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Full Length Research Paper

Husbandry practices and phenotypic characteristics of indigenous goat populations in Ethiopia

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The present study was conducted with the objective to characterize the husbandry practices and phenotypic characteristics of mature Bati, Borana and Short-eared Somali goats kept under traditional management systems so that the information generated can be used in designing breeding programs. The study covered five districts in four administrative zones of Ethiopia representing Bati (in Oromia Zone) and Kalu (in South Wollo Zone) for Bati goats; Yabello (in Borana Zone) for Borana goats and Shinille and Erer (in Siti Zone) for Short-Eared Somali goats. A total of 345 households interview was made and phenotypic measurements were taken on 601(468 females and 133 males) heads of adult goats with 4 pair of permanent incisors (PPI). However, because of difficulty of finding adequate number of 4PPI sample males, measurements were taken from 2PPI and above males. In this study, in number, goats accounted for 72.01, 50.93 and 47.38% of other livestock species in Siti, around Bati and Borana, respectively. The least square mean (±SE) of goat flock size (44.02±3.33) per household in Siti was significantly ($p<0.05$) higher than those observed in Borana (23.08±1.94) and Bati area (8.99±0.59). The major challenges of goat rearing in the studied areas include feed and water shortage, disease incidence and recurrent drought with different order of prioritization. Plain brown (deep and light) (51.85%) coat color was the predominant coat color observed on Bati goats of both sexes. Meanwhile, plain white coat color was most frequently observed on Borana goats (71.54%) and only 36.27% in Short-eared Somali goats. Though most quantitative traits showed slightly higher average values in the Bati goats, differences with Borana goats were not significant ($p>0.05$),whereas Short-eared Somali goats remained significantly ($p<0.05$) lower for most of the body measurement characteristics. The canonical analysis done on phenotypic measurements also put Bati and Borana goats closer by discriminating Short-eared Somali goat populations. The similarities between Bati and Borena goats and significance divergence of Short-eared Somali goats in phenotypic measurements suggested that the need of further molecular characterization study to validate information from phenotypic characterization. Correlation coefficient was consistently highest between live weight and chest girth in both sexes across the goat populations. Hence, linear measurements could be valuable to estimate live body weight for those farm communities where sensitive weighing scales are not readily available.

Key words: Ethiopia, husbandry practices, phenotypic characteristics, indigenous goats.

INTRODUCTION

Ethiopia has a large population of goats (approximately 29 million) mainly of indigenous breeds (CSA, 2015). FARM-Africa (1996) identified 12 goat types in the country. The description of goat type refers to goats which have certain phenotypic characteristics and geographic location. Alemu (2004) has also classified the indigenous goat types in to 8 distinct genetic units using genetic DNA markers, These are: Arsi-Bale, Gumez, Keffa, Woyto-Guji, Abergalle, Afar, Highland goats (Central and North West Highland goats) and the eighth unit (Hararghe Highland, Short-eared Somali and Long-eared Somali goats).

In Ethiopia, farmers/pastoralists kept goats for food, income generation, socio-cultural considerations and source of other valuable non-food products such as skin and manure (Tsegaye, 2009; Gebreyesus, 2010; Tadesse et al., 2013). Despite the large size, wide distribution and diversified functions, the Ethiopian goat population productivity is relatively low. This is due to different factors such as poor nutrition, prevalence of diseases, and lack of appropriate breeding strategies and poor understanding of the production system.

To increase and sustain the productivity of indigenous goats so as to respond to the growing domestic and foreign demands for live goats and products, improvement programs are necessary and should be crafted, especially for countries like Ethiopia where extensive system of husbandry is the commonest type. Characterization studies are essential for planning improvement, sustainable utilization and conservation strategies of a breed at local, national, regional and global levels (FAO, 2012). In the absence of baseline characterization information, some breed populations and unique characteristics they contain may decline significantly, or be lost, before their value is recognized and measures taken to conserve them (FAO, 2007).

In Ethiopia, various goat characterization studies for different goat populations had been executed (FARM Africa, 1996; Gebreyesus, 2010; Hassen et al., 2012). Despite the studies done, information on husbandry practices as well as phenotypic characteristics for indigenous goat populations such as Bati, Borana and short-eared Somali is still scanty. Some of the works published has also the disadvantage of having been carried out long years back where the results may not reflect the current situation. Husbandry practice involves management, feeding and breeding aspects and it is among the factors which cause the phenotypic characteristics of animals. This indicates that description of husbandry practices and phenotypic characteristics of a particular animal population/breed are complementary procedures to be addressed in a characterization study. Therefore, this study was designed to provide husbandry practice and phenotypic characteristic information of Bati, Borana and Short-eared Somali indigenous goat populations in Ethiopia so that the information provided through the characterization of husbandry practices and phenotypic characteristics of the indigenous goats enable the interested groups to make informed decisions on breeding programmes.

MATERIALS AND METHODS

Description of study areas

The study covered five districts in four administrative zones: representing Bati and Kalu for Bati goats in Oromia and South Wollo zones respectively; Yabello for Borana goats in Borana zone; Erer and Shinille from Siti (the previous Shinille) zone for Short-Eared Somali goats.

South Wollo and Oromia zones (referred to as 'Bati area' hereafter) have varied topography, from the dry plains 1000 m above sea level to the high peaks about 3500 m above sea level altitude. The main rain is in the '*kremt*' (July-September) and the mean rainfall is 726 mm per annum (LPAR, 2007). The annual temperature ranges from 23 to 32°C (BDARDO, 2014).

Borana zone is characterized by the predominant lowland (69.1%), some midland (28.5%) and less agricultural highland (2.4%). It lies at an altitude of less than 1500m above sea level. The minimum and maximum average annual rainfall ranges between 350 and 900 mm with considerable variability in quantities and distribution. The average maximum temperature is 29°C and the minimum average temperature is about 13°C (Lasage et al., 2010).

Siti zone is mostly lowland and is arid or semi-arid. The altitude ranges from 950 to 1350 m above sea level. The minimum and maximum annual mean temperature ranges between 22.5 and 32.5°C, depending on the location within the zone. The average annual rainfall ranges between 500 to 700 mm (Save the Children UK and Disaster Preparedness and Prevention Agency, 2008).

Data collection

Multi-stage sampling procedure was followed where the big sampling frames were administrative zones. After the rapid informal field survey and discussion with the zonal agricultural bureau officers and elders, representative districts were selected. By conducting further discussion with the districts' Development Agents (DAs) and leaders, a total of 14 peasant associations (5 in Bat area; 5 in Siti; 4 in Borana) were selected. During selection of districts and peasant associations, production potential of the targeted goat type and accessibility were considered. A total of 345 households (98 in Bati, 132 in Borana and 115 in Siti) were interviewed. Pre-tested semi-structured questionnaire adopting a questionnaire prepared by International Livestock Research Institute and Oromia Agricultural Development Bureau for survey of

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Table 1. Number of heads (least square mean ±SE) per household according to species and area of survey.

Specie	Bati area (N=98)		Borana (N=132)		Siti (N=115)	
	LSM±SE	%	LSM±SE	%	LSM±SE	%
Goat	8.99±0.59^{c}	50.93	23.08±1.94^{b}	47.38	44.02±3.33^{a}	72.01
Cattle	3.94±0.29^{b}	22.33	10.42±1.21^{a}	21.39	1.70±0.26^{c}	2.78
Sheep	1.27±0.22^{c}	7.16	7.82±0.82^{b}	16.05	11.89±1.14^{a}	19.45
Chicken	2.43±0.36^{a}	13.77	3.70±0.49^{a}	7.60	0.16±0.13^{b}	0.26
Camel	0.31±0.07^{b}	1.77	1.64±0.32^{a}	3.37	1.88±0.29^{a}	3.08
Donkey	0.40±0.08^{b}	2.27	0.78±0.17^{b}	1.60	1.47±0.14^{a}	2.40
Beehive	0.31±0.13^{ab}	1.77	1.27±0.49^{a}	2.61	0.01±0.01^{b}	0.07

N= Number of respondents; Means with different superscripts (^{abc}) within the same column are statistically different (at least $p<0.05$).

livestock breeds in Oromia (Ayalew and Rowlands, 2004), was used. Information on livestock composition, holding pattern, and goat flock structure, management practices, breeding system, feeding and watering strategies, production constraints and other related information were collected. Phenotypic Records were taken on 601(162 Bati (128 females and 34 males), 246 Borana (201 females and 45 males) and 193 Short-eared Somali (139 females and 54 males)) heads of adult goats with 4 pair of permanent incisors (PPI) using FAO (2012) descriptor list for morphological characterization of goats.

However, because of difficulty of finding adequate number of 4PPI sample males, measurements were taken from ≥2PPI. Qualitative traits such as: sex, coat color pattern, coat color type, horn shape; horn and ear orientation; facial and back profile; presence or absence of horn, wattles, beard and ruff were recorded. Quantitative records taken for both sexes were Body Length (BL), Chest Width (CW), Height at Wither (HW), Chest Girth (CG), Rump Length (RL), Pelvic Width (PW), Horn Length (HL), and Ear Length (EL). Scrotum Circumference (SC) was also measured for males. Body weight measurements were taken in the morning to avoid the effect of feeding and watering on the animal's size (FAO, 2012).

Statistical analyses

The percentage of each level of qualitative data was obtained using PROC FREQ procedure of SAS (2008). Indices were calculated according to a formula: Index = sum of (3 for rank 1 + 2 for rank 2 + 1 for rank 3) given for an individual attribute divided by the sum of (3 for rank 1 + 2 for rank 2 + 1for rank 3) for overall attributes. Proc mean of the SAS software packages (SAS, 2008) was used to analyze the quantitative data (separately for males and females) fitting goat type as fixed effect.

The magnitudes of quantitative variables were expressed as Least Squares Means (±SE). Tukey-Kramer test was used to separate least squares means with more than two levels. The following statistical model was used to analyze linear body measurements. Pearson's correlation coefficient was estimated between body weight and other linear body measurements for each goat type by sex.

$$Y_{ij} = \mu + \beta_i + \varepsilon_{ij}$$

Where: Y_{ij} = observed quantitative measurement of trait of interest ; μ = population mean ; β_i = i^{th} goat type effect (i = 1, 2, 3); ε_{ij} = random error associated with quantitative body measurements.

RESULTS

Livestock composition and holding pattern

In terms of numbers, goats were the predominant species in all surveyed areas accounting for 72.01, 50.93 and 47.38% of the total of livestock species in Siti, Borana and Bati area, respectively. The survey indicated significant variation ($p<0.05$) in the average goat possession per household across study areas (Table 1). The least square mean (±SE) goat flock size per household (44.02±3.33) in Siti was significantly ($p<0.05$) higher than those observed in Borana (23.08±1.94) and Bati (8.99±0.59) areas.

Flock structure

Does older than one year and kids less than 6 months represent the major proportion in the flock in all study areas. The LSM (±SE) number of breeding does per household was 9.30±0.78, 13.30±0.84 and 3.51±0.91 in Borana, Siti and Bati area, respectively. The proportion of adult females (30.23%) and kids less than 6 months old (29.62%) in Siti were slightly smaller than their counterparts in Bati area and Borana. On the other hand, comparing with Borana and Bati, the share of kids between 6-12 months age (23.86%) and intact males older than one year (12.64%) in the flocks of Siti were higher. The contribution of castrated males in Siti, Borana and Bati area were 3.65, 0.95 and 3.52%, respectively.

Breeding management

Sources of breeding buck and type of natural mating systems are shown in Table 2. Even though half of the respondents around Bati (50%) as well as 64.39 and 83.48% in Borana and Siti, respectively, had their own breeding buck, uncontrolled natural mating system was

Table 2. Frequency (N) and percent (in brackets) of type of natural mating systems and sources of breeding buck.

Particular	Bati area	Borana	Siti
Natural mating system			
Controlled	11(11.22)	2(1.52)	2(1.74)
Uncontrolled	87(88.78)	130(98.48)	113(98.26)
Source of breeding buck			
Own flock	49(50)	85(64.39)	96(83.48)
Others flock	49(50)	47(35.61)	19(16.52)

N= Number of respondents.

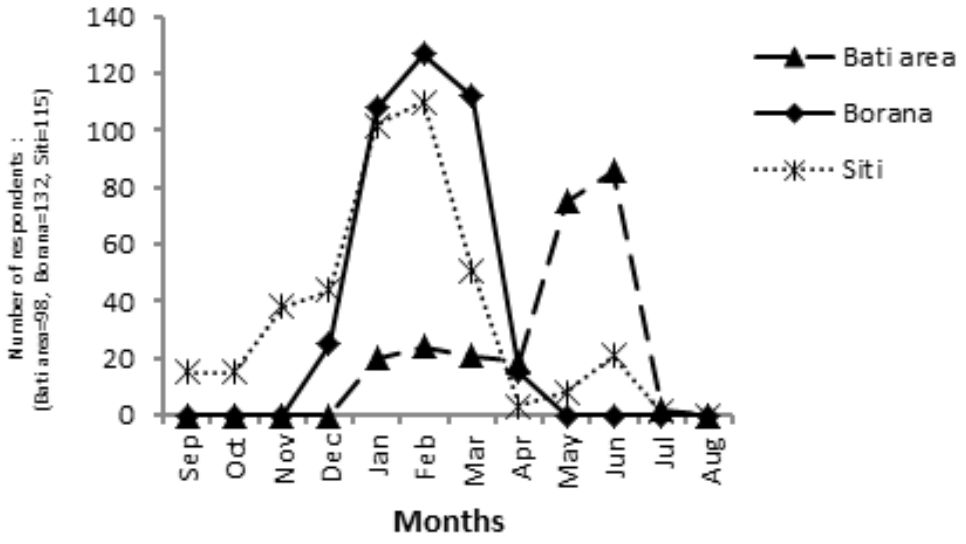

Figure 1. Feed shortage by months.

surpassed due to extensive communal production system in all the study areas. The average number of breeding bucks per flock within the interviewed households was 0.7, 1.1 and 1.4 for in Bati, Borana and Siti, respectively. Owners kept bucks on average until 2.4, 4.9 and 5.8 years of age in Bati, Borana and Siti, respectively, with a maximum stay of 4, 8 and 10 years in the same order.

Feeding and watering strategies

Natural pasture (shrubs and bushes) was the primary source of goat feed across the study areas during the dry and wet seasons of the year. Very few respondents also indicated the use of established forage, conserved hay and crop residues to feed their goats. Established forage trees such as sesbania (*Sesbaniasesban*), leucaena (*Leucaenaleucocephala*) and the commonly "kurkura" (*Ziziphisspina-christi*) planted on soil conservation structures and stock exclusion areas were reported as source of goat feed, used through cut-and-carry system around Bati. Feed shortage was reported in the three study areas, occurring in several months of the year (Figure 1) and by distinct causes. The major strategies for control of the feed shortage include collecting and providing of green leaves and pod from perennial plants, crop residues, collected and standing hay in Bati area, and migration of adult and healthy animals in Borana and Siti. About 55% of Bati area goat owners also reported supplementations based on availability of kitchen and milling residues, homemade grain, residues of local grain grinding houses and oilseed cake. In all the study areas, majority of the goat owners use mineral supplement (table salt) during wet season only when there is sufficient feed.

As presented in Table 3, the majority of goat owners in Bati area provides water to their goats every day and few individuals once in two days. Because of lack of surface water in Borana, almost all of the goat owners take their goats to the watering points once in three or two days.

Table 3. Watering frequency (N) and percent (in brackets) during dry season in the study areas.

Particular	Bati area	Borana	Siti
Frequency of watering			
Once a day	92(93.88)	3(2.27)	35(30.43)
Once in 2 days	6(6.12)	62(46.97)	67(58.26)
Once in 3 days	0(0)	67(50.76)	13(11.30)

N= Number of respondents.

Table 4. Goat production constraints as perceived by the respondents.

Constraint	Bati area				Borana				Siti			
	R1	R2	R3	Index	R1	R2	R3	Index	R1	R2	R3	Index
Drought	13	39	19	0.237	17	40	55	0.234	60	26	17	0.359
Feed shortage	46	21	14	0.338	26	60	38	0.296	20	41	28	0.245
Water shortage	0	3	5	0.019	5	11	16	0.067	17	6	28	0.131
Disease	26	20	31	0.260	84	18	14	0.379	19	43	31	0.251
Predator	4	4	7	0.047	1	4	7	0.023	0	2	2	0.009
Market	0	3	0	0.010	0	0	1	0.001	0	0	3	0.004
Labor problem	10	7	7	0.089	0	0	0	0	0	0	1	0.001

R = Rank.

However, in Siti, watering frequency ranged from every day to once in three days based on availability.

Major constraints associated with goat production

Though the major constraints facing goat production were mostly similar, their importance varied across the study areas (Table 4). In Bati area, feed shortage, disease occurrences and drought were ranked 1st, 2nd and 3rd, while disease occurrences, feed shortage and recurrent drought were the major constraints in Borana. Recurrent drought, disease, feed as well as water shortage have been perceived by the respondents as the most influencing constraints that hindering goat production in Siti. Almost all of the respondents did not rank lack of appropriate genotype as a constraint.

Qualitative characteristics

When appraised visually goats from the three areas were different (Figure 2).The frequency and percent for each level of qualitative traits of the three indigenous goat populations for both buck and does are presented in Tables 5 and 6. The observed overall coat color patterns for both sexes were 64.20% plain, 33.33% patchy/pied and 2.47% spotted in Bati; 72.36% plain, 23.98% patchy/pied and 3.66% spotted in Borana; and 45.08% plain, 39.90% patchy/pied and 15.03% spotted in Short-eared Somali goat populations. Most of Bati goats (87.5% females and 67.7% males) had straight head profile and about 14% (11.7% females and 23.5% males) were with slight concave head. Almost all (99%) of male and female Borana goats had straight head profile. From the total sampled Short-eared Somali goats, 41.7% females and 77.8% males had straight head profile. In studied populations the horned goats (does and bucks) accounted for 94.4, 78.9 and 80.8% of Bati, Borana and Short-eared Somali goats, respectively. The reminder proportions in each sampled population, except 8.9% of Borana does which displayed some rudimentary horns were polled.

The majority of Bati and Borana goats were characterized by lateral/sideway ear orientation accounting for a total of 59.9 and 78.9%, respectively, followed by hanged down ears observed in 35.8 and 12.5% of individuals in that order. Very small proportion of goats (4.3% Bati and 7.7% Borana) was also with forward erected ears. Large proportion (>84%) of forward and small proportion (15%) of lateral ear orientations distinguished Short-eared Somali goats from the two populations. Except for 2% of Borana and 4.7% of Short-eared Somali does, wattle was totally absent in all bucks of the three populations and in Bati does. It was found that about 56, 69 and 37% of Bati, Borana and Short-eared Somali bucks, respectively had ruff. Over 90% of Borana and Short-eared Somali and 67.7% of Bati bucks

Figure 2. Physical appearances of Ethiopian adult goats: Bati (left), Borana (middle) and Short-eared Somali (right).

had beard while about 25% of Bati and Borana as well as 17.9% of Short-eared Somali does were bearded.

Quantitative characteristics

Least square means for body weight, body condition score and other quantitative measurements of Bati, Borana and Short-eared Somali goats are presented in Table 7. Bati does were significantly ($p<0.05$) heavier (33.97±0.49 kg) and had widest chest (17.10±0.16 cm) among the three goat types. As compared with Borana does, Bati does varied significantly ($p<0.05$) in only three measurements (body weight, chest width and horn length) of the 9 measurements, otherwise they were comparable in most of their body dimensions (body length, height at wither, chest girth, rump length, pelvic width and ear length) and body condition score. The Short-eared Somali does remained significantly ($p<0.05$) smallest in body weight, body condition score and other body measurements except horn length.

Though most traits showed higher average values in Bati bucks, differences with Borana bucks were not significant ($p>0.05$) for most of body characteristics except pelvic width and horn length which were significantly ($p<0.05$) lower for Borana bucks. Most of the body measurements in Short-eared Somali bucks were significantly ($p<0.05$) lower as compared with their counterparts in Bati and Borana contemporaries. Despite the other measurements, the average values of pelvic width and horn length between Bati and Short-eared Somali; and body condition score in the three goat types were not different.

Differentiation between three goat types using discriminant analysis

The stepwise discriminant analysis procedure identified seven (HL, BW, EL, CG, HW, CW and PW) most significant discriminating traits between does while it was five (HW, HL PW, CG and EL) in bucks. The canonical analysis was carried out to observe the spatial distribution of sample populations on canonical variables by means of graph. It was conducted using those traits which shown significant discriminating power. The spatial distributions of the three populations for both sexes are presented in Figure 3. In both sexes, CAN1 discriminated Borana from Short-eared Somali goat populations effectively, keeping Borana and Bati populations closer on the right side of the X-axis. Though Bati goats put closer to Borana goats, they positioned more or less between Borana and Short-eared Somali goats. CAN2 is not effective in separating the three populations of both sexes except biasing Bati goats to the right side of X-axis.

Relationships between body weight and other linear body measurements

Coefficients of correlation between body weight and studied traits in this study varied from strong (0.86) to low (0.18) and highly significant ($p<0.01$) to non-significant

Table 5. Frequency (N) and percent (in brackets) of color and color pattern of indigenous goats by population.

Variable	Class level	Bati			Borana			Short-eared Somali		
		Female	Male	Total	Female	Male	Total	Female	Male	Total
		N (%)			N (%)			N (%)		
Coat color pattern	Plain	85(66.41)	19(55.88)	104(64.2)	144(71.64)	34(75.56)	178(72.36)	57(41.01)	30(55.56)	85(45.08)
	Patchy/pied	39(30.47)	15(44.12)	54(33.33)	48(23.88)	11(24.44)	59(23.98)	54(38.85)	23(42.59)	77(39.90)
	Spotted	4(3.13)	0	4(2.47)	9(4.48)	0	9(3.66)	28(20.14)	1(1.85)	29(15.03)
Coat color type	White	12(9.38)	6(17.65)	18(11.11)	140(69.68)	36(80.00)	176(71.54)	42(30.22)	28(51.85)	70(36.27)
	Dark red/brown	40(31.25)	8(23.53)	48(29.63)	1(0.5)	0	1(0.41)	5(3.60)	1(1.85)	8(4.15)
	Black	4(3.13)	0	4(2.47)	0	0	0	7(5.04)	1(1.85)	6(3.11)
	Gray	1(0.78)	0	1(0.62)	5(2.49)	0	5(2.03)	11(7.91)	2(3.70)	13(6.74)
	Light red	30(23.44)	6(17.65)	36(22.22)	2(1.00)	0	2(0.81)	9(6.47)	2(3.70)	11(5.70)
	White +Brown	15(11.72)	3(8.82)	18(11.11)	4(1.99)	0	4(1.63)	1(0.72)	4(7.41)	5(2.59)
	White +Black	3(2.34)	3(8.82)	6(3.7)	14(6.97)	3(6.67)	17(6.91)	30(21.58)	11(20.37)	41(21.24)
	White+ Light brown	23(17.97)	8(23.53)	31(19.14)	35(17.41)	6(13.33)	41(16.67)	34(24.46)	5(9.26)	39(20.21)

N= Number of goats.

Table 6. Frequency (N) and percent (in brackets) of incidence for some qualitative features of indigenous goats by population.

Variable	Class level	Bati			Borana			Short eared Somali		
		Female	Male	Total	Female	Male	Total	Female	Male	Total
		N (%)			N (%)			N (%)		
Facial profile	Straight	112(87.5)	23(67.65)	135(83.33)	199(99.00)	45(100)	244(99.19)	58(41.73)	42(77.78)	100(51.81)
	Slightly concave	15(11.72)	8(23.53)	23(14.2)	1(0.50)	0	1(0.41)	81(58.27)	12(22.22)	93(48.19)
	Slightly convex	1(0.78)	3(8.82)	4(2.47)	1(0.50)	0	1(0.41)	0	0	0
Horn	Present	126(98.44)	27(79.41)	153(94.44)	163(81.09)	31(68.89)	194(78.86)	128(92.09)	28(51.85)	156(80.83)
	Absent	2(1.56)	7(20.59)	9(5.56)	16(7.96)	14(31.11)	30(12.2)	11(7.91)	26(48.15)	37(19.17)
	Rudimentary	0	0	0	22(10.96)	0	22(8.94)	0	0	0
Horn orientation	Lateral	0	0	0	30(18.18)	2(6.45)	32(16.33)	9(7.03)	4(14.29)	13(8.33)
	Up ward	41(32.54)	2(7.41)	43(28.1)	37(22.42)	6(19.35)	43(21.94)	40(31.25)	4(14.29)	44(28.21)
	Back ward	85(67.46)	25(92.59)	110(71.9)	76(46.06)	22(70.97)	98(50.00)	77(60.16)	19(67.86)	96(61.54)
	Pointing forward	0	0	0	22(13.13)	1(3.23)	23(11.73)	2(1.56)	1(3.57)	3(1.92)

Table 6. Contd.

