoms were not observed on water-inoculated controls. In the pathogenicity assay of potato tubers and carrot roots, both groups 1 and 2 produced small water soaked lesions or soft rot symptoms by the end of 48 h after inoculation. Disease symptoms on bell pepper fruits initiated by water-soaked lesions, and crumpled skin near the inoculation site after 48 h that extended gradually. At the same period, water-soaked and dark-green lesions appeared on cabbage leaves that were rotten rapidly. Strains of both groups showed the same symptoms and severity in potato maceration and pathogenicity on host plants assays. No signs of HR reaction or tissue collapse were observed in areas injected with any of the Iranian *Pectobacterium* species on tobacco 24 h after inoculation. As expected, HR reaction signs were not visible on tobacco injected with distilled water as negative control.

Analysis of 16S rRNA gene of the isolates

To confirm the results obtained by physiological and biochemical assays, we analyzed the partial 16S rRNA gene sequence by PCR amplification using the primer sets described in materials and methods. The PCR amplified product had approximately the size of 1200 bp when electrophoresed on 1% agarose gel (Figure 1). When the PCR products obtained using specific primers were sequenced and analyzed by comparison with available sequences in the GenBank database, BpUr43 16S rRNA gene was 99% homologous to the *P. wasabiae* strain WPP163. The PtBo23 16S rRNA gene

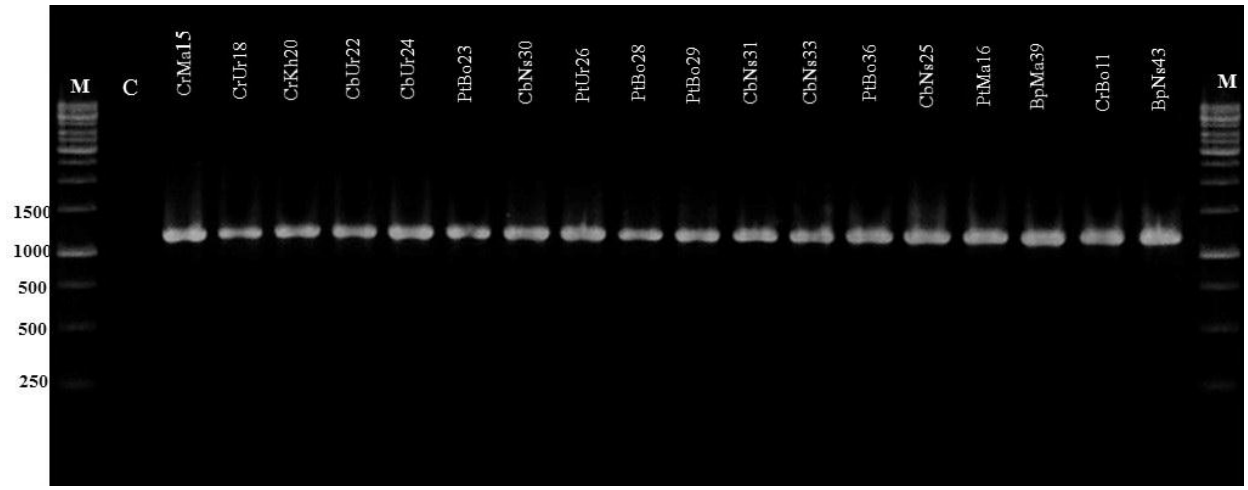

Figure 1. The 1200 bp fragment from the 16S rRNA gene of representative isolates in 1% agarose gel. C: Negative control. M: 1kb DNA ladder (Name of isolates have been given in Table 1).