Ear orientation	lateral	77(60.16)	20(58.82)	97(59.88)	156(77.61)	38(84.44)	194(78.86)	22(15.83)	7(12.96)	29(15.03)
	Forward erected	1(0.78)	6(17.67)	7(4.32)	16(7.96)	3(6.67)	19(7.72)	117(84.17)	47(87.04)	164(84.97)
	Hanged down	50(39.06)	8(23.53)	58(35.8)	26(12.94)	4(8.89)	30(12.20)	0	0	0
	Pendulous	0	0	0	3(1.49)	0	3(1.22)	0	0	0
Wattle	Present	0	0	0	4(1.99)	0	4(1.63)	9(6.47)	0	9(4.66)
	Absent	128(100)	34(100)	162(100)	197(98.01)	45(100)	242(98.37)	130(93.53)	54(100)	183(94.82)
Beard	Present	32(25)	23(67.65)	55(33.95)	51(25.37)	41(91.11)	92(37.4)	25(17.99)	49(90.74)	74(38.34)
	Absent	96(75)	11(32.35)	107(66.05)	150(74.63)	4(8.89)	154(62.60)	114(82.01)	114(82.01)	119(61.66)

N = Number of goats.

Table 7. Least square means for body weight (kg), body condition score and other body measurements (cm) for does and bucks as affected by goat type.

Sex	Trait	Bati (N=128)		Borana (N=201)		Short-eared N=139)		Over all mean	
		LSM±SE	CV	LSM±SE	CV	LSM±SE	CV	CV	R^2
Does	BC	2.65±0.08^{a}	35.0	2.62±0.07^{a}	38.0	2.32±0.07^{b}	33.7	36.1	0.02
	BW	33.97±0.49^{a}	16.2	31.49±0.36^{b}	16.4	24.67±0.28^{c}	13.2	15.9	0.38
	BL	62.97±0.27^{a}	4.9	62.48±0.23^{a}	5.3	57.85±0.41^{b}	8.3	6.2	0.23
	HW	68.74±0.29^{a}	4.7	68.91±0.22^{a}	4.5	62.88±0.25^{b}	4.7	4.6	0.44
	CG	73.55±0.36^{a}	5.6	73.59±0.27^{a}	5.1	67.27±0.28^{b}	4.9	5.2	0.38
	CW	17.10±0.16^{a}	10.4	16.37±0.12^{b}	10.6	15.35±0.14^{c}	10.7	10.6	0.13
	RL	15.25±0.08^{a}	6.3	15.10±0.07^{a}	6.3	14.07±0.08^{b}	6.7	6.4	0.22
	PW	14.36±0.09^{a}	6.9	14.17±0.07^{a}	6.9	13.73±0.13^{b}	11.0	8.3	0.04
	HL	13.87±0.24^{b}	19.0	8.59±0.26^{c}	40.8	17.51±0.34^{a}	22.0	26.7	0.56
	EL	15.65±0.12^{a}	8.3	15.34±0.12^{a}	10.7	12.99±0.10^{b}	8.9	9.6	0.39
Bucks	BC	3.06±0.16^{a}	30.1	3.02±0.11^{a}	23.9	3.22±0.10^{a}	23.1	25.2	0.01
	BW	41.30±0.85^{a}	11.9	40.04±1.21^{a}	20.3	30.62±0.67^{b}	16.1	17.0	0.39
	BL	65.59±0.59^{a}	5.2	65.13±0.63^{a}	6.5	57.28±0.69^{b}	8.9	7.1	0.45
	HW	76.09±0.68^{a}	5.2	74.84±0.66^{a}	6.0	64.98±0.67^{b}	7.6	6.4	0.57
	CG	81.25±0.95^{a}	6.8	79.49±0.78^{a}	6.6	71.24±0.73^{b}	7.6	7.0	0.42
	CW	18.12±0.29^{a}	9.5	18.49±0.41^{a}	15.0	16.37±0.30^{b}	13.4	13.1	0.15
	RL	16.41±0.21^{a}	7.5	16.22±0.16^{a}	6.8	15.44±0.23^{b}	11.1	8.9	0.09

Table 7. Contd.

PW	15.94±0.27[a]	9.9	14.73±0.20[b]	9.1	15.91±0.30[a]	13.6	11.4	0.09
HL	18.57±0.73[a]	21.3	13.05±0.75[b]	32.2	19.92±1.10[a]	30.2	28.1	0.29
EL	14.50±0.43[a]	17.3	14.31±0.27[a]	12.9	12.01±0.32[b]	19.6	16.7	0.22
SC	27.07±0.36[a]	7.8	27.02±0.30[a]	7.5	25.81±0.37[b]	10.6	8.9	0.06

Means with different superscripts ([abc]) within the same row are statistically different (at least p<0.05); BW = Body weight, BC = Body condition, BL = Body length, HW = Height at wither, CG = Chest girth, CW = Chest width, RL = Rump length, PW = Pelvic width, HL = Horn length, EL = Ear length, SC = Scrotum circumference; LSM = Least squares means, SE = Standard errors, CV = Coefficient of variations and R^2 = Magnitude of population effect.

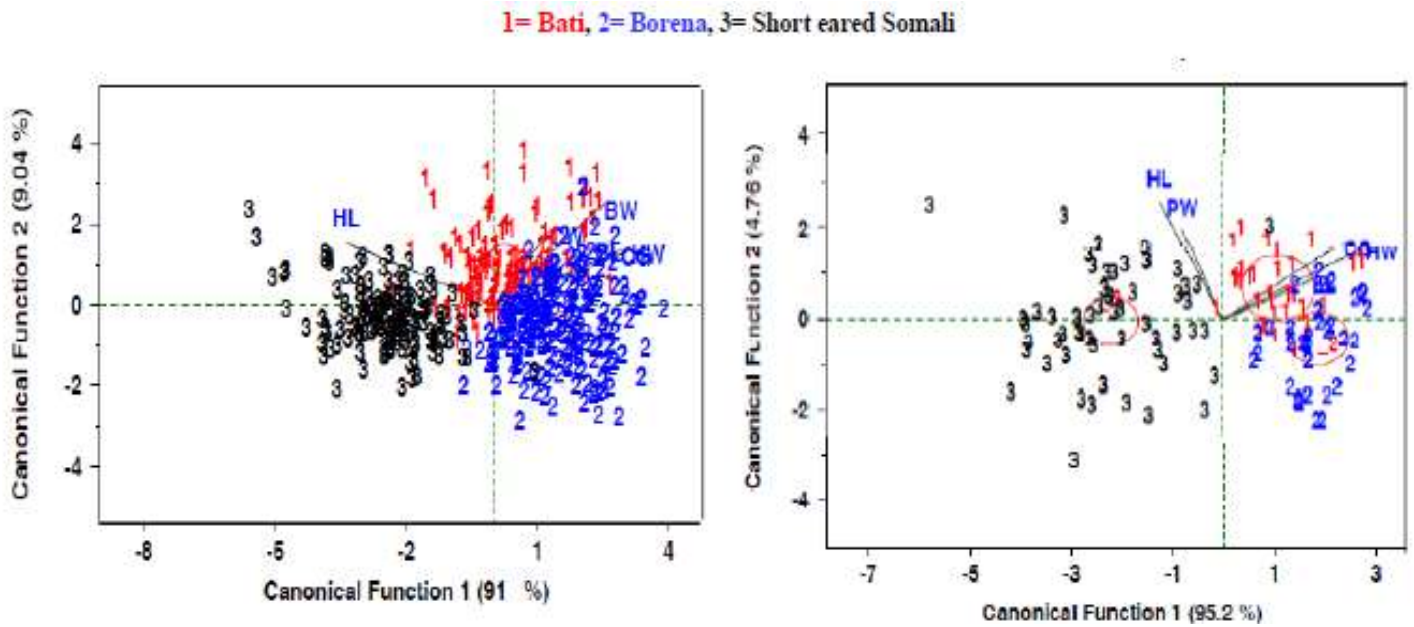

Figure 3. Spatial distributions of does (left) and bucks (right) on the first two canonical variants.

(Table 8). Most variables (BC, BL, HW, CG, CW, RL and PW) depicted positive and highly significant (p<0.01) correlation with live body weight. Correlation coefficient was consistently the highest between live body weight and chest girth in both sexes for the populations. However, for Short-eared Somali bucks equally the highest correlation coefficient was found for chest girth and height at wither with body weight. Even though the correlation of body weight with chest girth was positive and significant for both sexes, higher values were observed in bucks as

Table 8. Pearson's correlation coefficients of quantitative traits for bucks (above diagonal) and does (below diagonal).

Population	Trait	BW	BC	BL	HW	CG	CW	RL	PW	SC
Bati	BW		0.57**	0.61**	0.58**	0.85**	0.47**	0.55**	0.47**	0.55**
	BC	0.52**		0.59**	0.27^{NS}	0.54**	0.44**	0.14^{NS}	0.27^{NS}	0.34^{NS}
	BL	0.62**	0.35**		0.46**	0.77**	0.47**	0.41*	0.40*	0.40*
	HW	0.40**	0.17*	0.48**		0.54**	0.01^{NS}	0.44**	0.66**	0.54**
	CG	0.82**	0.34**	0.57**	0.51**		0.50**	0.71**	0.62**	0.56**
	CW	0.68**	0.30**	0.46**	0.33**	0.61**		0.40*	0.34*	0.31^{NS}
	RL	0.62**	0.29**	0.43**	0.41**	0.59**	0.59**		0.57*	0.60**
	PW	0.53**	0.32**	0.39**	0.32**	0.49**	0.52**	0.51**		0.60**
Borana	BW		0.36*	0.80**	0.79**	0.86**	0.55**	0.76**	0.78**	0.53**
	BC	0.40**		0.27^{NS}	0.51**	0.30*	0.10^{NS}	0.11^{NS}	0.22^{NS}	0.10^{NS}
	BL	0.67**	0.27**		0.77**	0.76**	0.28^{NS}	0.66**	0.71**	0.37*
	HW	0.50**	0.08^{NS}	0.50**		0.80**	0.43**	0.66**	0.75**	0.35*
	CG	0.82**	0.25**	0.56**	0.54**		0.40**	0.74**	0.75**	0.63**
	CW	0.71**	0.31**	0.56**	0.50**	0.67**		0.52**	0.53**	0.24^{NS}
	RL	0.57**	0.19**	0.58**	0.58**	0.60**	0.62**		0.76**	0.50**
	PW	0.54**	0.19**	0.53**	0.43**	0.58**	0.50**	0.54**		0.49**
Short-eared Somali	BW		0.69**	0.46**	0.79**	0.79**	0.53**	0.55**	0.26^{NS}	0.56**
	BC	0.62**		0.49**	0.44**	0.56**	0.49**	0.28*	0.25^{NS}	0.43**
	BL	0.25**	0.31**		0.51**	0.50**	0.25^{NS}	0.24^{NS}	0.20^{NS}	0.38**
	HW	0.37**	0.13^{NS}	0.17*		0.68**	0.40**	0.40**	0.24^{NS}	0.46**
	CG	0.73**	0.44**	0.25**	0.32**		0.61**	0.59**	0.43**	0.66**
	CW	0.40**	0.16^{NS}	0.07^{NS}	0.01^{NS}	0.53**		0.50**	0.12^{NS}	0.37**
	RL	0.34**	0.19*	0.11^{NS}	0.30**	0.45**	0.33**		0.42**	0.52**
	PW	0.24**	0.24**	0.03^{NS}	0.12^{NS}	0.40**	0.18*	0.26**		0.44**

BC = Body condition, BL = Body length, HW = Height at wither, CG = Chest girth, CW = Chest width, RL = Rump length, PW = Pelvic width, SC = Scrotum circumference; NS = Non Significant: *p < 0.05, ** p< 0.01.

compared with does within the population.

DISCUSSION

Livestock composition, holding pattern and flock structure

The major livestock species in the study areas were goats, sheep, cattle, camels and donkeys. Goats constitute the largest share (in number) among other livestock species in all study areas. According to Nega et al. (2009) the probability of keeping livestock is strongly correlated with agro-climatic conditions. In the present study, households in the lowland areas keep goats as the primary animal because of their ability to survive in a harsh environment. Gizaw et al. (2010) stated that flock sizes vary with the production system and the environment. Likewise, in this study, average flock size per household showed significant deviation ($p<0.05$) across study areas. The average number of goats holding per household found around Bati area (8.99±0.59) was comparable with the previous report of Getachew et al. (2006) in the same area (7.79± 4.54) and in Shewarobit area (9.6±2.68). On the other hand, the average number of goats per household in Siti area (44.02±3.33) was relatively higher than those reported by Tilahun et al. (2006) and Gebreyesus (2010) who reported 34±23.54 and 10.08±0.8 heads per household for the same goat type in rural peasant associations of Siti and Dire Dawa Administration Council, respectively. These results indicated the existence of variation in the number of goats per household among the districts, years and seasons implying the need of characterization study in short time interval for specific area.

The proportion of different classes of animals reflects the management decisions of the producers which in turn are determined by their production objectives (Gizaw et al., 2010). In our findings the breeding does were the major followed by kids less than 6 months in all goat populations. This is in agreement with findings of other researchers in Ethiopia (Tsedeke, 2007; Tsegaye, 2009).

Breeding management

Even though majority of the producers in the present study practiced breeding stock selection and possessed their own breeding buck, the traditional (communal) production systems in the study areas lead to uncontrolled mating making it difficult to control flock reproduction. According to Kosgey (2004), an advantage of natural uncontrolled mating is that it allows for all year round breeding. On the other hand, uncontrolled mating together with small flock sizes and poor/absent record keeping scheme on pedigree are expected to result in severe inbreeding which leads to poor growth rates (Saico and Abul, 2007). Use of bucks for long period in a flock in Borana and Siti areas depicts inbreeding problem in the flocks (Jimmy et al., 2010).

Feeding and watering strategies

Goat production in communal production systems is highly dependent on rangeland resources (Homann et al., 2007). In line with this statement, free natural pasture (shrubs and bushes) was the predominant feed resource among the other mentioned feed resources in both dry and wet seasons, particularly in Borana and Siti areas. The availability of water was not consistent particularly in the dry season. This also enforced the animals to stay for about three days without water as found in this study and also reported by Tadesse et al. (2013). According to Urge (2007), Short- eared Somali goats deprived water for about three days in dry season showed 22% milk yield reduction as compared to goats with water access every day. Therefore, watering is an important management component, which is often not addressed (Homann et al., 2007). Though, water shortage has impact on productivity of goats, in this study, Borana and Short-eared Somali goats showed relatively more drought tolerance and not affected adversely by water shortage as compared with Bati goats.

Major constraints

Major goat production and productivity challenges in the communal production systems include feed shortage, disease occurrences and water scarcity (Markos, 2006; Gizaw et al., 2010). The major constraints of goat rearing found in this study were similar with the constraints listed by the above authors, but their importance varied across the study areas.

Phenotypic characteristics

Phenotypic characteristics of a breed include qualitative, quantitative and economic traits (FAO, 2012). These characteristics are important in breed identification, classification, genetic improvement (selection) implementation and sustainable utilization and conservation (Salako and Ngere, 2002).Though the frequencies of some coat colors were small in a population, the current study demonstrated that the studied goat populations have a wide range of coat colors. Similarly, Hassen et al. (2012) and Gebreyesus (2010) reported wide range of coat colors for different Ethiopian goat populations. The availability of wide range of coat colors in a population might be attributed to lack of systematic selection program and would definitely offer opportunity for setting up breeding (selection) programmes. The most important coat color preferences in Bati area for both sexes were brown whereas plain white coat color was the most preferred by both Borana and Siti pastoralist and agro pastoralists. Not for their production or reproduction traits rather due to low price in the local markets, black coat color goats were not preferred by the producers in all study areas. The higher proportions of polled bucks than does across the studied goat types might be due to either producers' interest in polled bucks or the higher frequency of short-horned allele (HoP) for males. In this study, the presence of beard was dominant in bucks while the presence of wattle was rare for both sexes. Similar results were also reported by Gebreyesus (2010) for short eared Somali goats and Tsegaye et al. (2013) in Hararghe highland goats. According to Hagan et al. (2012), in addition to the thermoregulatory functions, the presence of wattle and beard is associated with reproduction traits such as higher prolificacy, higher milk yield, higher litter size, fertility and conception rate. Leng et al. (2010) also reported greater association ($p<0.01$) of heavier body weights and body measurements with the presence of wattles of Longling Yellow Goats in China. Therefore, the incidence of wattle and beard can be used as selection criteria by farmers for improved performance in the studied goat populations.

The similarity of most body measurements between Bati and Borana goat might be due to equivalence of measurements between populations since the probability of intermingling between the studied populations is very low due to big geographical distance between their habitats. As compared with the result found in the present study, slightly lower mean values of body weight, body length, height at wither and chest girth for mature Bati female goats were reported earlier by Hassen et al. (2012) and Getachew et al. (2006). The variations could be due to different age of animals included in the sample and season of measurement. The longer horn was observed in Short-eared Somali goats (19.92±1.10 cm in bucks and 17.51±0.34 cm in does). According to FAO (2012), size of horns is known to be relevant to the dissipation of excess body heat. Traits like BC, BW, CW and HL in females and BC, BW, CW, PW, HL and EL in bucks were found to have over 10% overall CV value for all three goat types. According to Mavule et al. (2013),

large variation observed in body measurements is a result of absence of selection, or the body parts are affected more by the environment than others.

Relationships between body weight and other linear body measurements

The observed positive and highly significant correlations between body weights and other linear body measurements indicates that traits in combination or individually could be measured to predict live body weight. Particularly, chest girth would provide a good estimate for predicting live body weight. However, Nsoso et al. (2003) noted inconsistencies between the relationship of body condition score and live body weight under extensive management system in dry and wet seasons. Therefore, body condition score appeared to be a more useful trait in assessing nutritional consequences than live weight body prediction under extensive management systems. In agreement with the present study, Hassen et al. (2012) and Gebreyesus (2010) for some Ethiopian goats; and Mavule et al. (2013) for sheep reported the highest correlation between body weight and chest girth. This shows that chest girth might be the best trait to predict live body weight for both goats and other livestock species.

Conclusion

Goats provided diversified functions for the small scale producers despite the presence of several constraints. The importance of identified constraints varied among the study areas as well as between seasons. Similarly, traits for selection preferred by goat producers in different areas varied. These aspects highlight the need to develop different strategies for the development of breeding programs according to the area with actions defined with the involvement of communities. The result in this study also revealed that the smaller least square mean values for most body measurements distinguished Short-eared Somali goats while it dictated the least differentiation between Bati and Borana goats. Therefore, molecular genetic approach is necessary to evaluate the phenotypic results. Diversity of qualitative traits like coat color, facial and back profile, presence or absence of horn, wattle, ruff and beard was observed among the three goat types. Since the breeders (producers) can easily distinguish desirable phenotypic characteristics, the variability of those traits could be useful in selection program. Due to high and positive correlation coefficients found between body weight and other linear body measurements (BL, HW, CG, CW, RL and PW), selection of one or more of these traits may increase live body weight of these goat populations and linear measurements could be used to estimate live body weight for those farm communities where sensitive weighing scales are not readily available.

Conflict of Interests

The authors have not declared any conflict of interests.

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Phosphorus fixation and its relationship with physicochemical properties of soils on the Eastern flank of Mount Cameroon

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The Eastern flank of Mount Cameroon is made up of human settlement and agricultural activities. Determination of phosphorus (P) fixation characteristics of soils around this region is important for a clean environment and economic fertilizer application. The objectives of this study were to evaluate P-fixation characteristics of soils within the region and identify factors contributing to P-fixation. Composite surface soil samples from five sites (between 36 to 1006 m) were collected and analyzed for physicochemical properties and P-fixation capacity. Fixation data were obtained by equilibrating the five soil samples with 25 ml of KH_2PO_4 in 0.01 M $CaCl_2$, containing 0, 10, 50, 100, 250 and 500 mg L^{-1}. Phosphorus-fixation data were fitted to Langmuir and Freundlich adsorption models and the relationship between P-fixation and soil properties was determined. The adsorption maximum (K_L) for Freundlich model was the highest for Limbe - Man O'War Bay Road (LMR, 174.58 mg P kg^{-1}), followed by SLR (34.12 mg P kg^{-1}), Dibanda-Mutengene Road (DMR, 24.72 mg P kg^{-1}), VAS and UPD (16.87 mg P kg^{-1}). The Langmuir adsorption maximum for Vasingi (VAS), Upper Boduma (UPD), Sasse-Limbe Road (SLR), DMR, and LMR were 0.01, 0.02, 0.01, 0.02 and 0.05 mg P kg^{-1}, respectively. The phosphate adsorption isotherm gave good fit adopting Freundlich (r = 0.98 to 0.99). K_F correlated with pH (r = - 0.87), Ca (r = - 0.87), exchange acidity (r = 0.87), and clay (r = 0.67). This study illustrated that P sorption isotherm in relation to soil properties can be used as a tool for P management in sustainable crop production.

Key words: Phosphorus fixation, adsorption isotherms, soil physicochemical properties, volcanic soils, Mount Cameroon.

INTRODUCTION

Phosphorus plays a very vital role in plant germination, growth and maturation. The chemistry of soil inorganic P strongly depends on the composition and crystalline nature of the solid phase of soils and the ionic nature of soil solution P (Mehmood et al., 2010). In most agricultural farms, the composition and crystalline nature

of the solid phase of soils, and the ionic nature of soil solution P vary greatly; this variation influences the chemistry of soil P and has drawn the attention of many researchers. Diverse techniques have been proposed to evaluate the status of P in the soil in a bid to advice on a better fertilizer management programme (Baskaran et al., 1994; Bolan and Baskaran, 1996; Muindi et al., 2015). The availability of P for plant uptake and its utilization are not a function of its concentration in the soil but rather a function of its release from soil surface into soil solution (Muindi et al., 2015). Fixation is said to have occurred when chemicals accumulate at the interface between solid phase and aqueous solution phase. This phenomenon determines the availability of native soil nutrients and the rates at which nutrients are applied to the soil as fertilizers. Phosphorus fixation has been defined by many researchers as a process in which phosphate ions are held on active sites of soil particle surfaces (Idris and Ahmed, 2012; Melenya et al., 2015). Phosphate ions are chemically unstable in soil solution, and readily react largely with oxides and hydroxides of aluminium (Al) and iron (Fe) found on clay surfaces for acidic soils and with the hydroxide of calcium (Ca) in calcareous soils to form less and more stable compounds (Bolan and Baskaran, 1996; Bolland et al., 2003).

Adsorption isotherms have been identified as an important criterion to estimate P concentration in aqueous phase of soil, energy of P adsorption and maximal adsorbed P by the soil (Gichangi et al., 2008; Hadgu et al., 2014), and identify the soil attributes responsible for P adsorption (Muindi et al., 2015). Isotherms can quantitatively describe the equilibrium relationship between the amount of adsorbed and dissolved phosphate at constant temperature (Muindi et al., 2015). Agbenin and Tiessen (1994) reported Langmuir maximum adsorption parameter, which significantly and positively correlated with soil properties such as clay, organic carbon, Fe and Al contents.

Several adsorption models (such as Langmuir, Freundlich, Tempkin, and Van Huay adsorption models) have been proposed to describe and quantitatively measure phosphate adsorption. The Freundlich and Langmuir models are the most popular models because they have been reported to give the best fits (Niang et al., 2002; Hadgu et al., 2014; Muindi et al., 2015).

Anghiononi et al. (1996) and Mehmood et al. (2010), reported positive correlations between adsorbed P and soil properties such as texture, organic matter, soil pH, aluminium saturation, cation exchange capacity (CEC), oxides of Al and Fe. Great attention should be given to any soil property that correlates positively with adsorbed P because increasing this soil property will increase adsorbed P and reduce plant available P.

Agriculture is the main activity carried out by most of the inhabitants of the Mount Cameroon region. The soils from this region are generally low in available P (Tening et al., 2013). Knowledge of the relationship between soil properties with P-fixation is necessary for the effective application of phosphorus fertilizer on varied soils on the Eastern flank of Mount Cameroon region. Tening et al. (2013) reported that P-fixation capacity correlated positively with clay content and pH, and negatively with organic carbon and available P in some soil horizons that were collected from a soil profile within this region. They also observed that between 52 and 99% of P is fixed by the surface soil horizon in this area a day after P application. Notwithstanding, very limited work has been carried out on varied agricultural surface soils to understand the relationship between the P-fixation capacity and specific soil properties.

The adsorption models have not been used around the Mount Cameroon region probably because no research work has focused at quantifying the amount of P retained by these soils. Here, attempt was made to use Langmuir and Freundlich adsorption models to characterize phosphorus fixation around the Mount Cameroon region and relate it to some soil attributes.

The objectives of this study were therefore to investigate the relationship between P-fixation capacity and soil properties within the sub region and to compare the applicability of Langmuir and Freundlich isotherm equations in describing the P-fixation in soils in the sub region.

MATERIALS AND METHODS

Location of study area

The study area lies on the lower slopes of the Eastern flank of Mount Cameroon, the highest mountain (4095 m) in West and Central Africa. Mount Cameroon, an active volcano with eight eruptions reported since the beginning of the 20th century, is located or lies within latitudes 4° 00'-4°13'N and longitudes 9°00' – 9°30'E (Figure 1). The climate of the Eastern slopes of Mount Cameroon is humid tropical. The former is characterized by a distinct dry season (mid-November to March) and rainy season (mid-March to November). Climatic conditions such as temperature (26 to 29°C), and annual rainfall (3000 to 4000 mm) (Manga et al., 2013) promote the growth of abundant vegetation on Mount Cameroon. The colonization of the Mount Cameroon basaltic deposits by grass, shrubs or trees, and a rain forest at about 2000 m is controlled by elevation and rainfall. A fivefold increase in weathering rates from high to low elevations in this area was observed by Benedetti et al. (2003). They attributed this to the role of vegetation, temperature and rainfall at lower elevations.

The soils of this region are developed from the weathering of a

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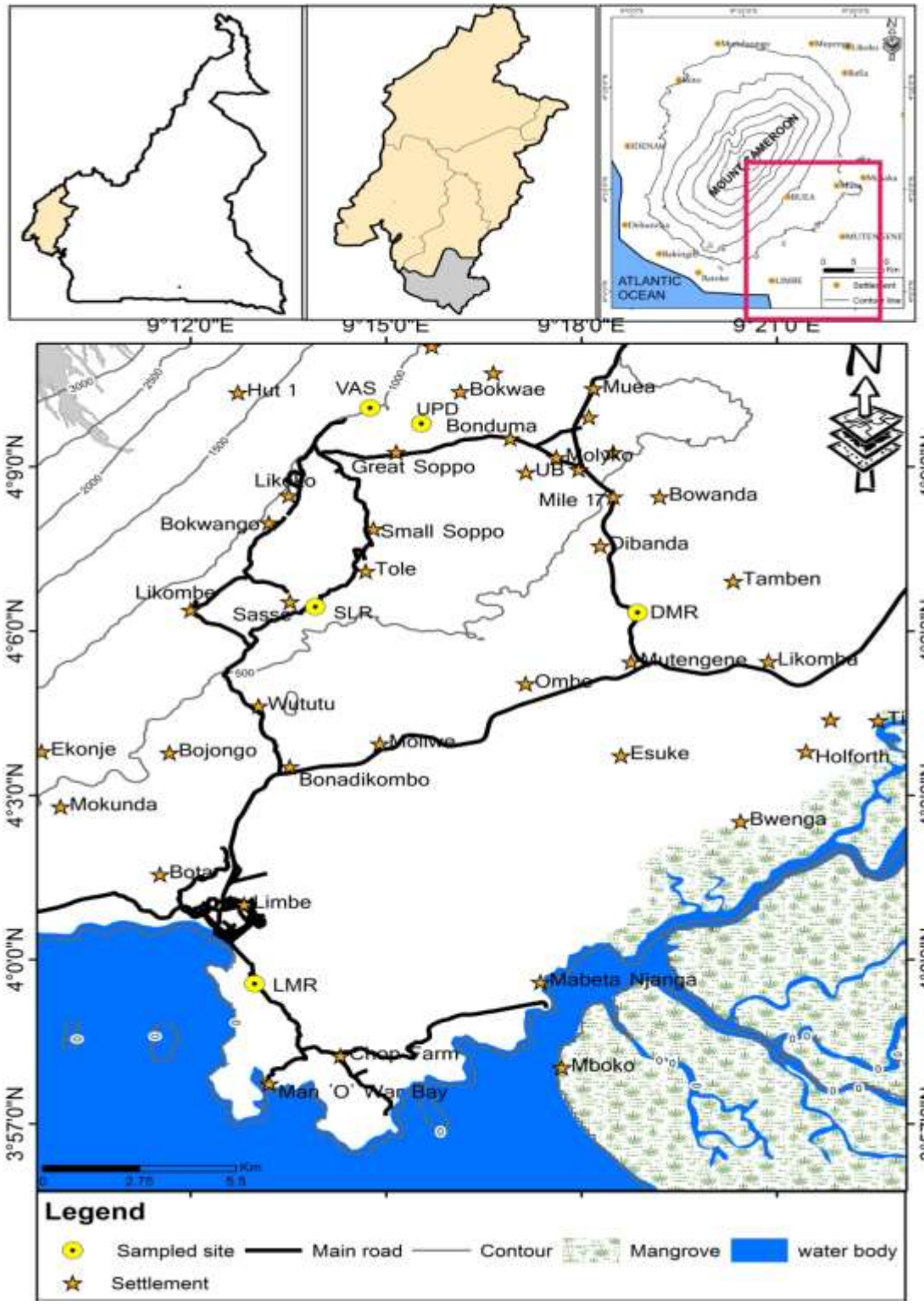

Figure 1. Location and sample distribution map. Inset is the map of Cameroon showing the SW Region, Mount Cameroon and Mount Cameroon with a zoom on the study area.

basaltic parent rock (Manga et al., 2013). These soils have been intensely weathered in some areas to produce well drained to clayey reddish brown and yellowish soils which are over 10 m thick. Yet in other areas, the soils are well drained, relatively young black soils developed from protracted weathering of basalticaa and pahoehoe lava flows. A lot of work has been carried out on the

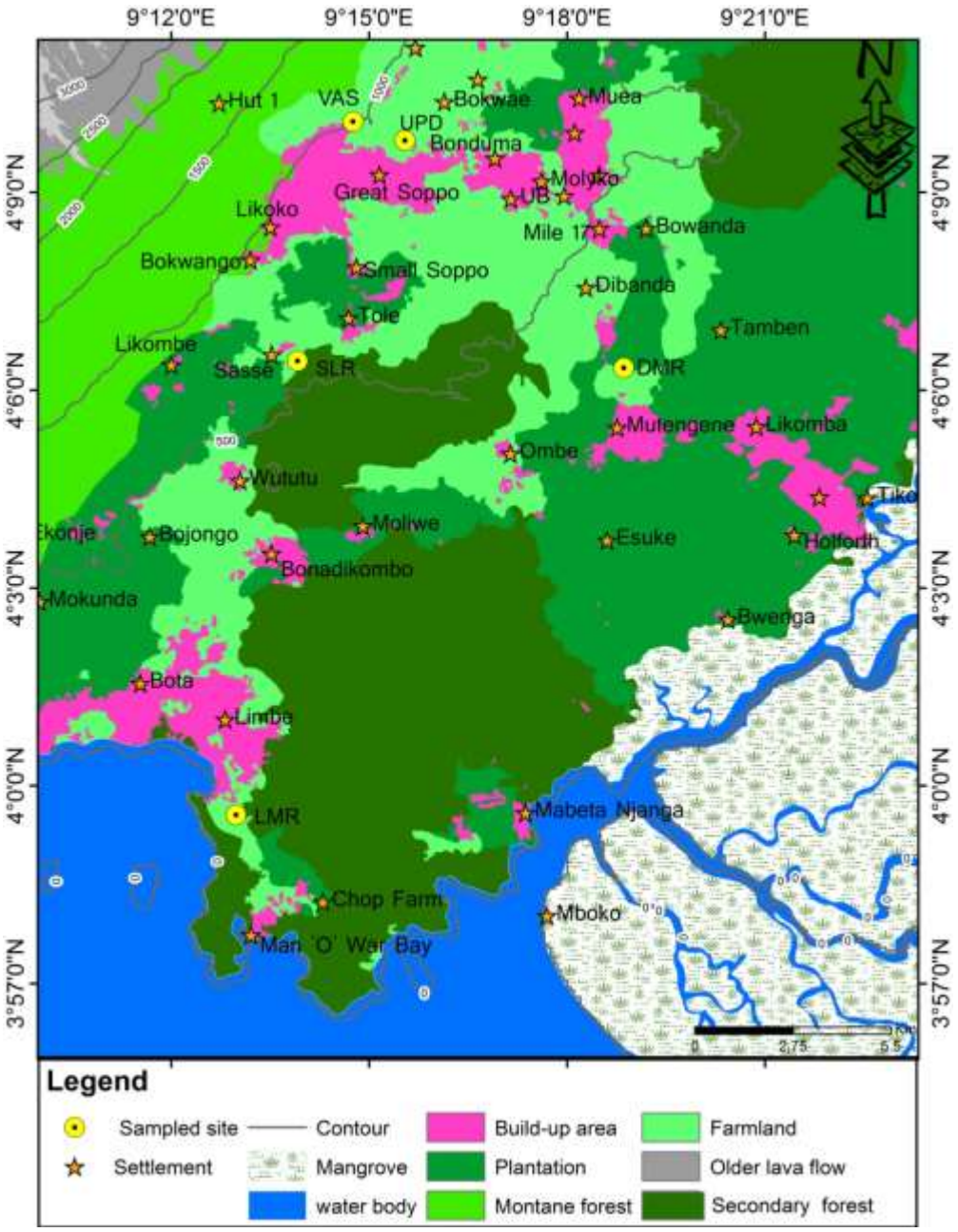

Figure 2. Land cover map of the study area. Note the presence of vast plantations and secondary forest.

toposequence of soils along the slopes of volcanoes to assess the effect of climate on weathering and mineral formation (Zehetner et al., 2003; Soubrand-Colin et al., 2007; Manga et al., 2013). The authors used parameters such as number of horizons, thickness of B-horizon characterized by altitudinal and climatic variability to evaluate degrees of soil formation. Manga et al. (2016) showed that the weathering status (derived from chemical weathering indices) of soils along the slopes of Mount Cameroon showed that the most weathered profiles are located at the higher elevations with lower mean annual precipitation.

Five sampling sites were selected at different elevations and agro-ecological zones along the Eastern slopes of Mount Cameroon (Figure 2 and Table 1). Surface soil samples (0 to 20 cm) were collected from these sites, air-dried, ground and passed through a 2-mm stainless steel sieve. Soil pH and electrical conductivity (EC) were measured in 1:5 soil to water ratio, bulk density and moisture content were determined following the oven-dry method. Organic carbon (%C) was determined by Walkley and Black wet combustion method described in Dhyan et al. (2005) and organic matter (OM) was calculated from the levels of organic carbon in the soil by multiplying by 1.72 as reported in Hazelton and Murphy (2007). Exchangeable bases were determined following the Schollenberger method using a 1 M ammonium acetate solution buffered at pH 7 described in Benton (1999). Exchange acidity was

Table 1. Location and altitudes of soil sampling sites within the Eastern flank of Mount Cameroon.

Sample site	Sample code	Latitude	Longitude	Altitude (m)	Human activity
Vasingi	VAS	04° 10'4.6"N	009° 14'45.3"E	1006	Plantain farm
Upper Boduma	UPD	04° 09'47.3"N	009° 15'32.5"E	786	Maize, beans and cassava farm
Sasse-Limbe Road	SLR	04° 06'26.6"N	009° 13'54.9"E	603	Maize, beans and cassava farm
Dibanda-Mutengene Road	DMR	04° 06'20.4"N	009° 18'51.7"E	351	Plantains, maize, beans and cassava farm
Limbe-Man O'War Bay Road	LMR	03° 59'33.5"N	009° 12'58.9"E	36	Cassava and maize farm

determined after the unbuffered KCl solution according to procedures described by Benton (1999). Cation exchanged capacity (CEC) was determined by a direct continuation of the Schollenberger's method using a 1 N KCl solution for displacement of ammonium ions. Effective cation exchange capacity (ECEC) was determined by summation (Sum of the exchangeable bases and exchange acidity). Available P was determined by Bray I method (Dhyan et al., 2005). Total nitrogen (%N) was determined by exploiting the Kjeldahl's distillation method (Dhyan et al., 2005). Total soil P was extracted using microwave assisted aqua regia digestion technique, the digested sample was mixed with Bray I extracting solution and total P in the extract was determined as reported by Murphy and Riley (1962). The distribution of particle sizes larger than 75 µm was determined by dry sieving and the distribution of particles smaller than 75 µm was determined by means of sedimentation process using a hydrometer method used to determine particle size according to ASTM D-422-63(1998) standard methods. To have an idea about the type of clay minerals present in these soil samples, soil activity was determined from Atterberg limits (liquid limit, plasticity limit and plasticity index) measured according to ASTM D 4318-10 (2010) standard methodology. Porosity was calculated from the bulk density and particle density (Brady and Weil, 1996):

$$f = 1 - (\rho b/\rho s) \quad (1)$$

Where f = total porosity, ρb = bulk density, ρs = particle size density (2.65 g/cm^3) a standard value as stated in Hazelton and Murphy (2007).

In the fixation study, about 5 g air-dried soil samples were weighed, in triplicates, into 50 ml polypropylene centrifuge tubes and equilibrated with 25 ml of KH_2PO_4 in 0.01M $CaCl_2$ containing 0, 10, 50, 100, 250, and 500 mg P/L. Two drops of chloroform were added to each tube to act as a microbial inhibitor. The tubes were set in a mechanical shaker for 16 h at 120 turns/min. Following equilibration, the soil suspensions were centrifuged at 4000 rotations/min for 20 min and filtered through Whatman No.5 filter paper to obtain a clear solution. The inorganic P was determined by the phosphomolybdate blue method of Murphy and Riley (1962). The amount of P fixed by the soil (P_{ads}) was calculated from the difference between the initial concentration (P_o) and the equilibrium concentration (P_{eq}) which is P remaining in soil solution. Phosphorus-fixation data for the soils used in this study were fitted into the linearized form of the Langmuir and Freundlich equations proposed by Muindi et al. (2015).

The Langmuir's equation described in its linear form is:

$$\frac{C}{X} = \frac{1}{K_L b_L} + \frac{C}{b_L} \quad (2)$$

The Freundlich's equation described in its linear form is:

$$\log X = \log K_F + n_F \log C \quad (3)$$

where C = Equilibrium concentration of phosphorus in solution (mg P L^{-1}), X = mg of P adsorbed (mg P kg^{-1}), b_L= Adsorption maximum for Langmuir model(mg P kg^{-1}), K_L= Bonding energy constant of Langmuir model (L mg^{-1}P), n_F = Empirical constant related to bonding energy of soil for phosphate, and K_F= Proportionality constant for Freundlich model (mg P kg^{-1}).

The phosphate adsorption isotherms of soils in the five study sites were determined by plotting the equilibrium concentration of phosphate (C) against the amount of phosphate fixed (X).

The sorption isotherms were examined according to the linear form of the Langmuir and Freundlich equations. Langmuir adsorption isotherms were plotted (Equation 2) by taking C (mg P L^{-1}) as X-axis against C/X (kg L^{-1}) as y-axis. The adsorption isotherm was equally examined by the linear form of the Freundlich equation (Equation 3) by plotting log C against log X.

RESULTS AND DISCUSSION

Physical properties

The five soils have a bulk density values varying from 0.67 to 1.01 g/cm^3. VAS had the lowest (0.67 g/cm^3), followed by Upper Boduma (UPD) and Sasse-Limbe Road (SLR, 0.84 g/cm^3), Limbe - Man O'War Bay Road (LMR, 0.96 g/cm^3), and the highest with Dibanda-Mutengene Road (DMR, 1.01 g/cm^3) (Table 2). Olafur (2008) reported that volcanic ash soils generally have bulk density less than 0.9 g/cm^3; therefore our values show that these soils are typically volcanic ash soils.

Particle size fraction indicates that the textural class of the surface composite soil samples ranged from loam (VAS), through clayey loam (UPD, SLR and LMR) to silty clay (DMR). In all the soils, silt fraction has dominant except for the LMR sample where the clay fraction dominated. Generally, we can say silt content > clay content > sand content (Table 2). This was clearly reflected by their corresponding total porosity values with the silty clay soil having the least porosity (0.62) and the loamy soil having the highest porosity (0.75). Clay is the most active part of the soil both chemically and physically (Page, 1952). The chemical properties of a soil are favorable when clays are abundant but its physical properties will either be good or poor depending on the arrangement of the soil particles (Page, 1952).

The moisture content of a soil is an important soil property in agriculture especially if it is interpreted with

Table 2. Some physical properties of soils from the five sites on the Eastern flank of Mount Cameroon.

Sample code	Soil colour	Bulk density (g/cm^3)	Moisture content (%)	Total porosity	Particle size distribution			Textural class*	Atterberg's activity
					Sand (%)	Silt (%)	Clay (%)		
VAS	2.5YR3/4	0.67	36.14	0.75	41.2	42.0	16.8	L	0.3
UPD	5YR3/2	0.84	31.78	0.68	24.8	45.8	29.4	CL	0.3
SLR	5YR3/2	0.84	21.79	0.68	23.0	41.9	35.1	CL	0.2
DMR	5YR3/2	1.01	19.01	0.62	19.6	40.0	40.4	SC	0.2
LMR	2.5YR8/6	0.96	28.28	0.64	29.4	32.1	38.5	CL	0.3

*CL: Clay loam; SC: Silty clay; L: Loam.

Table 3. Some chemical properties of soils from the five sites on the Eastern flank of Mount Cameroon.

Sample code	pH (H_2O)	EC	Total N	OM	Total P	Bray I P	Ca	Mg	K	Na	Exch. acidity	ECEC	Exch. Fe	CEC
		(uS/cm)	%		mg kg^{-1}		cmol(+) kg^{-1}							
VAS	5.6	38	0.22	5.04	602.4	7.9	8.65	4.32	1.91	0.11	0.68	15.67	0.17	21.82
UPD	5.3	19	0.14	3.01	813.2	6.8	4.52	1.65	0.07	0.13	0.14	6.51	0.15	17.07
SLR	5.2	22	0.20	4.40	800.2	7.2	3.96	1.77	0.07	0.10	0.87	6.77	0.16	14.44
DMR	4.8	24	0.15	3.30	629.5	3.6	3.13	2.01	0.09	0.10	1.13	6.46	0.16	14.54
LMR	4.7	18	0.16	3.47	1116.4	3.6	2.01	1.47	0.19	0.13	1.28	12.07	0.06	18.59

respect to the soil textural class. Of the five soil samples analyzed, that from Vasingi (VAS), a loamy soil, had the highest moisture content (36.14%) followed by UPD, a clay loamy soil (31.78%). These two sites had moisture contents, which were above field capacity when compared with the standard soil moisture range chart (Zotarelli et al., 2010), and if exceeded, can lead to landslides especially the sample UPD which is clay loam and had a total porosity of 0.684. LMR which is a clay loamy soil, had 28.28% moisture content and is known to be at field capacity with good agricultural potential. SLR a clay loamy soil with moisture content 21.79% is at its irrigation start point (Zotarelli et al., 2010). During farming seasons, irrigation is encouraged in this soil else plants will eventually start wilting. DMR is a clayey soil with moisture content 19.01% is at wilting point (Zotarelli et al., 2010), and hence immediate irrigation is recommended for farmers cultivating this area else crop will die at early stage.

Chemical properties

Soil pH (H_2O) of composite surface samples from this region ranged from 4.7 to 5.6 (Table 3). The LMR soil had the lowest pH of 4.7 while the VAS soil had the highest pH of 5.6. All composite surface soils from this region based on their pH (H_2O) are in the range of strongly acidic as per ratings by Benton (1999). The acidity of these soils may be associated with the high rain fall, coupled with the porous nature of the soils, resulting in leaching of bases. Based on the acidic nature of these soils, phosphate ions from P fertilizers often applied to soils of this nature could combine with Al and Fe to form aluminium and iron phosphates, respectively, which are known to be insoluble (Dhyan et al., 2005). There is an increase of pH with altitude which could be attributed to the decrease in temperature with altitude leading to decrease in rainfall. With low rainfall, the exchangeable bases are not washed away thereby increasing the pH.

Electrical conductivity (EC) ranged from 18 to 38 µS/cm (Table 3). According to the ratings established by Hazelton and Murphy (2007), EC was very low for all samples. Low range of EC indicates that tolerant crops and sensitive crops will not be affected if cultivated in these soils.

Vasingi (VAS) soils had the highest percent organic matter (5.04%) and DMR the lowest (3.30%). All the soil samples were in a high organic matter range as per ratings established by Hazelton and Murphy (2007). The soil organic matter content of the composite surface soils from the South Eastern flank of Mount Cameroon positively correlated to the available phosphorus ($r = 0.67$, $P < 0.05$) and exchangeable Fe ($r = 0.62$, $P < 0.05$) (Table 5). This positive correlation existing between organic matter and plant available phosphorus may be because soil organic matter (SOM) among other soil properties has been known to constituent the soil sorption complex which is responsible for binding of anions in the soil material (Tan, 1986). Cation exchange capacity

(CEC) in all soils was in the range of medium as per ratings established by Hazelton and Murphy (2007). CEC ranged between 14.44 cmol (+) kg^{-1} for SLR soil and 21.82 cmol (+) kg^{-1} for VAS soil (Table 3). CEC positively correlated to the effective cation exchange (ECEC) ($r = 0.70$, $P < 0.05$) and moisture content ($r = 0.80$, $P < 0.05$) (Table 5). The positive correlation between CEC and ECEC is due to the fact that CEC is the main constituent of ECEC. The positive correlation existing between CEC and moisture content may be due to the fact that a moist soil can easily leach its bases. The moderate CEC may be associated to the high organic matter content in these soils as organic matter is a source of negative charge in the soil. Landon (1991) attributed high soil fertility and high nutrient retention capacity to high CEC and low soil fertility, poor nutrient retention capacity to low CEC; therefore these composite surface soil samples are moderately fertile and moderate in nutrient retention capacity.

The ECEC ranged between 6.46 cmol (+) kg^{-1} and 15.67 cmol (+) kg^{-1} (Table 3) with VAS having the highest ECEC value (15.67cmol (+) kg^{-1}) and DMR the lowest (6.46 cmol (+) kg^{-1}). The desired range for ECEC is between 5 and 25 cmol (+) kg^{-1} Landon (1991). Therefore, ECEC was in the desirable range for all soils and is normally satisfactory for agriculture with average fertilizer application. One reason for the moderate ECEC may be that there is enough ionization of the functional groups of the OM to develop a great number of negative charges. Another possibility is that a great part of the organic charges might not have strongly interacted with the inorganic fraction, decreasing the effective negative charge. Both CEC and ECEC are moderate and positively correlated with each other ($r = 0.70$, $p < 0.05$). This may be due to the fact that very few portions of the exchangeable sites were occupied by exchangeable Al as a result of weathering and leaching that has occurred.

Exchangeable bases (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) in all composite surface soil samples showed the following trend: $Ca^{2+} > Mg^{2+} > K^{+} > Na^{+}$. Quantitatively, the concentrations of these cations vary as follows: Ca^{2+} (between 2.01 and 8.65 cmol (+) kg^{-1}), Mg^{2+} (between 1.47 and 4.32 cmol (+) kg^{-1}), K^{+} (between 0.07 and 1.91 cmol (+) kg^{-1}), and Na^{+} (between 0.10 and 1.13 cmol (+) kg^{-1}) (Table 3). These concentrations are quite low (especially for K^{+} and Na^{+}), low for Mg^{2+} and vary from low to medium for Ca^{2+} based on the ratings established by Hazelton and Murphy (2007). These low concentrations may be as a result of the porous nature of the composite surface samples that are prone to base leaching. These low concentrations can also be explained from the prevalence of pH values of less than 5.5, where these cations are deficient (Kim, 1998). Nevertheless, Ca^{2+} is in moderate concentrations for VAS soil with respect to the critical fertility levels.

Vasingi soils registered the highest value of available P (Bray 1) with 7.8 mg P kg^{-1} whereas DMR and LMR had the lowest 3.6 mg P kg^{-1} available P (Table 3). All samples from the study area were in the range of low available plant phosphorus as per ratings established by Hazelton and Murphy (2007). These low P concentrations may be associated with the acidic nature of the soils: all the soils studied are strongly acidic with pH values <5.5. Harrison (2007) reported that at this pH values, Al, Fe and Mg are highly soluble and will react with the phosphate ions ($H_2PO_4^-$) to form hydroxyl-phosphate which is insoluble, and unavailable for plants. The low contents of available P observed in the soils of the study area were in agreement with the studies made by Tening et al. (2013) on soils collected within this region.

From the Atterberg limits and soil activity index, which is only an indication of the type of clay mineral present, all samples are likely to be dominated by kaolinite clay minerals (1:1 clay type), inactive and non-expanding clays (Skempton, 1969), as a result, if these soils could retain P, fixation will be described as being more a physisorption than a chemisorption phenomenon.

Phosphorus adsorption

The graphical representation of equilibrium P concentration versus rate of P adsorbed on unit mass of soil colloid were used to calculate the maximum adsorption capacity of the soils and the affinity of the soil to retain P. In all five soils, the equilibrium P solution and the per unit P adsorption by soil colloids increased with increasing P addition. The variation of the equilibrium concentration and the P adsorbed at each different levels of P is as shown in Figure 3.The graphic representation of the adsorption isotherms (Figure 3) of the soils showed that all samples respected the first order isotherm. This is a good confirmation to the choice of our isotherm models (Langmuir and Freundlich isotherm models) which obey the first order isotherm.