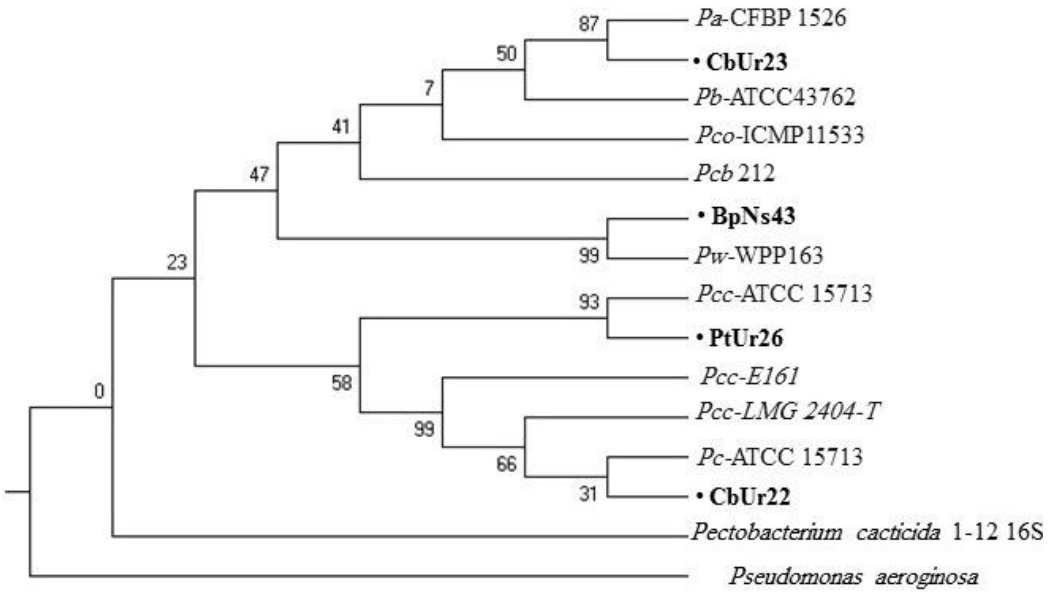

Figure 2. The relationship between Iranian *Pectobacterium* isolates (CbUr23, BpNs43, PtUr26 and CbUr22), and other *Pectobacterium* strains based on 16S rRNA nuclotide sequence data.

exhibited 99% homology to the 16S rRNA gene of *P. atrosepticum* strain CFBP 1526. Whereas the strains CbUr22 and PtUr26 16S rRNA gene exhibited 99% homology to the 16S rRNA gene of *P. carotovorum* strain ATCC 15713 and *P. carotovorum* subsp. *carotovorum* strain ATCC 15713 respectively. The phylogenetic tree of the isolates obtained by Neighbour-joining (NJ) and Maximum Likelihood (ML) methods using MEGA 6 software (Figure 2). *Pseudomonas aeruginosa* used as outgroup in this study. Partial sequence of PCR product from reaction of putative *Pectobacterium* spp. with 16S rRNA confirmed the results obtained from physiological and biochemical assays used for identification of the bacterium. Application of specific primers such as PCCSSF/PCCSSR successfully differentiated Iranian *P. wasabiae* and *P. carotovorum* subsp *carotovorum* isolates from other species and subspecies of *Pectobacterium*.