Linearizing the adsorption data into different isotherm models

The phosphorus adsorption maxima were determined by fitting the solution P concentrations and adsorbed P values in Langmuir and Freundlich equations. The highest P adsorption maxima (Table 4) for the five composite surface soils according to Langmuir (b_L) followed the order VAS (0.01 mg kg^{-1}) < SLR (0.01 mg kg^{-1}) < DMR (0.02 mg kg^{-1}) < UPD (0.02 mg kg^{-1}) < LMR (0.05 mg kg^{-1}) and according to Freundlich (K_F) followed the order VAS and UPD (16.87 mg kg^{-1}) < DMR (24.72 mg kg^{-1}) < SLR (34.12 mg kg^{-1}) < LMR (174.58 mg kg^{-1}). The phosphate adsorption isotherms gave a good fit in case of Langmuir ($r^2 = 0.91$ to 0.99) and Freundlich ($r^2 = 0.98$ to 0.99) obtained from their equations derived from Figures 4 and 5 are as shown in Table 4. The uniqueness

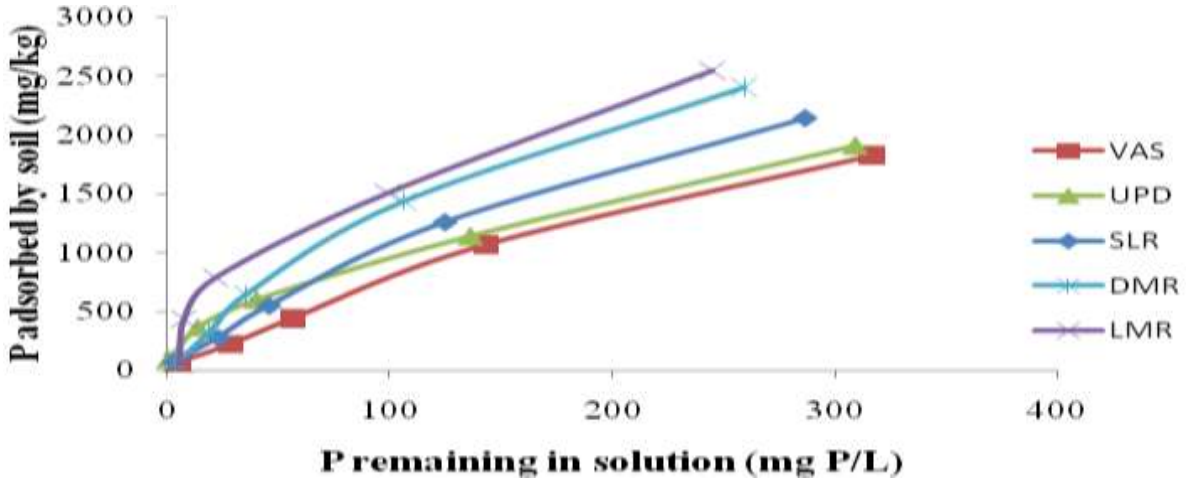

Figure 3. Phosphate adsorption plots of soils from the five sites on the Eastern flank of Mount Cameroon.

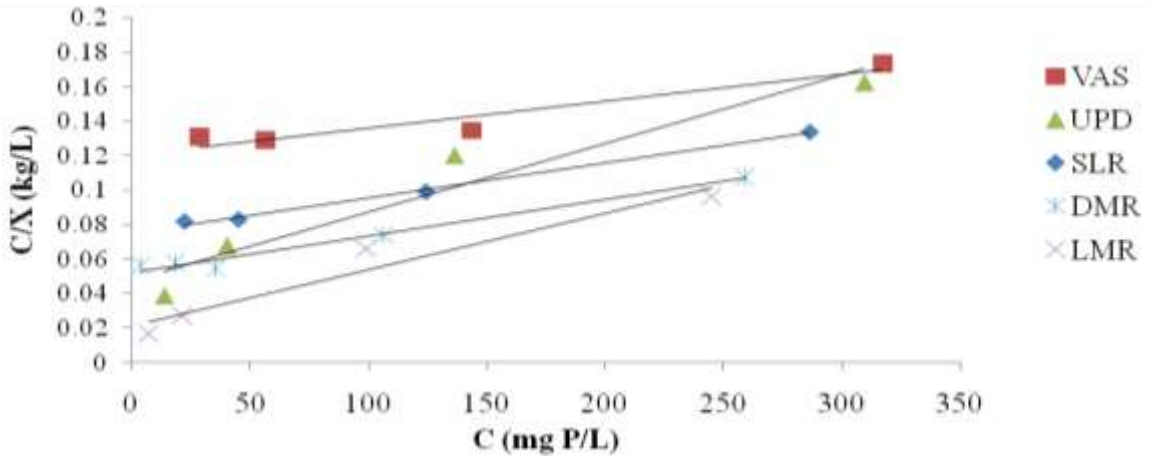

Figure 4. Phosphate adsorption fitted into Langmuir equation for soils from the five sites on the Eastern flank of Mount Cameroon.

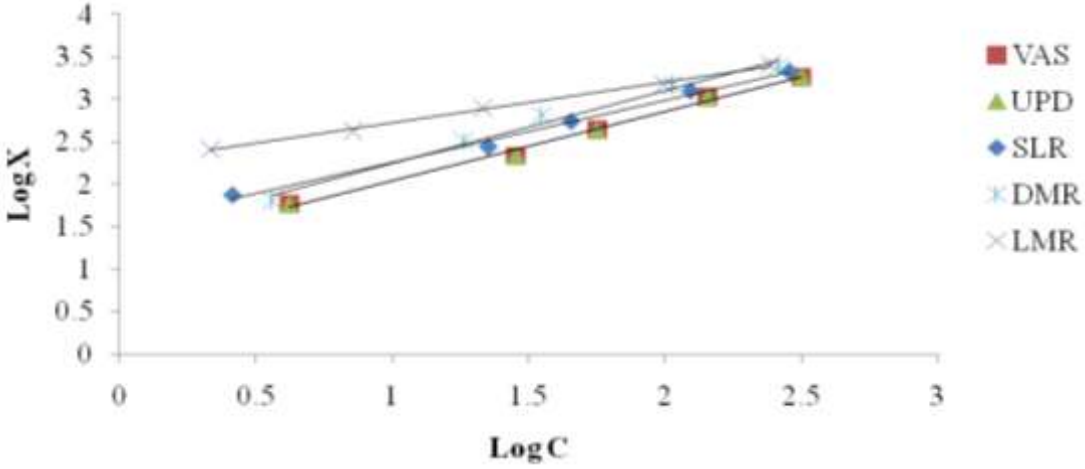

Figure 5. Phosphate adsorption fitted into Freundlich equation for soils from the five sites on the Eastern flank of Mount Cameroon.

Table 4. Parameters of fitted Langmuir and Freundlich adsorption models for five sites on the eastern flank of Mount Cameroon.

Parameter	Langmuir isotherm			Freundlich isotherm		
	b_L	K_L	R^2	K_F	n_F	R^2
VAS	0.008	1000	0.911***	16.866	0.813	0.993***
UPD	0.021	1000	0.930***	16.866	0.813	0.993***
SLR	0.014	1000	0.995***	34.119	0.729	0.993***
DMR	0.019	1000	0.978***	24.717	0.857	0.978***
LMR	0.050	1000	0.944***	174.582	0.480	0.997***

***Significant at P ≤ 0.001 level.

Table 5. Correlation coefficient (r) between some soil physicochemical properties.

Correlation	pH	EC	Total N	OM	Total P	Bray1 P	Ca	Mg	K	Na	Exch. acid	ECEC	CEC	Exch. Fe	clay	Bulk density	Moisture
pH	1.00																
EC	0.60*	1.00															
Total N	0.30	0.50	1.00														
OM	0.30	0.50	1.00**	1.00													
TotP	-0.60*	-1.00**	-0.50	-0.50	1.00												
Bray1P	0.87*	0.56	0.67*	0.67*	-0.56	1.00											
Ca	1.00**	0.60*	0.30	0.30	-0.60*	0.87*	1.00										
Mg	0.60*	1.00**	0.50	0.50	-1.00**	0.56	0.60*	1.00									
K	0.05	0.36	0.56	0.56	-0.36	0.11	0.05	0.36	1.00								
Na	0.00	-0.63*	-0.32	-0.32	0.63*	-0.16	0.00	-0.63*	0.16	1.00							
Exch. acid	-0.90**	-0.30	0.10	0.10	0.30	-0.67*	-0.90**	-0.30	0.31	-0.16	1.00						
ECEC	0.30	0.10	0.80*	0.80*	-0.10	0.56	0.30	0.10	0.67*	0.32	0.00	1.00					
CEC	0.30	0.10	0.30	0.30	-0.10	0.21	0.30	0.10	0.82*	0.63*	-0.10	0.70*	1.00				
Exch. Fe	0.67*	0.98**	0.62*	0.62*	-0.98**	0.71*	0.67*	0.98**	0.29	-0.65*	-0.36	0.21	0.05	1.00			
clay	-0.90**	-0.30	-0.40	-0.40	0.30	-0.87*	-0.90**	-0.30	-0.15	-0.32	0.80*	-0.60*	-0.50	-0.41	1.00		
Bulk density	-0.87*	-0.36	-0.56	-0.56	0.36	-0.95**	-0.87*	-0.36	-0.16	-0.16	0.72*	-0.67*	-0.41	-0.50	0.98**	1.00	
Moisture	0.70*	0.10	0.30	0.30	-0.10	0.62*	0.70*	0.10	0.41	0.63*	-0.60*	0.70*	0.80*	0.15	-0.90**	-0.82*	1.00
Total porosity	0.87*	0.36	0.56	0.56	-0.36	0.95**	0.87*	0.36	0.16	0.17	-0.72*	0.67*	0.41	0.50	-0.98**	-1.00**	0.82*

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

Table 6. Correlation coefficient between Freundlich parameters and soil physicochemical properties.

Correlation	k_F	n_F
pH	-0.87*	0.36
EC	-0.56	0.67*
Total N	0.10	-0.31
OM	0.10	-0.31
Total P	0.56	-0.67*
Bray1P	-0.53	0.00
Ca	-0.87*	0.36
Mg	-0.56	0.67*
K	-0.03	-0.03
Na	-0.08	-0.41
Exch. acidity	0.87*	-0.36
ECEC	0.05	-0.56
CEC	-0.31	-0.10
Exch. Fe	-0.53	0.53
clay	0.67*	0.05
Bulk density	0.55	0.13
Moisture content	-0.56	-0.15
Total porosity	-0.55	-0.13

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed). n_F = Empirical constant related to bonding energy of soil for phosphate; K_F = Proportionality constant for Freundlich model (mg P/kg).

of Langmuir bonding energy constant (K_L) for all the composite surface soils (Table 4) and (r^2 = 0.91 to 0.99) compared with the corresponding Freundlich parameters, it could be said that Freundlich model better described P fixation in these soils more than Langmuir. This assumption can further be confirmed based on the display of points in Figure 4 for Langmuir and Figure 5 for Freundlich plots. Figure 4 shows that except for the SLR soil sample, the linearized data according to Langmuir model produced curves than straight lines and this was contrary to Figure 5 where the linearized data according to Freundlich, both samples gave straight lines. The b_L values < 1.0 mg kg^{-1} and Freundlich bonding energy constant (n_F) varied between 0.48 and 0.86 L mg^{-1}. These n_F values were similar to those reported in Ali et al. (2013).

Relationship between adsorption and soil physicochemical properties

Significant correlations were observed between Freundlich maximal adsorbed P and some soil physicochemical properties. There a negative correlation between K_F and soil pH (H_2O) (r = - 0.87, p < 0.05), this may be due to the fact that an increase in pH in acidic soils reduce the solubility of exchangeable Al adsorption sites and will eventually reduce the amount of soil P adsorbed, hence K_F. K_F negatively correlated with calcium as a lone soil exchangeable base (r = - 0.87, p < 0.05), this may be because among soil exchangeable bases, only Ca can react with the phosphates to form calcium phosphates but this was not the case here since the soils were strongly acidic and the concentration of Ca was low in almost all the soils. There was a positive correlation between K_F and exchangeable acidity (r = 0.87, p < 0.05) (Table 6) similar to findings by Hoseini and Taleshmikaiel (2013). This was because the soils were moderate in exchangeable acidity and at this soil pH, aluminium was very soluble in soil solution and should be responsible for phosphorus adsorption. Soil clay fraction also correlated positively with K_F (r = 0.67, p < 0.05) illustrating that soil texture played a major role in P adsorption of these soils. This was in conformity with the findings of Tening et al. (2013) who reported a net negative significant correlation between percent fixed P and available P and attributed P-fixation to the soil pH and clay content. Clay minerals are not only important in cation exchange reactions in acidic soils but they are perhaps a major factor governing the availability of phosphate in many soils. This could be the case with these soils given that the major clay minerals were kaolinite as judged from the activity index. Clay content had a negative and significant correlation with soil pH (r = - 0.90, p < 0.01) and positive correlation with exchangeable acidity (r = 0.80 p < 0.05) (Table 5). This implies that the study area could be made up of clays containing high exchangeable aluminium (Al^{3+}) and

hydrogen (H^+) ions as found in most acid soils (Muindi et al., 2015).

Conclusion

The results depicted that Freundlich adsorption model is precise in predicting P-fixation in the soils of the Eastern flank of Mount Cameroon. These soils have the ability to fix P with the amount fixed increasing with increasing supply of P. Phosphorus fixation was site specific and revealed that the soils can adsorb up to174.58 $mgPkg^{-1}$.

Apart from Ca, exchangeable acidity, organic matter, clay and pH that influenced P fixation; other soil properties such as CEC, moisture content, and exchangeable Na also contributed significantly.

The study identified a wide range in P adsorbed on the soils. There is therefore need for more intensive plant growth experiments to understand the role and interactions of soil properties on P availability for each site in the area before valid and concise fertilizer recommendations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of cattle manure over the content, extraction and exportation of nutrients in snap bean

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The use of organic fertilizers is essential for the sustainable production of vegetables. In this context, the aim of this study was to evaluate the effect of different doses of cattle manure on plant nutrition, yield, extraction and exportation of nutrients in the snap bean cultivar "macarrão trepador". The experiment was held in EPAMIG-southeast, in Oratório-MG. The randomized block design was used, with four replications and five doses of cattle manure (0, 10, 20, 40 and 80 t ha^{-1}). The content of nutrient in the leaves, yield components and the extraction and exportation of nutrients were evaluated. The content of all nutrients are in the adequate range, except for K and Ca. The extraction and exportation of nutrients raised with the growing doses of cattle manure. The application of 67.5 t ha^{-1} of cattle manure provided the highest yield, in which the quantity of each nutrient exported by pods were 45.6 (N), 5.2 (P), 16.1 (K), 4.6 (Ca), 3.8 (Mg) and 2.02 (S) in Kg.ha^{-1} and of 88.8 (Zn), 89.5 (Fe), 100.5 (Mn), 11.4 (Cu) and 33.1 (B) in g.ha^{-1}. The greatest values for pod length (14.51 cm), number of pods per plant (58.34) and yield (14.86 t ha^{-1}) were estimated with 56.7, 66.6 and 67.5 t ha^{-1} of manure, respectively. The application of cattle manure fertilizer improves the nutritional state of plants and yield, which, in this study, soared from 7.56 t ha^{-1} in the control to 14.86 t ha^{-1}. These results are promising for the organic cultivation and sustainability of the production systems.

Key words: *Phaseolus vulgaris* L., organic cultivation, nutrition of plants, exportation of nutrients.

INTRODUCTION

Snap bean is the main vegetable of the family Fabaceae and belongs to the same species of common bean (*Phaseolus vulgaris* L.). Worldwide known, this vegetable also is one of the most popular in Brazil.

The main snap bean varieties cultivated in Brazil have pole-type habit, cylindrical or flat shaped pods, and yield on average, 25 t ha^{-1} ; bush-type varieties reach half of the yield compared to the pole type and have a reduced production cost because the plant staking is not required (Filgueira, 2008; Almeida et al., 2014; Trani et al., 2015).

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The snap bean plants are adapted to dry and hot weather, with temperatures ranging from 15 to 30°C and require high levels of promptly soluble nutrients for a short period during intense growth (Araújo et al., 2001). The amount of nutrients absorbed, accumulated and exported are essential information to understand the nutritional requirements of the culture, which, along the soil availability, will determine a strategy to supply any nutritional deficiency (Sampaio and Brasil, 2009).

Appropriate amounts of high quality manure can meet the needs of macronutrients for plants, especially potassium, which is the second nutrient extracted in large quantities by plants. Although exchangeable potassium represents the fraction available for plants, in some specific soils, non-exchangeable potassium can also contribute in the short-term supply of potassium (Raij et al., 1996).

In soils with reduced organic matter content, organic fertilizers are soil-conditioning agents, which improve crop conditions, increasing water retention and availability of macro and micronutrients absorbable by the roots (Galvão et al., 2008; Costa et al., 2013). The use of organic fertilizers in agricultural crops have already been investigated in many studies, with relevant results for quality and yield, for either organic and conventional farming (Araújo et al., 2001; Vidal et al., 2007; Oliveira et al., 2010; Silva et al., 2012b). Santos et al. (2001) analyzed and compared poultry, cattle and goat manure and earthworm humus as sources of organic matter in the snap bean culture and have concluded that cattle manure should be recommended as a source of animal organic matter in a crop fertilization program, due to the greatest yield and profitability over the other fertilizers.

The use of manure is a sustainable alternative that reduces the cost of crop fertilization, since the State of Minas Gerais has the second largest Brazilian herd of cattle and thus a high availability of this nutritional supplement. However, studies are necessary to indicate the appropriate doses of manure to each type of soil, because the application of elevated doses of organic fertilizers can cause imbalance of nutrients and lead to soil salinization (Rodrigues and Casali 1999), causing, occasionally, the unavailability of phosphorus. Therefore, there is a lack of information about the usage of organic fertilizers in snap bean. In this scenario, the aim of this study was to evaluate the effects of application of cattle manure on plant nutrition, yield, extraction and exportation of nutrients by the pods in the cultivation of snap bean cv. Macarrão Trepador (Favorito).

MATERIALS AND METHODS

The experiment was held at the Vale do Piranga experimental farm, belonging to EPAMIG, located in Oratorios-MG, during the months April to July 2012. The research unit is situated at latitude 20°30' S and longitude 43°00' W. The altitude is 400 m over the sea level, with an average annual maximum temperature of 21.8°C and minimum of 19.5°C, with an average annual rainfall of 1.250 mm. The soil is classified as cambic Red-Yellow Argisol, terrace phase, clayey. The soil chemical analysis in the 0-20 cm layer depicted the following characteristics: pH (water 1:2.5) = 6.0; organic matter = 21 $g.kg^{-1}$; in $mg.dm^{-3}$: P = 13.4; K = 142; Zn = 7.5; Fe = 173.2; Mn = 5.5; Cu = 5.9 and B = 0.5; in $cmol_c.dm^{-3}$: Ca^{2+} = 2.0; Mg^{2+} = 1.0; Al^{3+} = 0,0; $H^{+}Al$ = 2.48; CEC (t) = 3.36 and CEC(T) = 5.84; V = 58% and P-rem = 35 $mg.L^{-1}$.

The cattle manure was previously tanned and exhibited the following characteristics when applied over the crop: C.O. = 13.41 $dag.kg^{-1}$; pH = 7.60; C/N = 7.24; moisture = 50.45%; in $g.kg^{-1}$: N = 18.50; P = 8.10; K = 22.40; Ca = 16.80; Mg = 6.30 and S = 4.70.

The experiment was carried out in a randomized block design, with four replications and five treatments. The treatments consisted of the following doses of cattle manure: 0, 10, 20, 40 and 80 t ha^{-1}. The plots contained four three-meter lines, spaced 1 m between rows. Each row consisted of 40 plants and only the 16 central plants were harvested. The agricultural preparation of the experiment started by furrows, application and incorporation of half of each dose of cattle manure in the soil; then, small piles were formed where the coves were opened and two seeds of the commercial cultivar Macarrão Trepador (Favorito) were planted; 15 days later the plants were thinned, resulting in one plant per pit. The second half of the cattle manure doses were applied 30 days after planting (DAP).

The weed control consisted of two hand hoeing in the rows and three between them. The drip irrigation method was carried out utilizing pipes with drippers 10 cm apart, which were located in each row of snap bean. Pulverization with fermented cow urine were made biweekly during two months, at 1.0% concentration, until plants reached bloom stage. The chemical analysis of urine depicted the following characteristics, in percentage: N = 6.96; P = 0.00; K = 0.89; Ca = 0.00; Mg = 0.04; S = 0.03; organic compounds = 0.17; and, in particles per million: Zn = 0.00; Fe = 1.00; Mn = 0.00; Cu = 0.00 and pH = 8.50.

When the plants reached full-bloom, the reference leaf was collected (fourth uppermost completely expanded leaf) in the useful area of the plot; there were sampled six leaves (leaf blade and petiole) per treatment (Miyazawa et al., 2009). The collected material was stored in paper-bags and dried in a laboratory oven with forced air circulation at 65°C for 72 h or until it reached constant weight. Afterwards, the material was grinded in a Wiley mill and undergone to macro and micronutrients analysis: Nitrogen (N), phosphorus (P), potassium (K), calcium (C), magnesium (Mg) and sulfur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) (Miyazawa et al., 2009).

The harvest started 58 DAP, when the pods were completely developed, but before becoming fibrous and with salient seeds, which required weekly harvests. The total number and the fresh mass of commercial pods were evaluated by the harvest. Pods with injuries, tortuosity and damages caused by plagues and diseases were not considered for evaluation. Thus, the total number and the fresh mass of pods were obtained (Vidal et al., 2007). The yield was obtained by the fresh mass sum of commercial pods transformed in t ha^{-1}, due to the insignificant amount of defective pods.

In each harvest, samples of dry pods were grinded to determine the nutrient content, according to the method previously described (Miyazawa et al., 2009).

After harvesting the pods, the aerial part of the six plants in each treatment were cut off by the soil level and the material was used for mineral composition analysis and to determine fresh and dry mass. Afterwards determining the dry mass, the material was grinded and the macro and micronutrient content was assessed. The fresh mass of aerial part was estimated by summing the shoot fresh mass and the pod fresh mass. The extraction of nutrients by the shoot was obtained multiplying the content of each nutrient by the shoot dry mass and summing to it the content of this nutrient in the pods. The data was submitted to variance analysis (ANOVA)

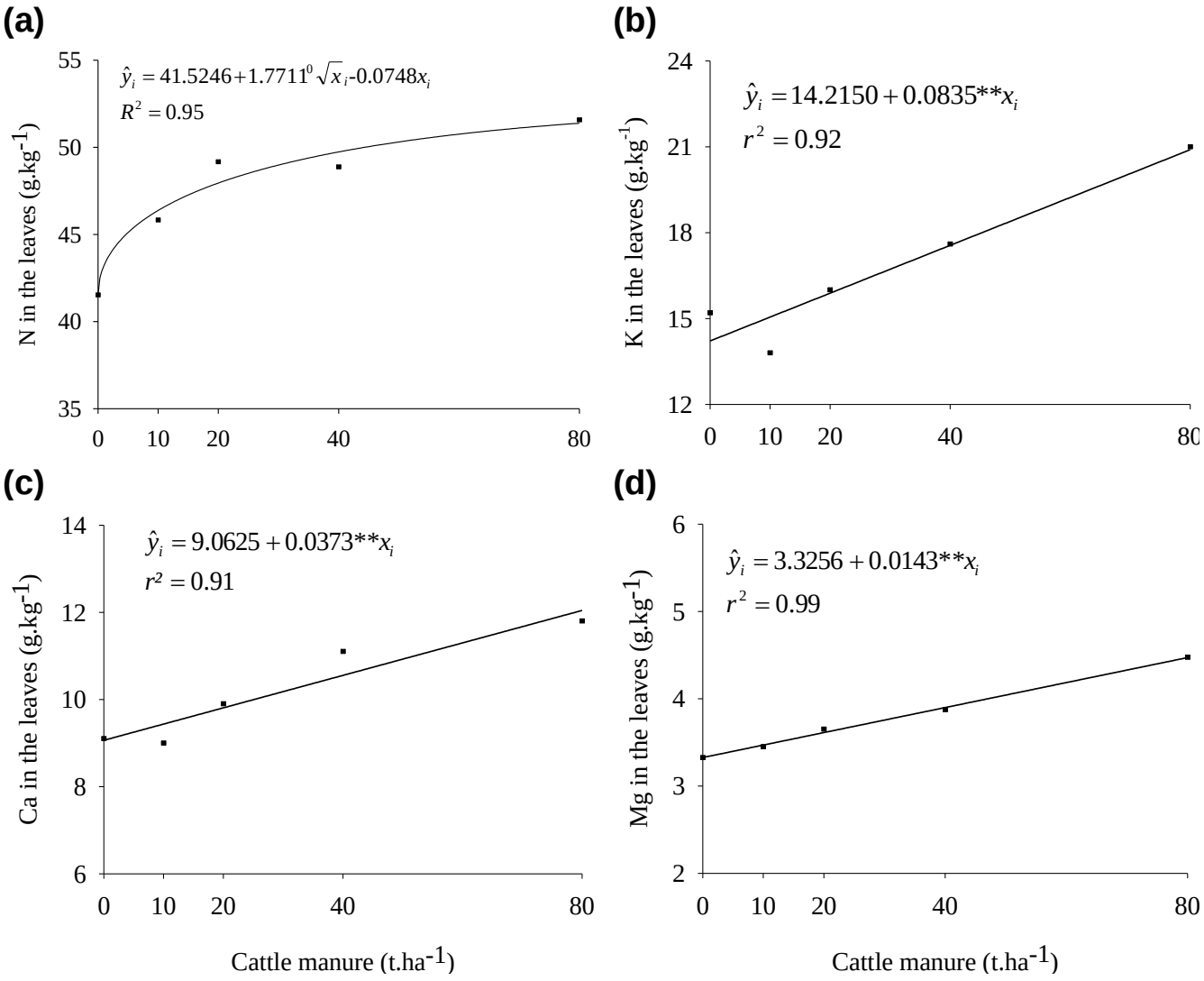

Figure 1. Content of nitrogen (a), potassium (b), calcium (c) and manganese (d) in the leaves of snap bean, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. **, ^{0} significant at 1 and 10% by the F test, respectively.

and the quantitative means were submitted to regression analysis, at 5%. The analysis was processed via software SAEG - Sistema para Análise Estatística e Genética (SAEG, 2007).

RESULTS AND DISCUSSION

The doses of cattle manure (CM) positively influenced the content of nitrogen, potassium, calcium, manganese and boron in the leaves, but negatively the content of Zinc, Iron and Copper, while no different was observed for the other nutrients. This result can be related to the fact that the addition of organic residuals increases the soil capability to donate and receive H^+ ions, increasing the buffering capacity and keeping the pH at values close to neutral. The organic residuals let N, K, Ca, Mg and B free in solution and reduce the availability of Zn, Fe and Cu to the plants (Pavinato and Rosolem, 2008; Silva et al., 2012a; Schoninger et al., 2012).

Positive and significant effect, square root function, was noted by the application of cattle manure over content of nitrogen in the leaves (Figure 1a). The nitrogen was in the adequate range for the culture (40-60 g ha^{-1}) in all doses of cattle manure, including the control. Probably, this is attributable to the fact that plants present symbiotic association with N_2-fixing bacteria, which allows the enhancement of soil fertility via the fixation of atmospheric N_2 at the vegetal mass, supplying the nitrogen necessary to the plant and improving the content of N in the soil (Pelegrin et al., 2009). Besides, the cattle urine pulverizations (6.96% of N) until bloom stage might had contributed to escalate the nitrogen content in the leaves.

Even though the content of K and Ca in the leaves had

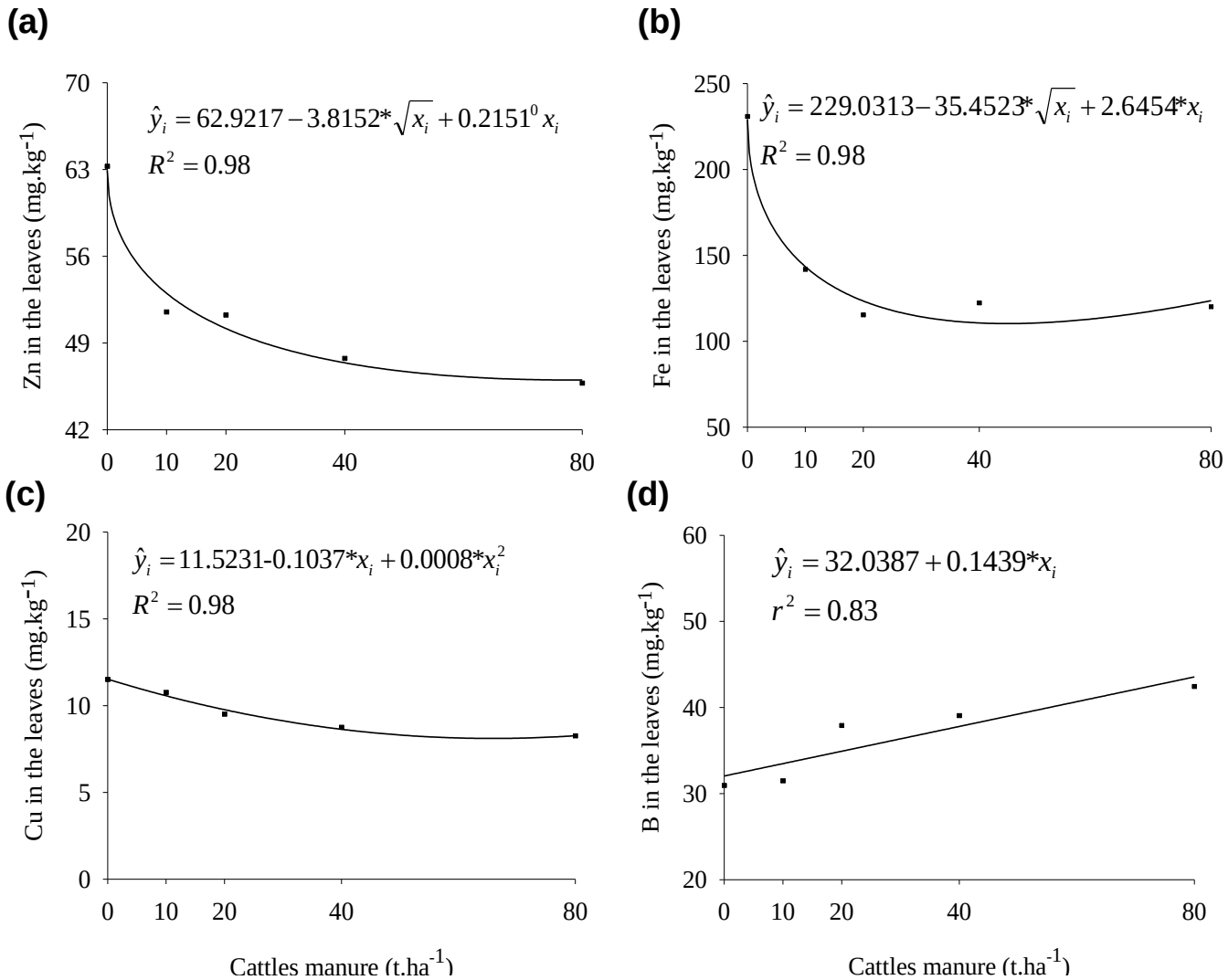

Figure 2. Content of zinc (a), iron (b), copper (c) and boron (d) in the leaves of snap bean, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. *, ^{0} significant at 5 and 10% by the F test, respectively.

heightened with increasing doses of cattle manure (Figure 1b and c), attaining 21 g.kg^{-1} of K and 11.8 g.kg^{-1} of Ca with the highest dose, these values are under the adequate range for the culture: 25-40 g.kg^{-1} of K and 15-30 g.kg^{-1} of Ca. It probably occurred since the high demand of these nutrients in a short period by the plants overcame the supply provided by the cattle manure applied. Similar results were depicted by Sediyama et al. (2015), in which the foliar content of K and Ca found was under the adequate range for the snap bean culture, utilizing doses of swine biofertilizer (up to 180 m^3.ha^{-1}).

The cattle manure doses had a positive linear relationship on the content of Mg (Figure 1d), reaching 4.5 g.kg^{-1}in the highest dose, which is in the adequate range to Mg (3-8 g.kg^{-1}). Oliveira et al. (2006) evaluated the effect of poultry manure, up to 28 t ha^{-1} , in the snap bean cultivation and observed the Mg content ranging from 4.4 to 5.6 g.kg^{-1} in the leaves, similarly to the present study.

The doses of cattle manure had a negative effect on the Zn, Fe and Cu contents in snap bean leaves (Figure 2a to c). However, meanwhile the contents of Zn and Fe were in the adequate range for the culture (30-100 mg.kg^{-1} for Zn and 50-300 mg.kg^{-1} for Fe), and the Cu content reduced to values slightly under the adequate range (10-30 mg.kg^{-1}) (Trani and Raij, 1996). It is believed that the organic fertilization might had reduced the concentration of exchangeable zinc, iron and, mainly, copper due to complexation of these elements in the organic matter and, therefore, resulted in reduced concentrations in the leaf tissue (Silva et al., 2012a; Schoninger et al., 2012).

The content of B chlorophyll in the leaves responded positively and linearly to increasing doses of cattle manure, attaining the adequate range for the culture (20-

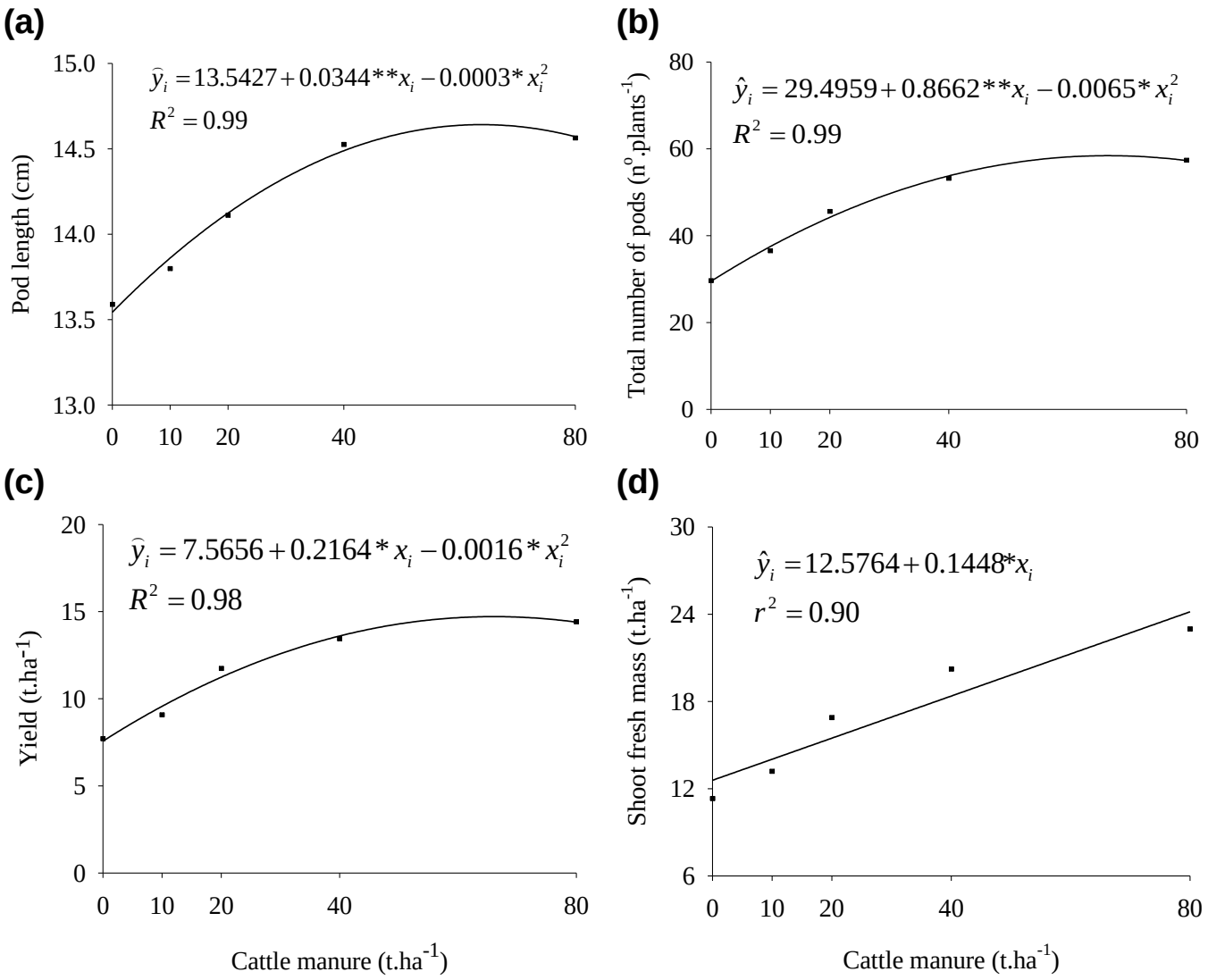

Figure 3. Length (a), total number (b) and yield (c) of pods and total fresh mass of the shoot (d) of snap bean plants, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. **, * significant at 1 and 5% by the F test, respectively.

60 mg.kg^{-1}) in all doses. Thus, the highest value, 42.4 mg.kg^{-1} was beheld in the greatest dose of cattle manure (Figure 2d).

The diameter, length and number of pods per plant took off significantly with the escalating doses of cattle manure applied. It was not possible to adjust a mathematical model to explain the response of the pod diameter over the application of cattle manure, which had an average value of 9.89 mm. The maximum length of pod (14.51 cm) was estimated with the application of 56.67 t ha^{-1} of cattle manure (Figure 3a). Santos et al. (2001) have also observed a rising response of pod length over different doses of cattle manure (0 to 40 t ha^{-1}) in the snap bean cultivar Macarrão Favorito.

The highest number of pods per plant (58.34) was estimated with the application of 66.6 t ha^{-1} of cattle manure, that is, an increase of 28 pods per plants over the control (Figure 3b). The number of pods per plant in every treatment, except the control, was superior to the values presented by Oliveira et al. (2005), who evaluated the response of snap bean plants to the application of P_2O_5 in sandy soil with reduced concentration of phosphorus (P = 11.06 mg.dm^{-3}). In the study, the application of 267 kg.ha^{-1} of P_2O_5 resulted in the largest number of pods (22 per plant). Hence, the greater number of pods obtained in this research can be a consequence of the higher content of phosphorus (13.4 mg.dm^{-3}) in the soil and the larger amount of P_2O_5 applied through the cattle manure (618 kg.ha^{-1} applied in the dose of greatest yield of pods - 66.6 t ha^{-1}), besides the concomitant supply of alternative nutrients. According to Primavesi (2002), the nutritional balance is more

important than bigger amounts of macronutrients singly applied to increase yield. Moreover, the cattle manure contributes to the improvement of soil characteristics, increasing the absorption of nutrients by the plant (Santos et al., 2001). The organic matter influences directly in the cation exchange capacity (CEC), retaining and providing nutrients, retaining moisture, structuring the soil, etc. Consequently, the organic matter contributes to reduce the fixation of phosphorus in the soil (Costa et al., 2013).

The average number of pods per plant contributed to step up yield and a positive correlation (r=0.99) was observed between these traits. In common bean, Soratto et al. (2010) observed that the number of pods per plant was the most correlated component with yield.

The doses of cattle manure did not influenced the fresh mass and the percentage of dry mass in the pods, which showed an average of 7.71 g and 8.98%, respectively. Oliveira et al. (2006), working with the same snap bean cultivar in tillage system and different cover crops, evaluated the fresh mass of pods and did not observed any significant differences either.

The greatest yield of pods (14.86 t ha^{-1}) was predicted by the application of 67.5 t ha^{-1} of cattle manure, that is, an increment of 7.29 t ha^{-1} in comparison to control (Figure 3c). The increase in yield was a consequence of the biggest number of pods per plant, stimulated by the better nutritional conditions. Santos et al. (2001) studied the effects of different doses of cattle manure over snap bean cultivar Macarrão Trepador and observed an escalating yield with increasing doses up to 24 t ha^{-1}. Peixoto et al. (2001), working with 30 inbred lines of pole-type snap bean in Anápolis-GO, have also observed yields between 9.5 and 21.3 t ha^{-1}.

There was a significant linear effect between the total fresh mass of the shoot and the cattle manure doses (Figure 3d). Therefore, the total fresh mass of the shoot picked up from 12.58 to 24.16 t ha^{-1} with the growing supply of nutrients, through the escalating doses of cattle manure.

The amount of macronutrients extracted by the snap bean plants climbed with the heightening doses of cattle manure applied (Figure 4a and f), in which the nitrogen was the most extracted nutrient. The nitrogen, followed by the potassium, were the most extracted nutrients in cowpea, showing a significant gap from the other nutrients (Sampaio and Brasil, 2009). These results corroborates with Oliveira et al. (2007), who have studied the application of growing doses of K_2O and verified that potassium is the second most extracted nutrient in the snap bean culture.

The extraction of micronutrients analysis depicted a significant increase in the quantity of Fe and B extracted, in which the greatest values were attained with the highest doses of cattle manure (Figure 5a and b). The doses of cattle manure had no significant effect over the extraction of Zn, Mn and Cu, whose average values were 83.1, 340.2 and 14.8 g.ha^{-1}, respectively.

The supply of larger amounts of nutrients, with the growing doses of cattle manure, provided an increase of 11.58 t ha^{-1} in the total fresh mass of the shoot (Figure 3d). Thus, besides escalating the content of nutrient in the leaves, there was also an increase of nutrients extracted from the soil, according to the plant growth and pod yield. Although the plants had a major development with the cattle manure doses, the amount of Zn, Mn and Cu did not differ significantly, which means the plants that yielded 7.7 t ha^{-1} as well as the plants that yielded 14.4 t ha^{-1} extracted the same amount of these micronutrients. This fact can be due to the decline in the content of Zn and Cu in the leaves. The content of Mn in the leaves have also plummet from 229 to 124.8 mg.kg^{-1} with the rising doses of cattle manure; however, the high coefficient of variation (49.13%) proportioned no significant difference.

The doses of cattle manure had a significant positive linear relationship over the exportation of macronutrients (Figure 6a and e), Fe and B (Figure 7A and b) by the pods. The amount of macronutrients exported with the application of 67.5 t ha-1 of cattle manure, which provided the greatest yield (14.86 t ha^{-1}), were 45.6 (N), 16.1 (K), 5.2 (P), 4.6 (Ca), 3.8 (Mg) and 2.02 (S) kg.ha^{-1}. The quantity of micronutrients exported were 88.8 (Zn), 89.5 (Fe), 100.5 (Mn), 33.1 (B) and 11.4 (Cu) g ha^{-1}. In a work carried out by Sediyama et al. (2015) utilizing the same snap bean cultivar and doses of swine biofertilizer up to 180 L ha^{-1}, the same order of macronutrients extracted was detected.

The micronutrients Mn and Cu did not had significant differences over the quantities exported by the pods.

The percentage of macro and micronutrients exported by the pods had no significant difference in relation to the nutrients extracted by the plant. Therefore, the average percentage of nutrients exported by the pods in relation to the total extracted by the shoot were 53 of N, 57 of P, 43 of K, 19 of Ca, 40 of Mg, 38 of S, 50 of Zn, 9 of Fe, 23 of Mn and 37 of B. It demonstrates equilibrium in the distribution of nutrients in the shoot, which kept the same proportion of nutrients translocated to the reproductive part, despite the development of the plant.

The Cu was the sole nutrient to express an alteration in its distribution in the plant with growing doses of cattle manure, declining its percentage from 67% in the control to 38% in the highest dose of cattle manure (Figure 8).

The content of nutrients in the pods with increasing doses of cattle manure disclosed significant difference only to P, Ca, Zn and B (Figure 9a and d). The average nutritional content of the other nutrients were 299.72 of N; 106.41 of K; 26.27 of Mg; 17.99 of S; 0.63 of Fe; 0.78 of Mn and 0.14 Cu (mg.100 g^{-1} of pods).

Altogether, the content of phosphorus in the pods rose with the escalating doses of cattle manure applied (Figure 9a), while the content of Ca and Zn reduced in comparison to control. However, the content of B

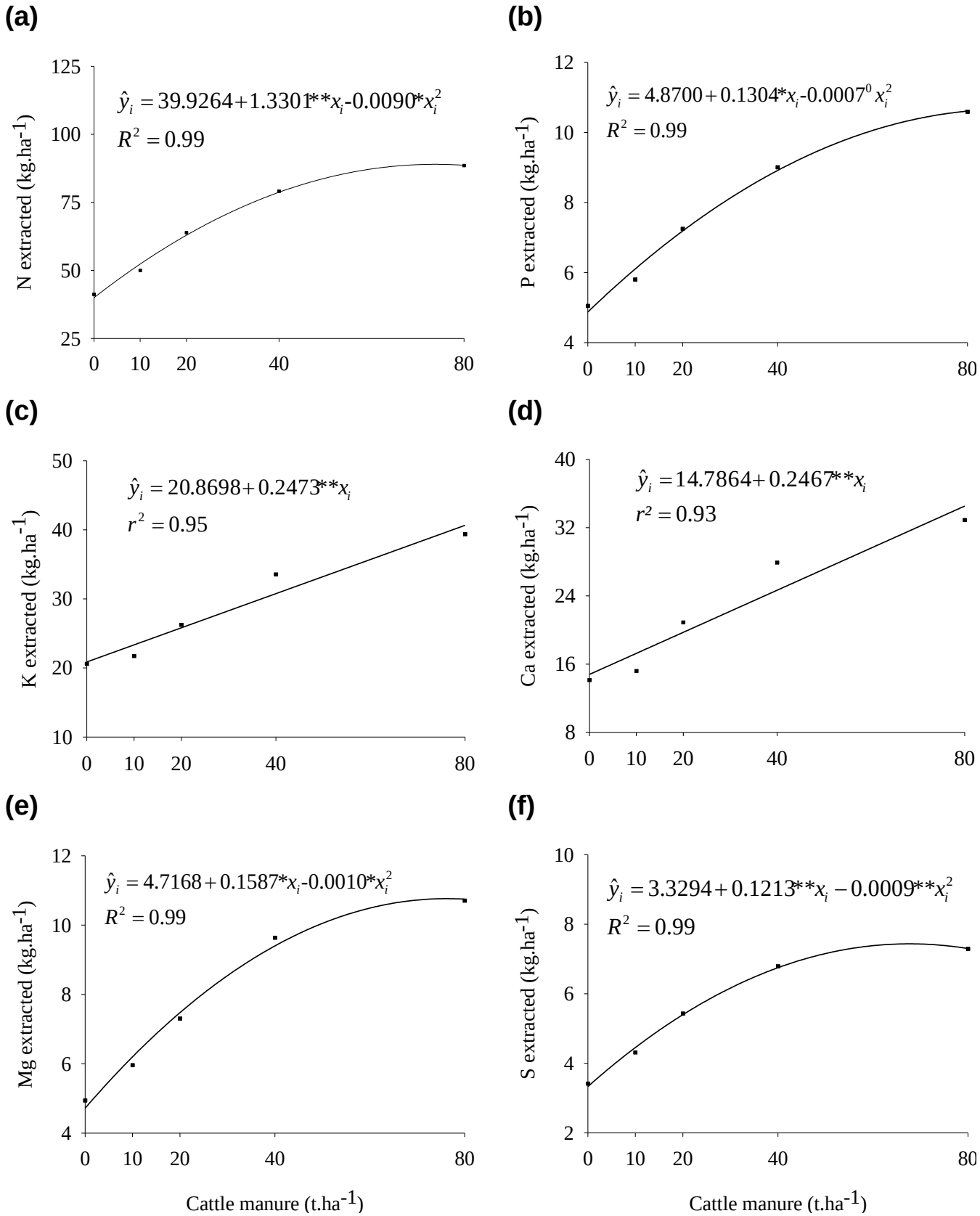

Figure 4. Extraction of macronutrients by snap bean plants, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. **, * and ^{0} significant at 1, 5 and 10% by the F test, respectively.

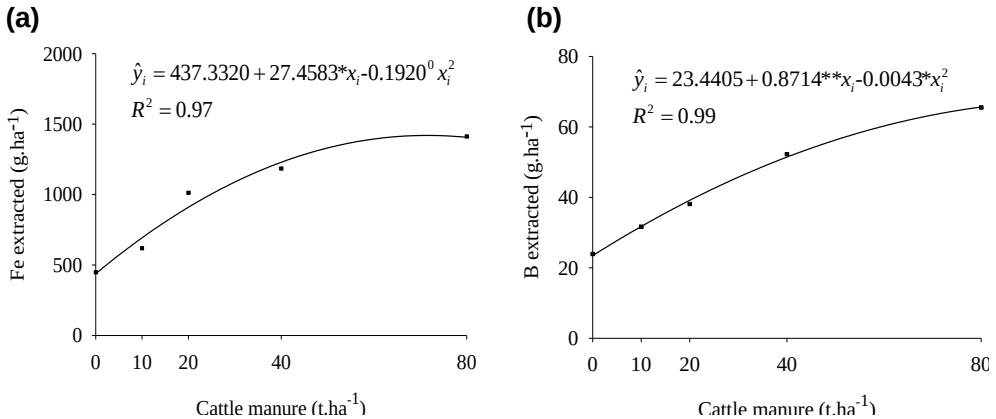

Figure 5. Extraction of micronutrients: Iron (a) and boron (b) by snap bean plants, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. **, * and ^{0} significant at 1, 5 and 10% by the F test, respectively.