Rep-PCR genomic fingerprinting of isolated Pcc strains

DNA amplification of *Pcc* strains and standard isolate

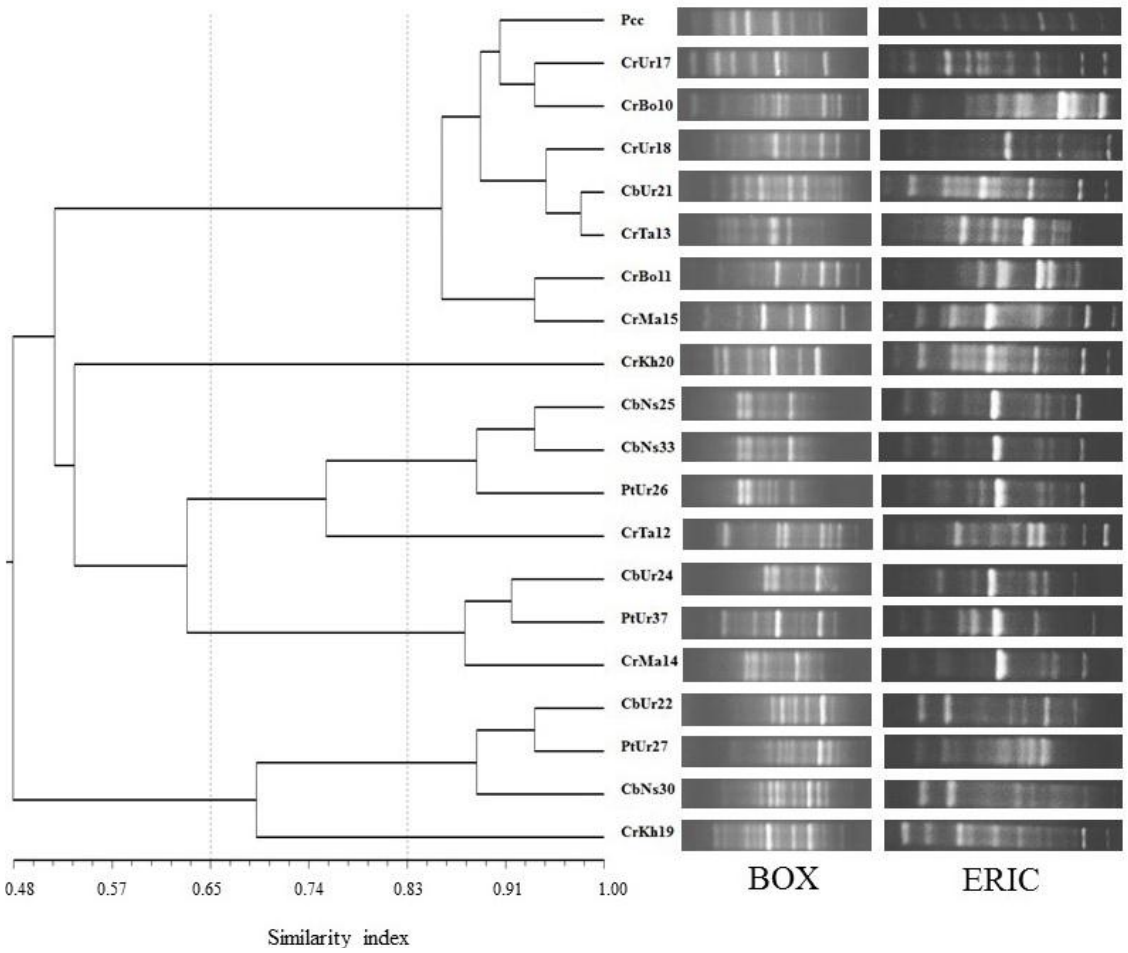

Figure 3. Cluster analysis of combined ERIC and BOX-PCR genomic fingerprint patterns of the Pcc strains. The UPGMA algorithm was applied to the similarity matrix generated with Jaccard's coefficient (Name of isolates have been given in Table 1).

were performed by rep-PCR method using two BOX and ERIC primers and they were assessed for their abilities to differentiate between isolated strains of *P. carotovorum* subsp. *carotovorum*. The rep-PCR banding patterns of each gel were normalized by Alpha Ease Fc 4.0 (Alpha Innotech of San Leandro, Calif.). Normalized bands were scored as present (1) or absent (0) for each rep-PCR. Individual and combined finger prints of two rep-PCR were analyzed by Pearson correlation and UPGMA using NTSYSpc software (ver 2.0; Exeter Software, USA). On this basis, with the use of BOX-PCR amplified DNA, fingerprints ranged from nine to 18 bands with sizes between 200 and 2,500 bp. While ERIC-PCR produced 11 to 20 bands with sizes between 180 and 4,500 bp (Figure 3).

Based on UPGMA, BOX-PCR and ERIC-PCR individual profiles revealed three main clusters at 58 and 55.5% similarity value respectively and were subdivided the *Pcc* strains to five clusters with a similarity coefficient of 65%. Whilst cluster analysis of combined finger-print patterns of two rep-PCR revealed three clusters with a similarity coefficient of 53%, which were subdivided to five and seven clusters with a similarity coefficient of 65 and 83% respectively (Figure 3).

DISCUSSION

Our research goal was to identify *Pectobacterium* spp. as the causal organisms of soft rot through phenotypic and molecular techniques. A polyphasic approach including physiological, biochemical and molecular characteristics was used in the present investigation to identify isolated strains of soft rot causing *Pectobacterium* species from carrot, potato, bell pepper, and cabbage in north-west of Iran.

We examined two methods to differentiate the *Pectobacterium* isolates and found that phylogenetic analysis of the 16S rRNA gene was the most accurate method to characterize our set of pectolytic enterobacteria.