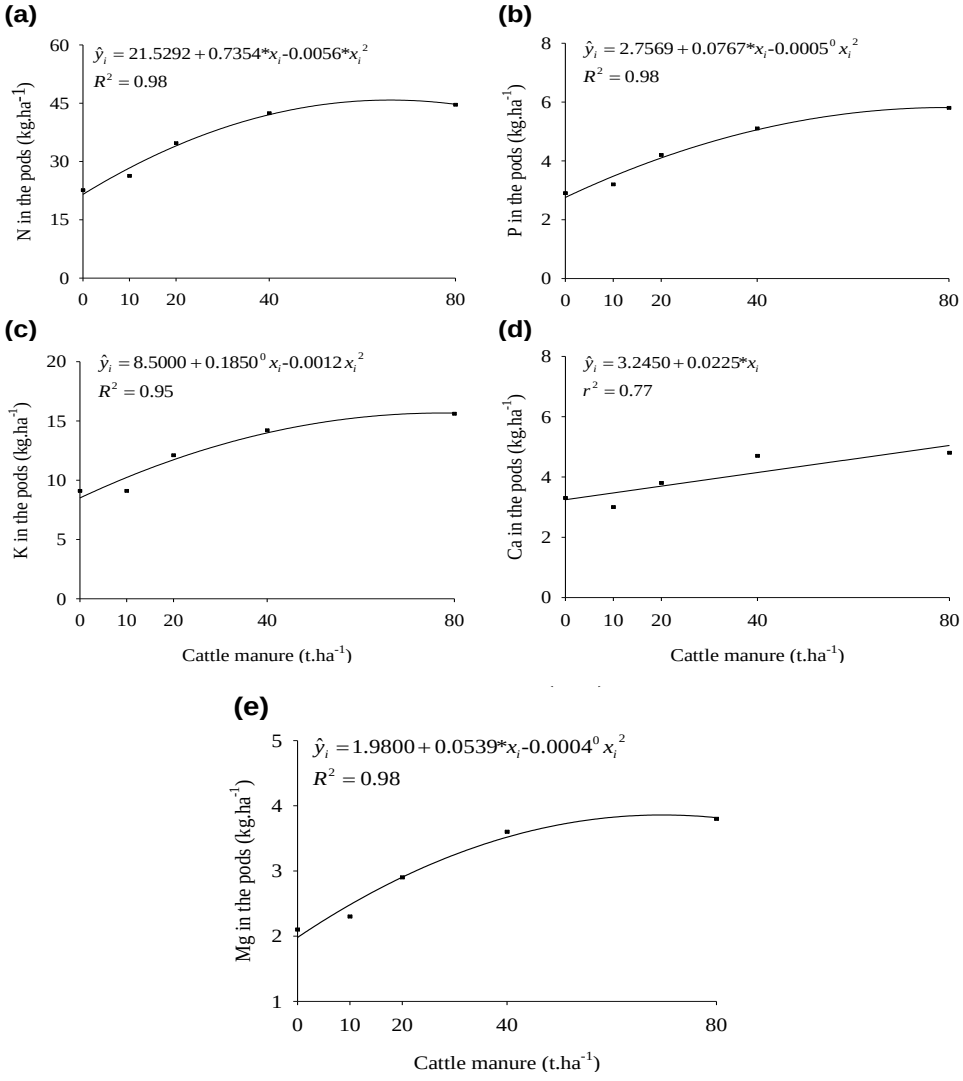

Figure 6. Exportation of macronutrients by the pods of snap bean, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. **, * and ^{0} significant at 1, 5 and 10% by the F test, respectively.

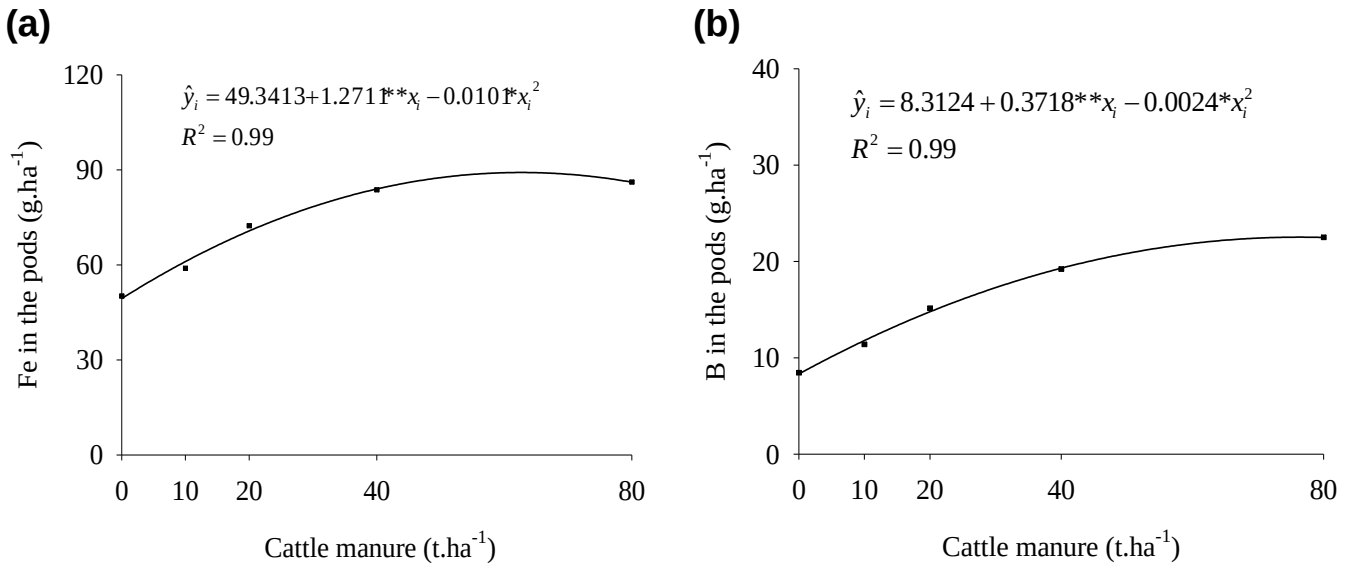

Figure 7. Exportation of micronutrients: Iron (a) and boron (b) by the pods of snap bean, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. ** and * significant at 1 and 5% by the F test, respectively.

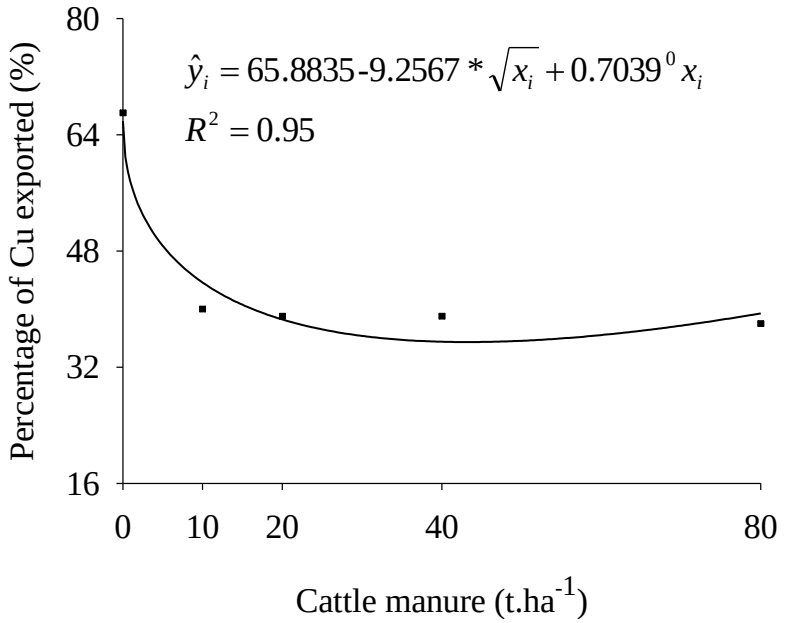

Figure 8. Relation between copper exportation by the pods with the extraction of copper by shoots of snap bean plants, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. * and ^{0}, significant at 5 and 10% by the F test, respectively.

chlorophyll in the pods expressed an increasing linear relationship with the growing doses of cattle manure (Figure 9d). According to Ribeiro (2010), the chemical composition of bean seeds can vary as a response to soil conditions, such as type of soil, fertilization, texture, organic matter, etc.

Conclusion

The application of cattle manure enhances the nutrition of plants, especially, the content of N, K, Ca, Mg and B in the leaves of snap bean, providing an increase in yield. The extraction of nutrients by the shoot and the

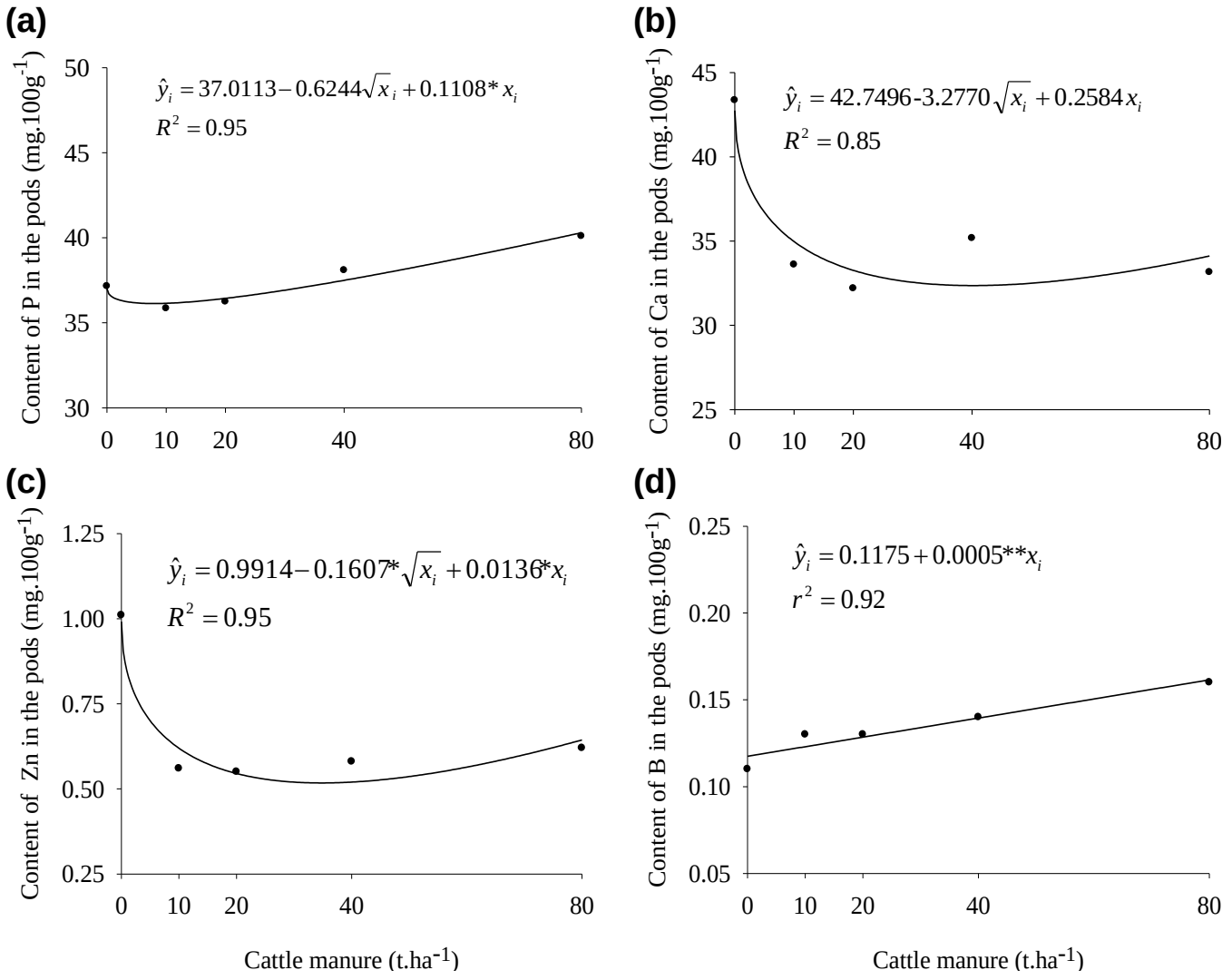

Figure 9. Content of phosphorus (a), calcium (b), zinc (c) and boron (d) in the pods of snap bean, cultivar Macarrão Trepador (Favorito), fertilized with cattle manure. Oratórios, EPAMIG, 2012. ** and * significant at 1 and 5% by the F test, respectively.

exportation of macronutrients, Fe and B by the pods escalated with the growing doses of cattle manure. The amount of nutrients exported by the pods step up with the increasing doses of cattle manure applied. The application of 67.5 t ha-1 of cattle manure yielded 14.86 t ha-1 of pods and provided the following exportation of nutrients: 45.6 (N), 5.2 (P), 16.1 (K), 4.6 (Ca), 3.8 (Mg) and 2.02 (S) kg.ha^{-1} and 88.8 (Zn), 89.5 (Fe), 100.5 (Mn), 11.4 (Cu) and 33.1 (B) g.ha^{-1}. The percentages of nutrients exported by the pod compared to the total extracted were 53 of N, 57 of P, 43 of K, 19 of Ca, 40 of Mg, 38 of S and 50 of Zn, 9 of Fe, 23 of Mn and 37 of B.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Genetic diversity of rice from Iran region assessed by simple sequence repeat (SSR) markers

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Simple sequence repeat (SSR) markers (36 microsatellite pairs) were used to assess genetic diversity among sixteen rice (*Oriza sativa* L.) cultivars from Iran, four from Uzbekistan, and one from Afghanistan, and to compare this diversity with that of three control cultivars including Nipponbare, Jasmine 85 and Basmati 370. Among the 36 microsatellite pairs, 31 produced polymorphisms ranging from 1 to 7 alleles (average = 3.72 per microsatellite). There were 134 alleles detected using all the SSR primers, 103 of which (76.9%) showed polymorphisms, while 31 did not. The genetic similarity coefficient among the 24 rice cultivars was 0.601. The genetic diversity revealed by this survey will be useful to designate the most appropriate parental cv. to initiate a breeding program aimed at developing new rice varieties with traits adapted for Middle Eastern agriculture.

Key words: Rice cultivars (cvs.), simple sequence repeat (SSR) marker, polymorphism information contents (PIC) value, and genetic similarity (GS), microsatellite.

INTRODUCTION

Cultivated rice (Oryza sativa L.), which is grown worldwide is one of the most important cereals for human nutrition (Huang et al., 2012). Among major crops grown in Iran, rice is second crop after wheat. The prevalence of rice cultivation is due largely to the affordability of rice for people of all economic classes. To meet an anticipated future increase in demand, a rice breeding program is underway, focused on traits particularly desirable in Iranian cultivars. According to the International Rice Research Institute (http://irri.org/rice-today/irri-in-iran), breeding goals for Iranian rice include introduction of salt tolerance, resistance to sheath blight caused by *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (A.B. Frank Donk), elimination of chaulkiness in the kernels, and increased yield, especially for long grain cultivars and aromatic types called Sadri rice.

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In the past, Iranian plant breeders chose parental materials for rice hybridization based on morphological and agronomic characteristics. Molecular marker technology, however, has improved breeders' ability to accurately identify and characterize genetic resources. DNA markers use nucleotide sequences to identify species (Ganie et al., 2015) and microsatellites or single sequence repeats (SSRs) are extensively employed in plant genetics studies, using both low and high throughput genotyping approaches (Vieira et al., 2016). Polymorphic DNA was chosen to characterize rice germplasm because it satisfies these criteria. All molecular markers technique can be used for several different applications including: germplasm characterization, genetic diagnostics, characterization of transformations, study of genome organization, marker assisted selection (MAS) and phylogenic analysis (Mishra et al., 2014). In this study, genetic diversity was determined among 24 rice cultivars using simple sequence repeats (SSRs) to identify heterotic groups favorable for use in rice cross-hybridization.

Newly developed single nucleotide polymorphism (SNP) markers are effective in detecting genetic diversity (Ren et al., 2013). SSR and single nucleotide polymorphic (SNP), the two most robust markers for identifying rice varieties were compared for assessment of genetic diversity and population structure (Singh et al., 2013). Both genetic diversity and population structure analysis improved varieties from landraces and local selections (Kumbhar et al. 2015). The study of genetic diversity is important on crop breeding program for selection of suitable diverse parents (Tidke et al. 2014). The objectives were determined genetic diversity using SSR markers to investigate interrelationship among the genotypic variation.

MATERIALS AND METHODS

Plant materials

This study was based on multiple sources of germplasm: sixteen Iranian cvs. [Fajr, Shafagh, Pouya, Shiroudi, Tabesh, Nemat, Neda, (from Mazandaran province), Kadus, Saleh, Dorfak, Sepidroud, Khazar (from Gilan province), Zayandehroud, Sazandegi (from Isfahan province), Doroudzan, and Ghasredashti (from Fars province)], one Afghan cv. [Pashadikonar], four Uzbek cvs. [Gulnar, Shortanby, Nokos-2, and Nokos-70], one Japanese cv. [Nipponbare], one Indian cv. [Basmati], and one Thai cv. [Jasmine 85]. The Iranian cvs. were provided by the Rice Research Institute Iran (RRII), located in Amol City near the Caspian Sea.

DNA extraction and SSR primers

Young leaves (100 mg/sample) were ground to fine powders in liquid nitrogen. DNA was extracted following the CTAB protocol introduced by Doyle and Doyle (1987). Primers (Table 1) for 36 microsatellites were downloaded from: http://www.gramene.org/db/markers/marker_view and used to assess genetic diversity among rice cultivars.

PCR analysis

PCR was performed in a 12.5 µL reaction mixture containing 7.7 µL double distilled water, 0.5 unit *Taq* DNA polymerase, 10 ng genomic DNA, 1.25 µL 10X buffer, 1 µL 50 mM $MgCl_2$, 1 µL of 5 mM DNTPs, 5 pM of each primer. PCR conditions were as follows: denaturation at 94°C for 5 min followed by 35 cycles (94°C for 1 min, 55°C for 1 min, 72°C for 2 min), and final elongation at 72°C for 5 min. PCR products were separated by electrophoresis on a 1.5% agarose gel. One Kb mass Ruller^{TM} was used to estimate the fragment sizes. The gel was stained with ethidium bromide for 20 min, and then photographed under UV light.

Data analysis

In this study, microsatellite band fragments were analyzed by the binary procedure. The presence and absence of SSR amplified bands characteristic of each genotype were scored 1 for presence and 0 for absence of the band. The polymorphism information content (PIC) value was calculated for each microsatellite locus using the following formula (Nei, 1970):

$$PIC = 1 - \sum x_i^2$$

where x_i is the frequency of i allele. Genetic similarity (GS) was calculated using this formula (Nei and Li. 1979):

$$GS_{ij} = 2N_{ij} / N_i + N_j$$

Where N_{ij} is the number of bands present in both genotypes i and j, N_i is the number of bonds present in genotype i, and N_j is the number of bonds present in genotype j. Microsatellite markers were clustered using the unweighted pair group method (UPGMA) with Statistical software.

RESULTS

SSR analysis

Allele variation of SSR markers was determined among the 24 rice cvs; 31 polymorphic bands, which ranged from 1 to 7 alleles (average = 3.72) per microsatellite, were detected by the 36 microsatellite marker pairs. These SSRs detected a total of 134 alleles, of which 103 (76.9%) showed polymorphisms, while 31 alleles (23.1%) did not (Table 2).

The polymorphism information content (PIC), is defined as the expected fraction of informative offspring from a cross. The higher the PIC, the more likely the gene for a particular trait would be co-inherited with the SSR marker. PIC values were calculated for the rice cvs. and the SSRs used in this study. The values ranged from 0.008 to 0.99 (average = 0.3) for those SSRs that produced polymorphic bands, while the non-polymorphic markers that produced a PIC value of 0. cvs. with the lowest PIC values were RM332 (0.008), RM1 and RM173 (0.02), RM30 (0.04), and RM126 (0.06); cvs. With the highest PICs were RM51and RM261 (0.77), RM204 (0.86), RM124 (0.87), and RM130 (0.99). The data are shown in Table 3.

Table 1. Microsatellite sequence used to evaluate genetic diversity.

Primer name	Chromosome location	Sequence (F) 5´-3´	Sequence (R) 5´-3´
RM1	1	gcgaaaacacaatgcaaaaa	gcgttggttggacctgac
RM5	1	tgcaacttctagctgctcga	gcatccgatcttgatggg
RM6	2	gtcccctccacccaattc	tcgtctactgttggctgcac
RM13	5	tccaacatggcaagagagag	ggtggcattcgattccag
RM20	12	atcttgtccctgccaggtcat	gaaacagaggcacatttcattg
RM21	11	acagtattccgtaggcacgg	gctccatgagggtggtagag
RM30	6	ggttaggcatcgtcacgg	tcacctcaccacacgacacg
RM31	5	gatcacgatccactggagct	aagtccattactctcctccc
RM51	7	tctcgattcaatgtcctcgg	ctacgtcatcatcgtcttccc
RM60	3	agtcccatgttccacttccg	atggctactgcctgtactac
RM82	7	tgcttcttgtcaattcgcc	cgactcgtggaggtacgg
RM84	1	taagggtccatccacaagatg	ttgcaaatgcagctagagtac
RM126	8	cgcgtccgcgataaacacaggg	tcgcacaggtgaggccatgtcg
RM130	3	tgttgcttgccctcacgcgaag	ggtcgcgtgcttggtttggttc
RM136	6	gagagctcagctgctgcctctagc	gaggagcgccacggtgtacgcc
RM137	8	gacatcgccaccagcccaccac	cgggtggtccccgaggatcttg
RM142	4	ctcgctatcgccatcgccatcg	tcgagccatcgctggatggagg
RM173	5	cctacctcggcatccccccctc	ccatgaggaggaggcggcgatc
RM177	4	ccctcttagacagaggccagaggg	gtagccgaagatgaggccgccg
RM202	11	cagattggagatgaagtcctcc	ccagcaagcatgtcaatgta
RM204	6	gtgactgacttggtcataggg	gctagccatgctctcgtacc
RM207	2	ccattcgtgagaagatctga	cacctcatcctcgtaacgcc
RM214	7	ctgatgatagaaacctcttctc	aagaacagctgacttcacaa
Rm215	9	caaaatggagcagcaagagc	tgagcacctccttctctgtag
RM219	9	cgtcggatgatgtaaagcct	catatcggcattcgcctg
RM222	10	cttaaatgggccacatgcg	caaagcttccggccaaaag
RM228	10	ctggccattagtccttgg	gcttgcggctctgcttac
RM230	8	gccagaccgtggatgttc	caccgcagtcacttttcaag
RM232	3	ccggtatccttcgatattgc	ccgacttttcctcctgacg
RM240	2	ccttaatgggtagtgtgcac	tgtaaccattccttccatcc
RM244	10	ccgactgttcgtccttatca	ctgctctcgggtgaacgt
RM245	9	atgccgccagtgaatagc	ctgagaatccaattatctgggg
RM247	12	tagtgccgatcgatgtaacg	catatggttttgacaaagcg
RM261	4	ctacttctccccttgtgtcg	tgtaccatcgccaaatctcc
RM313	12	tgctacaagtgttcttcaggac	gctcacctttgtgttccac
RM332	11	gcgaaggcgaaggtgaag	gctcacctttgtgttccac

F, Forward; R, reverse.

Table 2. Parameters of the SSR analysis.

Parameter	Microsatellites
Number of paired primers	36
Total amplified Alleles	134
Polymorphism alleles	103 (76.9%)
Average alleles per locus	3.72
PIC average	0.3

PIC, Polymorphism information content; microsatellites, SSR markers.

Table 3. Allele variation and PIC value for 36 pairs of SSRs for 24 rice genotypes.

SSR marker	Alleles / polymorphism alleles	PIC value
RM1	5/4	0.02
RM5	4/3	0.08
RM6	6/4	0.27
RM13	5/5	0.26
RM20	3/3	0.27
RM21	6/6	0.13
RM30	5/3	0.04
RM31	7/4	0.16
RM51	3/3	0.77
RM60	2/no	0
RM82	5/4	0.54
RM84	1/no	0
RM126	5/4	0.06
RM130	2/1	0.99
RM136	6/4	0.29
RM137	5/3	0.37
RM142	5/4	0.48
RM173	6/4	0.02
RM177	2/no	0
RM202	4/4	0.51
RM204	1/1	0.86
RM207	1/no	0
RM214	5/4	0.87
Rm215	6/4	0.32
RM219	2/2	0.49
RM222	5/5	0.65
RM228	4/4	0.32
RM230	2/1	0.30
RM232	1/1	0.16
RM240	7/7	0.21
RM244	2/2	0.14
RM245	1/1	0.16
RM247	2/2	0.52
RM261	5/4	0.77
RM313	1/no	0
RM332	2/2	0.008

PIC, Polymorphism information content.

Genetic similarity

The 36 SSRs were used to estimate genetic similarity among 24 rice genotypes (Table 4). The average value was 0.601. The minimum and maximum genetic similarity coefficients were 0.401 and 0.885, respectively. The highest similarity coefficients are: Tabesh - Neda (0.707), Pashadikonar - Golnar (0.721), Golnar - Shortanby (0.741), Shortanby - Nokos-2 (0.777), Pouya - Shiroudi (0.781), Neda - Dorfak (0.785), Doroudzan - Ghasredashti (0.804), Nokos-2 - Nokos-70 (0.808), Fajr – Shafagh (0.812), Shiroudi - Tabesh (0.818), Pouya - Shafagh (0.821), Saleh – Dorfak (0.837), Zayandehroud - Sazandegi (0.853), and Kadus - Saleh (0.885). The lowest genetic similarity coefficients are: Tabesh - Shortanby (0.496), Fajr - Nemat (0.491), Pashadikonar - Nokos-70 (0.483), Nokos-70 - Basmati370 (0.479), Kadus - Nokos-70 (0.476), Ghasredashti - Nokos-70 (0.473), Shafagh - Nemat (0.468), Doroudzan - Nokos-70 (0.440), Zayandehroud - Nokos-70 (0.435), Sepidroud - Nokos-70 (0.430), Saleh - Nokos-70 (0.412) and Sazandegi - Doroudzan (0.401). The smaller the genetic

Table 4. Genetic similarity coefficient among the 24 genotypes.

Genetic similarity	Fajr	Shafagh	Pouya	Shiroudi	Tabesh	Nemat	Neda	Kadus	Saleh	Dorfak	Sepidroud	Khazar	Zayandehroud	Sazandegi	Doroudzan	Ghasredashti	Pashadikonar	Gulnar	Shortanby	Nokos-2	Nokos-70	Nipponbare	Basmati 370	Jasmine 85
Fajr	0	0.812	0.790	0.629	0.704	0.491	0.691	0.678	0.674	0.723	0.659	0.628	0.603	0.616	0.567	0.614	0.679	0.633	0.546	0.626	0.504	0.579	0.653	0.583
Shafagh		0	0.821	0.657	0.739	0.468	0.715	0.725	0.721	0.769	0.681	0.643	0.666	0.630	0.630	0.616	0.678	0.608	0.507	0.614	0.512	0.569	0.579	0.588
Pouya			0	0.781	0.766	0.523	0.766	0.716	0.701	0.725	0.728	0.648	0.634	0.628	0.623	0.620	0.646	0.653	0.588	0.605	0.503	0.520	0.634	0.550
Shiroudi				0	0.818	0.666	0.689	0.610	0.605	0.592	0.595	0.620	0.581	0.567	0.543	0.567	0.581	0.625	0.547	0.601	0.555	0.598	0.593	0.588
Tabesh					0	0.645	0.707	0.647	0.630	0.666	0.695	0.648	0.609	0.585	0.526	0.561	0.597	0.544	0.496	0.604	0.516	0.601	0.633	0.548
Nemat						0	0.614	0.466	0.412	0.427	0.503	0.592	0.535	0.472	0.492	0.462	0.488	0.581	0.604	0.607	0.620	0.660	0.647	0.571
Neda							0	0.764	0.738	0.785	0.695	0.648	0.658	0.670	0.622	0.561	0.634	0.598	0.526	0.575	0.516	0.601	0.645	0.592
Kadus								0	0.885	0.758	0.694	0.635	0.658	0.647	0.635	0.610	0.658	0.614	0.517	0.565	0.476	0.523	0.634	0.533
Saleh									0	0.837	0.680	0.604	0.583	0.619	0.631	0.594	0.642	0.596	0.481	0.503	0.421	0.489	0.618	0.532
Dorfak										0	0.691	0.630	0.595	0.595	0.596	0.605	0.654	0.569	0.613	0.587	0.500	0.555	0.606	0.561
Sepidroud											0	0.606	0.565	0.565	0.566	0.565	0.619	0.526	0.457	0.515	0.430	0.553	0.662	0.541
Khazar												0	0.662	0.634	0.608	0.631	0.593	0.671	0.596	0.633	0.514	0.612	0.661	0.637
Zayandehroud													0	0.853	0.790	0.690	0.658	0.639	0.571	0.561	0.435	0.559	0.596	0.474
Sazandegi														0	0.401	0.654	0.573	0.653	0.541	0.532	0.419	0.559	0.571	0.488
Doroudzan															0	0.804	0.550	0.640	0.558	0.542	0.440	0.561	0.560	0.478
Ghasredashti																0	0.631	0.688	0.571	0.643	0.473	0.586	0.607	0.521
Pashadikonar																	0	0.721	0.556	0.575	0.483	0.517	0.596	0.488
Gulnar																		0	0.741	0.737	0.598	0.666	0.597	0.593
Shortanby																			0	0.777	0.602	0.625	0.507	0.538
Nokos-2																				0	0.808	0.694	0.558	0.654
Nokos-70																					0	0.640	0.479	0.681
Nipponbare																						0	0.571	0.614
Basmati 370																							0	0.515
Jasmine 85																								0

distance value, the more closely related the parents of a cross are.

Cluster analysis

Cluster analysis, based on informative SSR alleles, classified 24 rice genotypes into seven groups (Figure 1). Group 1: Fajr and Shafagh, Group 2: Kadus, Saleh and Dorfak, Group 3: Pouya, Tabesh, Neda, and Sepidroud, Group 4: Shiroudi and Nemat, Group 5: Gulnar and Shortanby, Group 6: Nokos-2, Nokos-70, Jasmine 85 and Nipponbare, Group 7: Zayandehroud, Sazandegi and Doroudzan. In this comparison, four cvs. did not fall into any Group: Basmati 370 from India, Pashadikonar from Afghaniztan, and Khazar and Ghasredashti, which are from two different regionsin Iran. Native Iranian cvs. were distributed across all seven groups.

Cluster analysis revealed that aromatic cvs. from Iran are genetically different from standard aromatic cvs. Yet, the Iranian aromatic cvs. themselves clustered in different groups. For example, Zayandehroud, Sazandegi (from one region in Iran) clustered in Group 7, whereas Fajr and Shafagh (from the same region) clustered in Group 1. Basmati 370 was genetically distinct from all other cvs., since it did not fall into any of the Groups, even though it is considered a

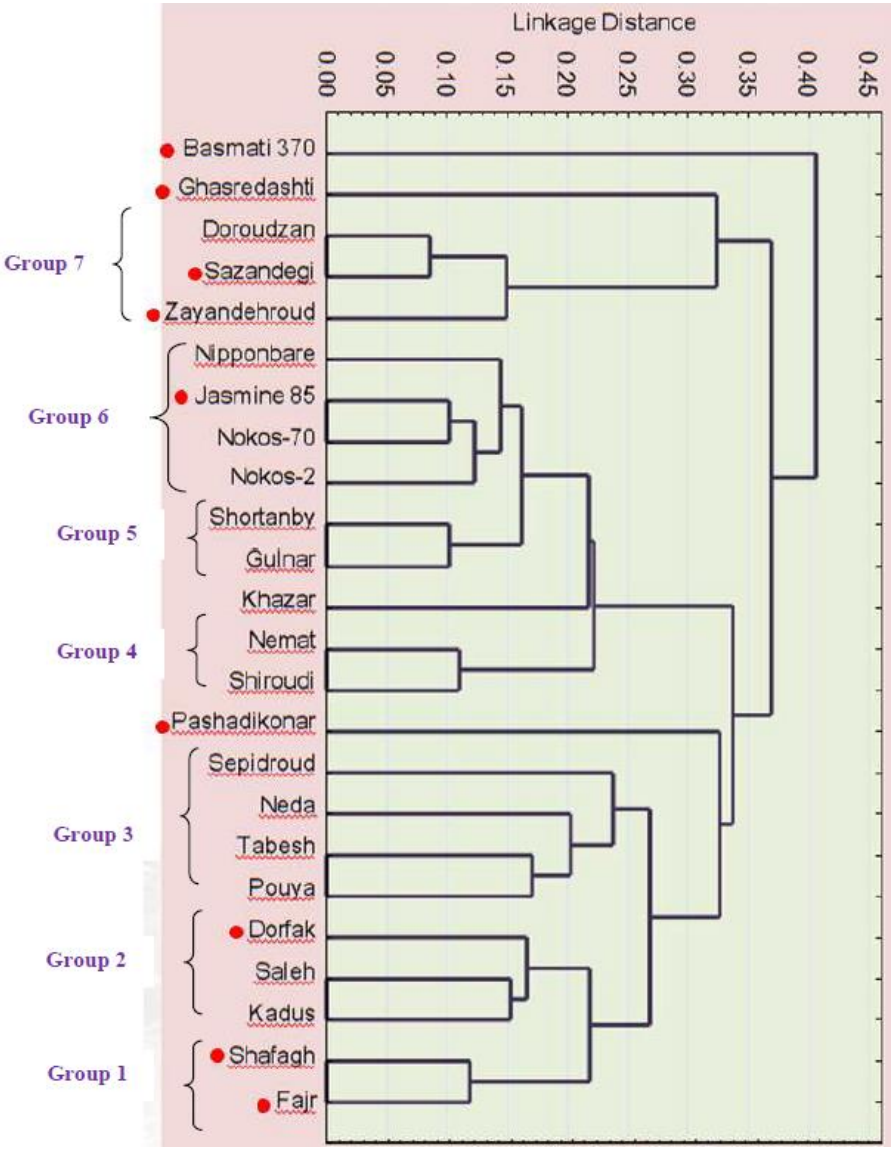

Figure 1. Dendrogram showing clustering of rice cvs., as determined by SSR analysis. ●: Aromatic varieties.

standard aromatic variety. Two other aromatic cvs. Ghasredashti and Pashadikonar (an aromatic rice from Afghanistan), also failed to be grouped (Figure 1).

DISCUSSION

Many previous studies have shown that both morphological and SSR markers are useful for analyzing genetic diversity in rice and other crops. Single-nucleotide polymorphisms have become the genetic markers of choice in various genetic, ecological, and evolutionary studies (Tang et al., 2016). In the present study, SSR markers were used to detect genetic variation among 24 rice genotypes; in a separate analysis (data not shown here), morphological features were combined with the SSR results, and it was found that there was general agreement between the two sets of data with respect to pair-wise genetic distances among the rice cvs.

In cases of disagreement between the datasets, greater significance was attached to the SSR marker analysis. There are several reasons for this: (1) morphological features are phenotypes, whereas SSRs are genotypes; morphological similarities may obscure genetic differences. (2) Many morphology traits are controlled by multiple genes; hence a morphological variation alone does not indicate the number of genes involved, or the effects of pleiotropy, epitasis and the environment. (3) SSRs are distributed across the entire

genome, whereas morphological features are lesscomprehensive. (4) SSRs are more easily scorable than most morphological features, (5) SSR detection is highly reproducible and can reveal intraspecific homology. (6) The abundance of SSRs enhances the likelihood of linkage to specific agronomic traits; for instance, SSR results revealed large genetic distances between certain landraces of rice, thus evaluation of genetic diversity and genetic structure in crops has important implications for plant breeding programs and the conservation of genetic resources (Ren et al., 2013). The result of cluster analysis revealed that there was to significant correlation between the aromatic feature and genetic similarity.

DNA polymorphisms have proven to be powerful tools for genotyping, and for estimating genetic diversity. Among molecular markers, SSRs have been used to indicate genetic diversity of crop germplasms and have been widely applied in the genetic diversity analysis (Salgotra et al. 2015). The importance of plant genetic diversity is now being recognized as a specific area since exploding population with urbanization and decreasing cultivable lands are the critical factors contributing to food insecurity in developing world (Govindaraj et al., 2015). SSR-based analysis of genetic diversity can be used to identify duplicate germplasms in morphological similar accessions.

This study was set in the context of previously reported crop breeding programs. Genetic diversity is the main source of variability in any crop improvement program and it serves as a reservoir for identifying superior alleles controlling key agronomic and quality traits through allele mining association mapping (Nachimuthu et al., 2015). The microsatellite DNA marker has been the most widely used, due to its easy application by simple PCR, followed by a denaturing gel electrophoresis for allele size determination, and to the high degree of information provided by its large number of alleles per locus (Mishra et al., 2014). The goal of our work was to identify genetic variation within a rice germplasm collection, so that desirable parents could be chosen for rice breeding programs in Iran. The relatively low genetic similarity (average = 0.601) that we found indicated high genetic diversity in our collection; thus these rice cvs. are a good source from which to choose genetically dissimilar parents to be cross-bred in a program aimed at improving commercial Iranian rice. For example, cvs. Khazar and Ghasredashti, which are genetically far apart, might be favorable parental materials for making new varieties.

It is worth mentioning that cross hybridization is pivotal between genotypes of different clusters to improve desirable commercial varieties. Genome level profiling of germplasm collections in crop species is essential to identify accessions for their efficient use in crop improvement programs (Choudhury et al., 2014). Kioko et al. 0(2015) mentioned that the assessment of genetic diversity is crucial in germplasm characterization. Toward this end, the present results emphasize the importance of Iranian rice germplasm collection, conservation and maintenance. The focus should be on introduction of more diverse cultivated rice and any other heterotic materials that can be used for hybridization. As Iranian rice breeding programs are mobilized, it should be emphasized that each succeeding breeding generation should be monitored by molecular analysis; this will require the use of more primer combinations and introduction of additional molecular markers.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Differential reaction of cowpea genotypes to brown blotch disease (*Colletotrichum capsici*) in Burkina Faso

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Thirty six *Colletotrichum capsici* (L.) single spore isolates associated with brown blotch disease in cowpea were collected from three agro-ecological zones of Burkina Faso from October to November 2014. To identify the most virulent strains, cowpea genotypes KVx61-1, KVx396-4-5-2-D and Moussa Local was inoculated with each isolate. The results showed that isolates 096-SA-2, 071-FA-6 and 079-PM-2 were the most virulent, respectively, in North Soudanian, Soudanian and Sahelian zones. To identify brown blotch disease resistant cowpea, each of the isolates was used to inoculate 41 different cowpea genotypes. Inoculated cowpea plants were evaluated for brown blotch disease severity at 7, 14 and 21 days after inoculation. Highly significant differences ($P < 0.001$) were found among genotypes, isolates and their interactions. Seven cowpea genotypes including KN-1, Moussa Local, Donsin Local and Melakh were identified as resistant and present specific resistance to the isolates. These genotypes can be used to improve cowpea resistance to brown blotch disease in Burkina Faso.

Key words: Cultivars, pathotypes aggressiveness, disease resistance.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain legume crop in Sub-Saharan Africa (Adegbite and Amusa, 2008). The crop is grown worldwide with an estimated cultivation area of about 12 million hectares annually with the production of over 10 million metric tons a year (FAOSTAT, 2011). About 65 to 70% of the world's cowpea grain production occurs in the west and the central part of Sub-Saharan Africa. Cowpea provides

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food and nutrition for humans and feed for livestock. However, in Burkina Faso and other African countries, cowpea production is subject to many biotic constraints such as soil-borne and seed-borne fungal pathogens including *Colletotrichum capsici,* the causal agent of brown blotch disease, which can result in yield losses of between 42 and 100% in tropical agriculture (Sereme, 1999; Banerjee et al., 2007; Adegbite and Amusa, 2008; Torres-Calzada et al., 2011). *C. capsici* is a major constraint to many crop production causing severe losses both in pre- and postharvest decay (N'Guettia et al., 2013; Saxena et al., 2014; Chandra et al., 2009).

Infection by *C. capsici* in cowpea is particularly devastating due to its hemibiotrophic nature (Hyde et al., 2009). Additionally, the pathogen occurs in different races. For instance, Emechebe (1986) identified eight races of *C. capsici* associated with brown blotch disease in Nigeria; four out of the eight races were specific to Guinea and Sudan Savanna while the remaining four were to the rainforest area. Sereme (1999) reported 12 pathogenic groups of *Colletotrichum* spp. including *C. capsici* associated with brown blotch disease in Burkina Faso. Based on molecular characterization with a specific primer pair, Thio et al. (2016) identified four variants of *C. capsici* in Burkina Faso.

Several *C. capsici* management techniques including cultural control, use of plant extracts (Sereme, 1999; Mark et al., 2015; Mark and Channya, 2016), use of chemicals, biological control (Chacko and Gokulapalan, 2015) and use of tolerant and resistant cultivars (Adebitan et al., 1992; Amusa et al., 1994) have been recommended. However, no single specific management program can eliminate brown blotch disease in cowpea (Obi and Barrusa-Vargas, 2014). Breeding for resistance is also the most economical for growers as well.

Therefore, host plant resistance remains the most viable option in managing the brown blotch disease in cowpea (Adebitan et al., 1992; Fery and Singh, 1997; Enyiukwu et al., 2014; Obi and Barrusa-Vargas, 2014).

In this study, spraying method was used to study the pathogenicity and resistance of cowpea genotypes to *C. capsici strains* originating from different locations in Burkina Faso.

MATERIALS AND METHODS

Collection of isolates of *Colletotrichum* spp.

Cowpea plant parts (leaves, stems, pods) naturally infected by *Colletotrichum* spp. were collected from farmers' fields in three agro-ecological zones of Burkina Faso during the period from October to November 2013. The locations included Saria, Kamboinse and Kouaré (North Soudanian zone), Farako Bâ and Gaoua (Soudanian zone), and Pobe Mengao (Sahelian zone). The cowpea plant materials were surface-sterilized in 70% (v/v) ethanol for 1 min followed by immersion in sodium hypochlorite (NaOCl) 1% (v/v) for 5 min and three successive rinses in sterilized distilled water. After disinfection, the materials were allowed to dry under laminar flow hoods for 15 min. Tissues of approximately 4 mm^2 in size were placed in Petri dishes containing moistened blotting paper. Plates were incubated in alternating cycles of light/darkness (12 h/12 h) for 7 to 9 days at 28°C. The *Colletotrichum* species were identified based on the growth habit characteristic and morphology of acervuli and conidia and on available identification keys (Marthur and Kongsdal, 2003).

Sporulating cultures of *C. capsici* were aseptically transferred in Petri dishes containing potato dextrose agar (PDA), which was previously autoclaved for 15 min at 121°C and then supplemented with streptomycin (0.3 µg/l of PDA) to prevent bacterial contamination. The Petri dishes were incubated at 24°C under UV light in alternating cycles of light/darkness (12 h/12 h) for 7 days.

Single spore production

A pure single spore culture was obtained from each *C. capsici* isolate after 7 days of growth on PDA. 100 ml of distilled water were added to the fresh fungal culture, and 200 µL of the suspension was spread on PDA media culture and incubated for 12 to 24 h depending on cell germination and growth. Three to five single cells or mycelium from each isolate were transferred to new Petri dishes containing PDA. After 7 days of growth, pure cultures of single spore were stored at 20°C before use.

Pathogenicity tests

A total of 36 single spores *C. capsici* (Table 1) were used to carry out pathogenicity tests in greenhouse conditions. These isolates were used for artificial inoculation of three differential cowpea genotypes namely KVx61-1, KVx396-4-5-2-D and "Moussa Local". The response of these three cowpea lines have been identified by Sereme (1999). Cowpea seeds were sterilized in 1% sodium hypochlorite (NaOCl) for 5 min, rinsed three times successively with distilled water, and dried on laminar flow hoods for 24 h. Fifteen to twenty seeds were sown in duplicates in 2.5 L pots containing sterilized soil mixture of sand and forest soil in a 1:2 ratios (v/v). Fourteen days after sowing (DAS), cowpea plants were inoculated (the whole surface of plants) with a concentration of approximately 10^6 spores/ml of each *Colletotrichum* isolate. To favor spores penetration inside the plant, the inoculated plants were maintained at 22 ± 1°C in humid (RH > 78%) conditions for 5 days. Then, the cowpea plants were examined for brown blotch disease symptoms after 7, 14 and 21 days after inoculation (DAI).

The disease severity index was based on a 1 to 5 scale, where 1 = no symptoms, 2 = small spots of brown blotch in the stem, 3 = coalescent spots on the stem, 4 = coalescent spot with presence of acervuli but surviving plant, 5 = withered stem and plant death (Figure 1) (Sereme, 1999), and on the formula proposed by Allen et al. (1981) as follows:

$$I = \frac{\Sigma (Xi - 1)\, ni}{[E (Xi) - 1]\, N} \times 100$$

Where: Xi = the note of disease for each plant; ni = individual number of category Xi; N = total number of observed plant; E (Xi) = scale range.

Analysis of variance (ANOVA) was performed using Gen Stat 12^{th} edition on disease incidence and severity data. All the data for disease severity were subjected to Arc sine transformation before

Table 1. List of *C. capsici* single spores isolates used for the pathogenicity test in this study.

Name of *Colletotrichum* isolates	Organ	Cowpea genotype	Sites of collection	Agro ecological zone
003-KO-1	Pod	KVx61-1	Kouare	North Soudanian
006-KB-1	Pod	KVx61-1	Kamboinse	North Soudanian
009-KB-2	Pod	KVx396-4-5-2D	Kamboinse	North Soudanian
013-KB-3	Stem	KVx780-8	Kamboinse	North Soudanian
017-KB-4	Stem	KVx780-8	Kamboinse	North Soudanian
020-KB-5	Stem	Komcalle	Kamboinse	North Soudanian
021-KB-5	Stem	Komcalle	Kamboinse	North Soudanian
023-GA-1	Stem	KVx61-1	Gaoua	Soudanian
024-GA-2	Stem	KVx61-1	Gaoua	Soudanian
026-GA-3	Pod	KVx61-1	Gaoua	Soudanian
030-GA-4	Pod	KVx61-1	Gaoua	Soudanian
035-GA-5	Pod	KVx61-1	Gaoua	Soudanian
036-GA-5	Pod	KVx61-1	Gaoua	Soudanian
039-GA-6	Pod	KVx396-4-5-2D	Gaoua	Soudanian
043-GA-7	Pod	KVx396-4-5-2D	Gaoua	Soudanian
044-GA-8	Pod	KVx396-4-5-2D	Gaoua	Soudanian
045-GA-8	Pod	KVx396-4-5-2D	Gaoua	Soudanian
047-GA-9	Stem	IT98K-205-8	Gaoua	Soudanian
053-GA-10	Stem	Komcalle	Gaoua	Soudanian
054-FA-1	Leaf	Variety hybride 1	Farako-Ba	Soudanian
057-FA-2	Leaf	Variety hybride 1	Farako-Ba	Soudanian
059-FA-3	Leaf	Variety hybride 1	Farako-Ba	Soudanian
060-FA-3	Leaf	Variety hybride 1	Farako-Ba	Soudanian
064-FA-4	Stem	KVx61-1	Farako-Ba	Soudanian
069-FA-5	Stem	KVx61-1	Farako-Ba	Soudanian
071-FA-6	Pod	KVx61-1	Farako-Ba	Soudanian
074-BA-1	Pod	Local variety	Bani	Sahelian
077-PM-1	Pod	KVx61-1	Pobe-Mengao	Sahelian
079-PM-2	Pod	KVx61-1	Pobe-Mengao	Sahelian
084-PM-3	Stem	KVx61-1	Pobe-Mengao	Sahelian
086-PM-4	Stem	KVx61-1	Pobe-Mengao	Sahelian
090-PM-5	Stem	KVx61-1	Pobe-Mengao	Sahelian
092-SA-1	Stem	Variety hybride 2	Saria	North soudanian
093-SA-1	Stem	Variety hybride 2	Saria	North soudanian
096-SA-2	Stem	Variety hybride 2	Saria	North soudanian
097-SA-2	Stem	Variety hybride 2	Saria	North soudanian

analysis. The Fisher's least significant difference test was used to compare mean values of disease severity. XLSTAT 2013 3.4 software was used for ascendance classification.

Seedling resistance evaluation of brown blotch disease

Forty one cowpea lines were screened for their resistance or susceptibility to brown blotch disease under greenhouse conditions. The seeds were previously surface sterilized in sodium hypochlorite (NaOCl) 1% (v/v) for 5 min and air dried for 24 h before sowing. A mixture of sand and field soil (v/v=1:2) was autoclaved at 121°C for 1 h and used to fill up 75% of the 10 L pot . Two replicates were set up for each cowpea accession and for each *C. capsici* isolates in a split plot design. The most virulent strain of *C. capsici* from each Agro-ecological zone of the country, designated Ccap-PO, Ccap-FA and Ccap-SA were used for plant inoculation (Figure 2).

About 15 to 20 seeds were sowed in each pot for plant growth. The spore suspension from fresh fungi culture of 7 days was prepared and adjusted to a concentration of 10^6 spores/ml. The *C. capsici* suspension was applied by spraying over the entire surface of the individual plant and plant incubated for symptoms notation. Disease incidence and severity were determined using the same methods described for the pathogenicity test. Seedlings were also scored for disease severity using a modified scale (Sereme, 1999) as shown in Table 2.

Figure 1. Brown blotch disease annotation symptoms on 1 to 5 scale.

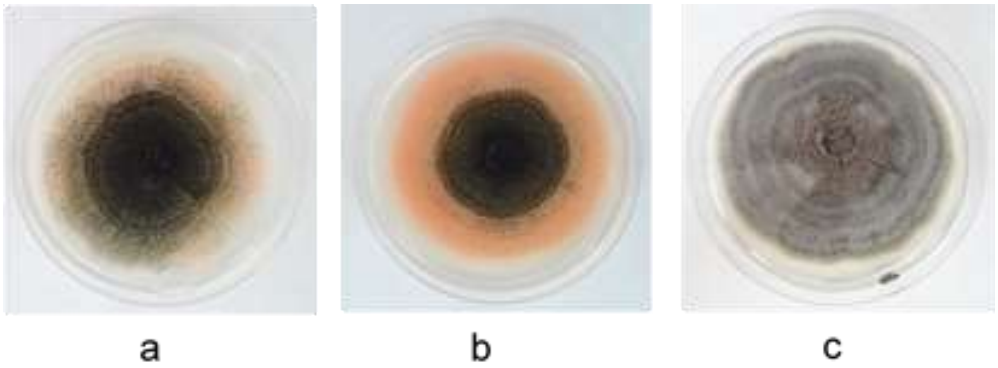

Figure 2. Colony surface of strains: a: Ccap-PO; b: Ccap-FA and c: Ccap-SA, in PDA media, after 7 days of growth.

Table 2. Designation of resistance level based on disease severity score.