Our results have demonstrated that all of the isolated bacteria from infected tissues (35 isolates) belonged to *Pectobacterium* spp. Most of the isolates were identified as *P. carotovorum* subsp. *carotovorum* using biochemical and physiological experiments. The existence of some unusual *Pcc* strains from potato, carrot and cabbage (group 1) that were unable to grow at 37°C and their inability to elicit HR on tobacco leaves persuaded us to check these isolates with analysis of the 16S rRNA gene. We applied PCR-based technique with specific primer for *Pectobacterium* sp. to enhance detection simplicity and rapidity in comparison to physiological and biochemical assays. However, all the strains were able to amplify specific bands relevant to Pectobacterium spp. Diversity in phenotypic characteristic among isolated *Pcc* strains from different plant hosts within the same geographical regions have been commonly reported in Golkhandan et al. (2013); Baghaee et al. (2011); Toth et al. (2011); Hu et al. (2008); Duarte et al. (2004) and Gardan et al. (2003).

Pectolytic Erwinias do not have specific host range and they can cause soft rot disease in broad host range and vast geographical distribution (Barras et al., 1984). The pathogenicity of obtained isolates from one host on another plants confirmed this subject. The virulence of representative strains of carrot and bell pepper were higher than other isolates.

P. carotovorum subsp *carotovorum* was previously reported as the major soft rotting causal agent on vegetable crops and ornamental plants in Iran (Soltani-Nejad et al., 2005; Firoz et al., 2007; Baghaee et al., 2011). Recently in parts of Iran another species of soft rotting bacteria was observed which was different from *Pcc* in some aspects (Baghaee et al., 2011). These isolates, unlike typical *Pcc* strains could not grow at 37°C and elicit HR on tobacco leaves.

In agreement with Yap et al. (2004); Kim et al. (2009); Pitman et al. (2009) and Baghaee et al. (2011), the members of group 1 including atypical *Pcc* strains from potato were confirmed as *P. wasabiae* by using a combination of physiological, biochemical and phylogenetic analysis. This is the first time that the presence of *P. wasabiae* strains from bell pepper in Iran has been described. Baghaee et al. (2011) isolated *P. wasabiae* strains from potato which elicited HR on tobacco, while our findings were in agreement with many other studies by Moleleki et al. (2012) and Glasner et al. (2008) that showed *P. wasabiae* was not able to elicit HR on tobacco. Even though in this aspect putative *P. wasabiae* strains were similar to reference strains of *P. atrosepticum* (SCRI 1043), and some atypical *Pcc*, they were differentiated from these two species of *Pectobacterium* by utilization of raffinose and lactose. Similar to other researches, *P. wasabiae* isolates of this study were able to cause soft rot of tubers as well as stem lesions (Yap et al., 2004; Pitman et al., 2009; Baghaee et al., 2011). Therefore, it could be hypothesized that different pectolytic species may use different mechanisms to overcome plant barriers.

Genetic diversity of *Pcc* strains in this study was also performed based on rep-PCR. Comparing the results of genetic fingerprint BOX-PCR and ERIC-PCR, some similarities and differences were observed in their classification. In both cases, strains were placed in three main fingerprinting groups. There was no complete correspondence between the BOX-PCR results and ERIC-PCR and even those strains which showed the same genotype in BOXPCR showed different genotypes in ERIC-PCR. Geographically, the groups created by BOX-PCR were closely related to their sample collection areas. In ERIC-PCR, these correlations were defined. Linkage between rep-PCR results and the geographic origin of bacterial strains has been recognized in various studies (Scortichini et al., 2001; Mkandawire et al., 2004). Louws et al. (1994) believe that one of the important reasons for this phenomenon is that the selection for one geographically suitable area can have influence on the genetic map of bacterium and also dispersion of these repetitive units in the genome of bacterium. This work supports this idea and in some cases artifacts, these relations were observed. No doubt rep-PCR is a reliable tool for epidemiological studies of diseases and one can use the information as a device for detection of pathogens. Despite this, more recent work with ISSR-PCR of bacteria such as *Clavibacter michiganensis* subsp. *michiganesisis* proved the greater sensitivity, specificity and reliability of this technique as another helpful informative tool in epidemiological studies (Baysal et al., 2011).

Moreover, in this study, we have shown that a polyphasic approach including physiological, biochemical and molecular characteristics is relatively a reliable method to classify strains of *Pectobacterium* spp. However, it seems that phylogenetic analysis with housekeeping genes can be the most accurate method to characterize strains of pectolytic enterobacteria. In summary, we can conclude that the damaging enterobacteria isolates from bell pepper storages in north-west of Iran belong to *P. wasabiea*. While, in this area the most of damaging enterobacteria strains isolated from carrot, potato and cabbage belong to *P. carotovorum subsp carotovorum*.

Conflict of Interests

The authors have not declared any conflict of interests.

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