Severity scale	Severity (%)	Resistance level
0	S = 0	Symptomless (SL)
1	1 ≤ S ≤ 6	Highly resistant (HR)
2	6 < S ≤ 10	Resistant (R)
3	10 < S≤ 20	Moderately resistant (MR) or moderately susceptible (MS)
4	20 < S ≤ 50	Susceptible (S)
5	S > 50	Highly susceptible (HS)

RESULTS AND DISCUSSION

Pathogenicity testing

Thirty six single spore isolates of *C. capsici* from natural infected cowpea plants have been used for pathogenicity test in greenhouse conditions. 14 days after inoculation, all the plants presented symptoms of brown blotch disease. The aggressiveness of brown blotch disease depended on the isolate and cowpea line. Seven

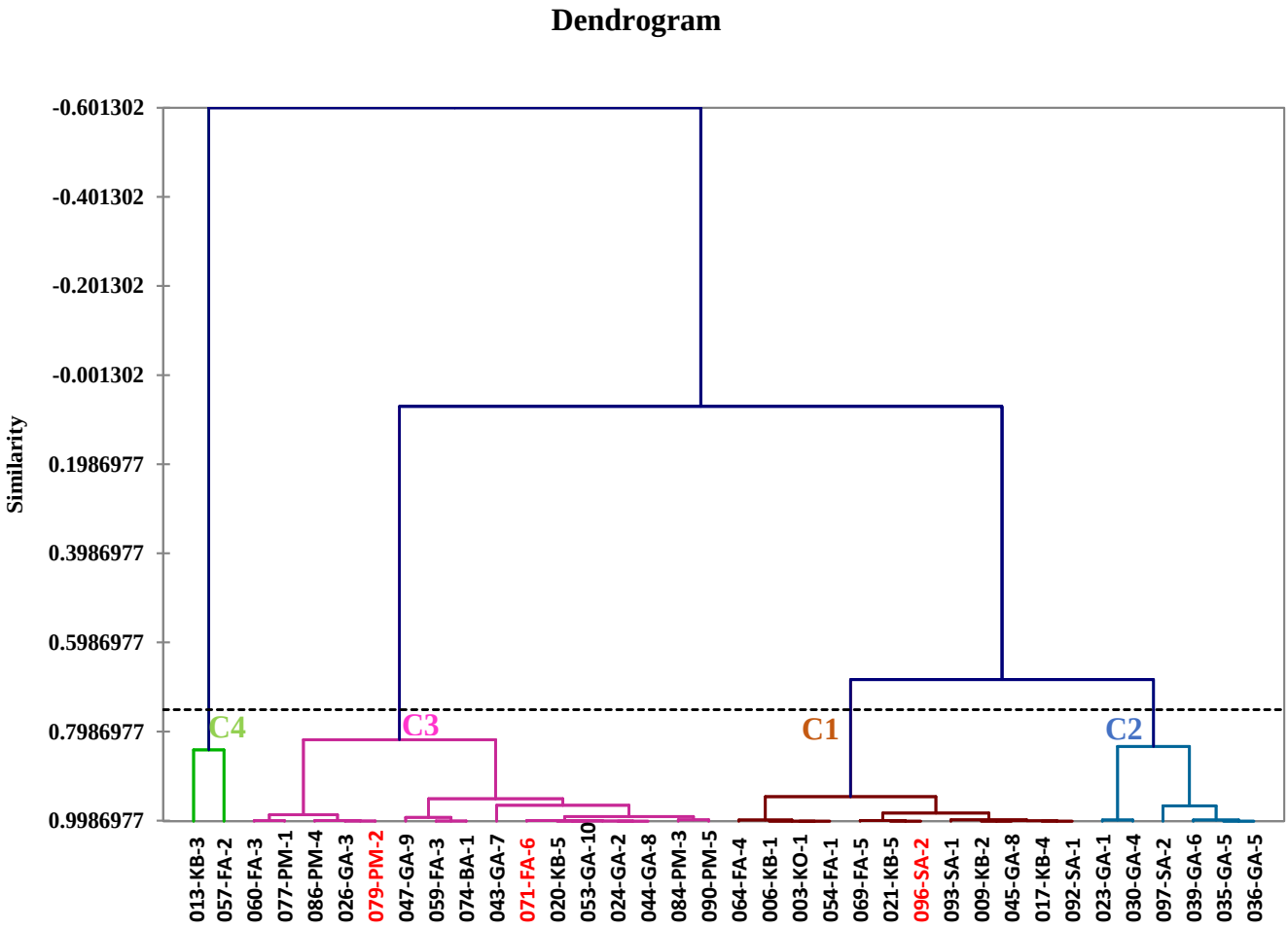

Figure 3. Ascendance hierarchical classification of pathotypes (four classes were grouped at 75% of Pearson correlation coefficient).

pathogenic groups or races of *C. capsici* were identified based on their virulence or aggressiveness. Therefore, Agglomerative Hierarchical Clustering (AHC) analysis was used to predict 4 classes of pathogens in terms of disease severity at 75% of Pearson correlation coefficient (Figure 3). This analysis also indicated a high similarity coefficient within class at 84 (49%) against 15 (51%) for between classes. Thus, the Classes 1 and 2 contain the most pathogenic strains of *C. capsici.* All of the isolates of Saria were identified as the most virulent and are associated with the cowpea genotype KVX396-4-5-2D (Tables 3 and 4). Among these isolates, the strain 096-SA-2 was the most devastating with 78, 82 and 10% of severity, respectively, for KVX61-1, KVX396-4-5-2D and Moussa Local (Table 3). Isolates 096-SA-2, 079-PM-2 and 071-FA-6 were the most aggressive for the North Soudanian, Sahelian and Soudanian agro-ecological zone, respectively. Disease severity and incidence varied across the three agro-ecological zones. These three isolates are newly designated as Ccap-SA (096-SA-2), Ccap-PO (079-PM-2) and Ccap-FA (071-FA-6).

The analysis of disease incidence and severity due to the virulent pathogens showed a highly significant difference ($P < 0.001$) among the isolates, genotypes and their interactions (Table 5). Among the three cowpea lines used for the test, KVX61-1 (28% severity) was the most susceptible followed by KVX396-4-5-2D (18%) while Moussa Local (7%) seemed to be resistant.

Sereme (1999) reported that the most pathogenic groups of *Colletotrichum* spp. associated with brown blotch disease of cowpea were from Soudanian zone and were collected on the site of Farako Ba. The results of this study indicate a progression of *C. capsici* virulence around the country. The most pathogenic strains belonged to the *C. capsici* variant 1 which were identified by using internal transcribed spacer (ITS) phylomarkers in a previous study (Thio et al., 2016). The strains 035-GA-5 and 020-KB-5 presented the lowest values of aggressiveness and correspond, respectively to variants 2 and 4 of *C. capsici*.

Table 3. *C. capsici* strains virulence associated to brown blotch disease on three cowpea genotypes at 14 days after inoculation.

Singlespore isolate	Cowpea cultivar			Severity	Pathogenic group
	KVx61-1	KVx396-4-5-2D	Moussa Local		
096-SA-2***	64	71	2	46[a]	1 (S S R)
097-SA-2	38	89	0	42[a]	1
092-SA-1	51	61	0	37[a]	1
093-SA-1	52	69	0	40[a]	1
090-PM-5	54	2	10	22[b]	2 (S R R)
003-KO-1	30	39	0	23[b]	1
079-PM-2**	25	0	12	13[cde]	2
009-KB-2	26	25	1	17[bc]	1
071-FA-6*	28	0	2	10[cdefg]	2
077-PM-1	24	0	5	10[cdefg]	2
084-PM-3	34	0	15	16[bcd]	2
086-PM-4	3	1	15	16[bcd]	3 (R R R)
060-FA-3	26	0	4	10[cdefg]	2
074-BA-1	26	5	0	10[cdefg]	2
006-KB-1	25	22	2	17[bcd]	1
059-FA-3	35	4	0	13[cde]	2
024-GA-2	32	0	0	11[cdef]	2
021-KB-5	20	5	0	8[defghi]	2
057-FA-2	17	1	30	16[bcd]	4 (S R S)
030-GA-4	0	21	5	9[cdefghi]	5 (R S R)
043-GA-7	2	0	3	2[ghi]	3
039-GA-6	7	0	5	4[efghi]	3
045-GA-8	1	0	0	0[i]	3
036-GA-5	5	0	0	2[ghi]	3
044-GA-8	4	2	0	2[fghi]	3
054-FA-1	0	5	3	3[fghi]	3
026-GA-3	7	1	5	5[efghi]	3
047-GA-9	2	0	0	1[hi]	3
064-FA-4	0	0	0	0[i]	3
069-FA-5	2	2	0	2[ghi]	3
013-KB-3	1	0	6	2[fghi]	3
017-KB-4	0	4	0	1[ghi]	3
020-KB-5	0	0	0	0[i]	3
023-GA-1	0	6	0	2[fghi]	3
053-GA-10	5	0	0	2[ghi]	3
035-GA-5	0	0	0	0[i]	3
No treatment	0	0	0	0[i]	3
Pathotype virulence mean	**18[a]**	**12[b]**	**3[c]**		

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$. ***Most pathogenic strain from agro-ecological zone.

A specific reaction of cowpea genotypes to *C. capsici* isolates

The screening of 41 cowpea cultivars in greenhouse condition involves identifying potential source of cowpea resistance. The results indicated highly significant difference ($P < 0.05$) in disease severity among cultivars, isolate effects and their interactions (Table 6). These results indicate high genetic variability in cowpea cultivars to brown blotch disease. All cowpea cultivars present approximately the same incidence at 14 DAI which is not significant. The observations also showed that the cowpea cultivars were more severely affected 21 days after inoculation than 14 DAI. The current study indicated

Table 4. *C. capsici* strains virulence associated to brown blotch disease on three cowpea genotypes at 21 days after inoculation.

Single spore isolate	Cowpea cultivar			Severity	Pathogenic group
	KVx61-1	KVx396-4-5-2D	Moussa Local		
096-SA-2***	78	82	10	57[a]	1 (S S R)
097-SA-2	58	98	0	52[a]	1
092-SA-1	68	88	0	52[a]	1
093-SA-1	62	86	5	51[a]	1
090-PM-5	81	5	11	33[b]	2 (S R R)
003-KO-1	51	44	0	32[b]	1
079-PM-2**	48	5	31	28[bc]	4 (S R S)
009-KB-2	34	41	3	26[bc]	1
071-FA-6*	39	19	18	25[bc]	6 (S S S)
077-PM-1	50	2	22	25[bc]	4
084-PM-3	54	7	15	25[bcd]	2
086-PM-4	46	1	26	25[bcd]	4
060-FA-3	40	10	24	24[bcd]	4
074-BA-1	46	17	8	24[bcde]	1
006-KB-1	34	29	6	23[bcde]	1
059-FA-3	45	9	0	1[8cdef]	2
024-GA-2	44	1	0	15[defg]	2
021-KB-5	20	21	1	14[efgh]	1
057-FA-2	10	1	31	14[efgh]	7 (R R S)
030-GA-4	2	22	5	10[fghi]	5 (R S R)
043-GA-7	18	4	1	10[fghi]	2
039-GA-6	8	11	5	8[fghi]	3 (R R R)
045-GA-8	10	12	0	8[ghi]	3
036-GA-5	5	15	0	7[ghi]	3
044-GA-8	14	2	1	6[ghi]	3
054-FA-1	8	7	3	6[ghi]	3
026-GA-3	8	1	5	5[ghi]	3
047-GA-9	11	4	0	5[hi]	3
064-FA-4	6	5	0	4[hi]	3
069-FA-5	6	6	0	4[i]	3
013-KB-3	1	3	6	3[i]	3
017-KB-4	4	4	2	3[i]	3
020-KB-5	9	0	0	3[i]	3
023-GA-1	0	6	1	3[i]	3
053-GA-10	8	0	0	3[i]	3
035-GA-5	1	4	0	2[i]	3
Pathotype virulence mean	**28[a]**	**18[b]**	**7[c]**		

Means followed by the same letter in a column are not significantly different at $P \leq 0.05$ *** or ** or *= most pathogenic strain from agro-ecological zone; R= Resistant, S= Susceptible.

that 76% of cultivars tested were susceptible to brown blotch disease. The results revealed that among the 41 cowpea lines screened, the cultivar KN-1 showed a specific resistance to the three pathotypes. It was consistently brown blotch disease resistant and should be a good source of resistance genes for susceptible cowpea lines. However, KN-1 cultivar did show symptoms of brown blotch on the stem. No other genotype was completely resistant to the disease. MELAKH, Moussa Local, Donsin Local, Djouroum Local, Pobe Local and 58-57 appeared to have some level of tolerance (MR/MS) to one or two of the three isolates (Table 7). Cultivars KN-1 and Moussa Local showed a stable level of resistance to brown blotch disease during

Table 5. ANOVA of mean disease incidence in cowpea at 14 days after inoculation due to *C. capsici* virulence.

Source of variation	*df*	Mean square	F value	Pr > F (LSD 5%)
Cultivars	2	34907.8	260.12	< 0.001***
Isolates	36	3142.7	23.42	< 0.001***
Cultivars X Isolates	72	1575.5	11.74	< 0.001***
Grand mean	24			
CV%	47.3			

***Highly significant difference; CV = Coefficient of variation; *df* = Degree of freedom; Pr = Probability of F value.

Table 6. ANOVA of genetic variability of cowpea cultivars in brown blotch disease severity at 21 days after inoculation.

Source of variation	*df*	Mean square	F value	Pr >F (LSD 5%)
Genotypes	40	729.3	3.01	< 0.001**
Isolates	3	6877.1	28.35	< 0.001**
Genotypes X Isolates	120	371.4	1.53	0.006*
Grand mean	20			
CV%	77.3			

**Highly significant difference; CV = Coefficient of variation; *df* = Degree of freedom; Pr = Probability of F value.

Table 7. Differential reaction of 41 cowpea cultivars at 14 and 21 days after inoculation in *C. capsici* pathotypes.

Cowpea line	Resistance level at 14 DAI			Resistance mean	Resistance level at 21 DAI			Resistance mean
	Ccap-PO	*Ccap-FA*	*Ccap-SA*		*Ccap-PO*	*Ccap-FA*	*Ccap-SA*	
KN-1	HR	HR	HR	2^{a}	R	HR	R	6^{a}
Moussa local	MR/MS	HR	HR	5^{a}	MR/MS	R	R	8^{b}
MELAKH	HR	HR	HR	6^{a}	HR	MR/MS	R	7^{b}
Donsin local	HR	SL	HR	4^{a}	MR/MS	HR	R	8^{b}
Djouroum local	R	HR	HR	5^{a}	R	R	MR/MS	10^{b}
Pobé local	R	R	HR	5^{a}	MR/MS	MR/MS	HR	10^{b}
58-57	HR	R	HR	3^{a}	HR	S	R	9^{b}
IT99K-573-2-1	HR	MR/MS	HR	6^{a}	HR	S	HR	11^{c}
503/46-13	SL	MR/MS	MR/MS	8^{b}	SL	S	MR/MS	15^{c}
IT95K-499-35	HR	S	MR/MS	9^{b}	R	S	MR/MS	12^{c}
Komcallé	HR	S	MR/MS	11^{c}	R	S	MR/MS	14^{c}
KVx61-1	HR	S	R	11^{c}	MR/MS	S	R	15^{c}
KVx65-114	R	MR/MS	MR/MS	9^{b}	MR/MS	MR/MS	MR/MS	14^{c}
KVx780-1	HR	S	HR	9^{b}	MR/MS	S	R	14^{c}
IT95M-190	HR	MR/MS	R	7^{b}	MR/MS	MR/MS	MR/MS	13^{c}
KVx780-9	MR/MS	S	S	19^{c}	MR/MS	S	S	21^{d}
Gorom local	HR	HR	MR/MS	5^{a}	MR/MS	MR/MS	S	14^{c}
Gourgou	HR	S	MR/MS	17^{c}	MR/MS	S	MR/MS	19^{c}
HTR	R	MR/MS	HR	8^{b}	MR/MS	S	MR/MS	17^{c}
NS-1	HR	MR/MS	MR/MS	8^{b}	HR	MR/MS	S	13^{c}
TN88-63	R	S	HR	9^{b}	MR/MS	S	MR/MS	14^{c}
TVU14676	HR	MR/MS	R	7^{b}	R	MR/MS	MR/MS	11^{c}
503/46-48	MR/MS	S	MR/MS	20^{c}	S	HS	S	36^{d}
503/46-72	S	S	MR/MS	25^{d}	HS	S	S	29^{d}
524B	R	S	MR/MS	13^{c}	MR/MS	S	HS	27^{d}

Table 7. Contd.

B301	HR	HR	HS	15[c]	R	MR/MS	HS	27[d]
CB46	MR/MS	S	MR/MS	15[c]	MR/MS	S	S	23[d]
IT81D-994	HR	MR/MS	S	15[c]	R	MR/MS	HS	26[d]
IT82D-849	HR	S	MR/MS	16[c]	R	S	S	28[d]
IT93K-503-1	MR/MS	R	S	18[c]	MR/MS	S	HS	26[d]
IT95K-627-4	R	MR/MS	S	13[c]	R	S	S	23[d]
IT98K-205-8	MR/MS	S	S	16[c]	MR/MS	S	HS	30[d]
KVx30-309-6G	HS	R	HR	18[c]	HS	MR/MS	HR	22[d]
KVx396-4-5-2D	R	MR/MS	HS	24[d]	R	S	HS	32[d]
KVx404-8-1	MR/MS	S	S	24[d]	S	S	HS	36[d]
KVx421-2J	S	S	S	22[d]	S	S	S	27[d]
KVx525	S	S	S	23[d]	S	S	S	29[d]
KVx745-11P	S	S	S	29[d]	HS	HS	S	43[d]
KVx771-106	S	S	S	22[d]	S	S	S	35[d]
KVx775-33-2G	S	S	S	20[d]	S	S	S	27[d]
Bambey-21	S	S	MR/MS	22[d]	S	S	S	26[d]
Pathotype virulence mean	**12[b]**	**20[a]**	**18[a]**	13	17[b]	26[a]	29[a]	24

SL= Symptomless; HR=Highly Resistant; R= Resistant; MR/MS= Tolerant =Moderately Resistant or moderately susceptible; S= Susceptible; HS= Highly Susceptible; DAI= Day after inoculation; Means followed by the same letter (a, b, c and d) in a resistance mean column belong to the same susceptibility class.

the last decade (Sereme, 1999).

They might have revealed a polygenic resistance to the disease. All of the new improved varieties; Gourgou, KVx98K-205-8, Komcalle, Tiligre (KVx775-33-2G), Nafi (KVx771-11P) and IT93K-503-1 from IITA were susceptible to brown blotch. The cultivars IT99K-573-2-1 and 503/46-13 showed highly specific resistance, respectively, to two (Ccap-PO and Ccap-SA) and one (Ccap-PO) of the three pathotypes. They could be also used for resistance gene incorporation to susceptible genotypes depending on agro-ecological zone. The cultivar IT81D-994, IT82D-849 and KVx61-1 were previously reported susceptible to brown blotch disease (Adebitan et al., 1992; Sereme, 1999) corresponding with the results.

Disease severity appeared highly dependent on plant age or period of disease evaluation. According to Health (1996), age-related resistance of certain cultivars of cowpea to disease appears to be due to a delay in parasite-specific resistant genes activation. Early evaluation of disease incidence and severity could influence susceptibility class designation. 21 days after inoculation is allowed presented the best reaction of the interaction, genotype-pathotypes in term of disease severity. In conclusion, the study clearly demonstrated that resistance or susceptibility depends on cowpea cultivars, the pathotypes specific resistance and the stage of cowpea plant development and may be consistent with the presence of different genes; recessive, dominant or both can control resistance (Pakdeevaraporn et al., 2005; Mahasuk et al., 2009).

The identification of new sources of resistance from cowpea cultivars is a decisive step in managing the brown blotch disease in Burkina Faso. These results will significantly contribute in designing good programs for marker assisted selection in cowpea resistance to *C. capsici*.

In this study, potential cowpea cultivars presenting a specific resistance to the pathotypes of *C. capsici* and associated with the three agro-ecological zones of the country were identified.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Coffee production through wet process: Ripeness and quality

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Coffee has the characteristic of ripening unevenly. Production through wet process favors ripe berries. The fraction of green and green-cane fruit on the coffee tree is normally treated as an inferior quality product. The objective of this work was to evaluate the physical and sensorial aspects of the quality of green-cane and cherry coffee produced through wet process. Batches of coffee were separated according to ripeness and pulped. Drying was carried out on raised patios until reaching 11% wb. After processing, the beans were selected and classified by size. The sensorial analysis was performed through cupping using the scale of the Brazilian Specialty Coffee Association (BSCA). The experiment was carried out at DBC with seven blocks each containing four plots, the source of variance being the ripeness on two levels: cherry and green-cane. The data was subjected to variance analysis (ANOVA) and the averages compared using F test at 5%. Green-cane coffee presented better yield both in sieving and dry mass, as well as a higher quantity of defects. The final cup quality rating did not differ in regard to ripeness. The pulped green-cane coffee may have added value for its beverage quality.

Key words: Sensory analysis, processing, classification, grains, yield.

INTRODUCTION

In the coffee producing regions of Brazil, the dry period, typically occurring in winter, promotes the differentiation and development of flower buds, with the opening of the flowers beginning with the first rains of spring. From September to December, the coffee tree may present various flowerings, making the development and maturation of the fruits uneven, with green, ripe or dry berries occurring on the same plant (Carvalho et al., 2014; Ságio et al., 2013).

Ripeness is a factor of great importance to coffee quality and a large part of the defects known as blacks, greens or rancids (BGR) can be attributed to the harvesting of unevenly ripened fruit. The cherry stage is considered the ideal point for quality (Folmer, 2014).

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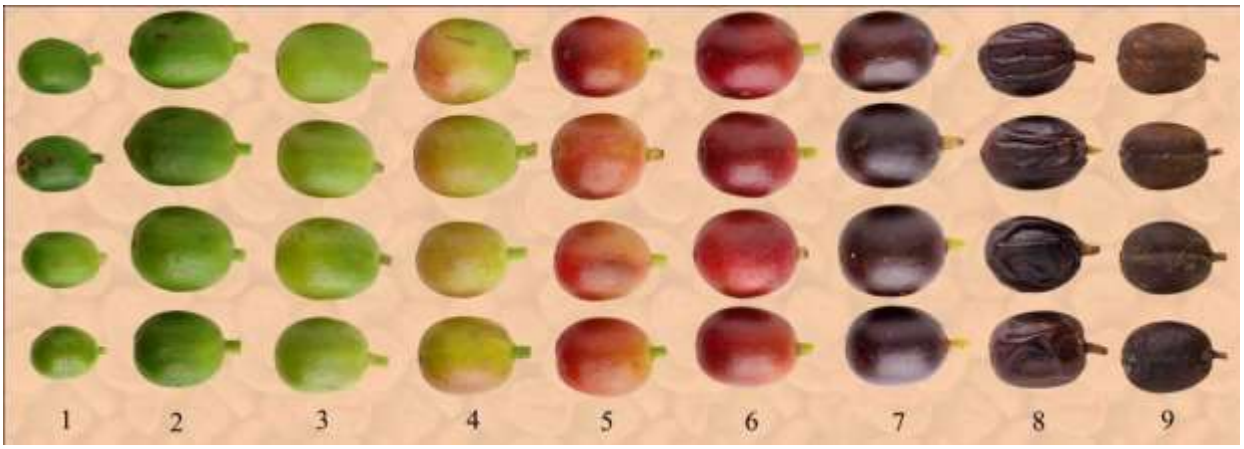

Figure 1. Coffee fruit ripeness scale: 1 - Unripe, 2 - Green, 3 - Green-cane I, 4 - Green-cane II, 5 - Cherry I, 6 - Cherry II, 7 - Post-Cherry, 8 - Raisin and 9 - Dry.

Table 1. Characterization of sample composition of coffee according to fruit ripeness.

Sample	Cherry (%)	Green-cane (%)	Green (%)
1	72.15	6.32	21.53
2	76.12	7.18	16.70
3	68.45	15.60	15.95
4	44.58	18.01	37.41
5	73.20	14.96	11.84
6	50.05	18.62	31.33
7	39.23	14.07	46.70

However, selective harvesting of ripe fruit is labor intensive, which increases the production cost and often makes its practice non-viable (Silva et al., 2013).

Significant contributions to the improvement of quality and consequential remuneration are attributed to the production of coffee through wet process. In this system, after washing, the dry green fruits are separated from the ripe fruits known as cherries and are pulped. The fraction of immature fruits removed from each batch of coffee can generally be differentiated into green and green-cane categories. Morais et al. (2008) demonstrated, through a phenological scale, that the ripeness of coffee begins with green berries, which pass to green-cane and subsequently cherry. The green-cane stage, for being close to maturity, already presents characteristics which enable pulping.

The present research has the objective of determining, in comparison to cherry fruits, the potential of the physical and sensorial quality of coffee harvested at green-cane stage when produced through wet process.

MATERIALS AND METHODS

Samples obtained from seven batches of recently harvested Arabica coffee (*Coffea arabica*) were used. Batches 1, 2, 3, 4 and 5 were composed of fruits predominantly from the Catuaí cultivar, harvested on properties in the region of the Viçosa municipality, whose average altitude varies from 673 to 803 m. Batches 6 and 7 were harvested from plantations of the Catuaí cultivar located in the Vargem Alta municipality - ES, at 870 and 1000 m in altitude, respectively. The batches were sent to The Coffee Processing Unit of the Federal University of Viçosa.

For the characterization of the batches as to ripeness, the classification of the coffee berries was performed based on Figure 1. In Table 1 we can find the characterization of the batches as to percentage of cherry, green-cane and green fruits.

Production

The batches were submitted to wet process production. The green and green-cane coffee berries were mechanically separated from the cherry fruits in the pulping machine (DC-12-SDV-11: Pinhalense-SA). The equipment was adjusted so that fully mature berries were pulped during the first run, with a tolerance of up to 5% of the cherry fruit being made up of green and green-cane fruit. The cherry coffee was pulped while the green and green-cane berries continued onto a second pulping machine (DC-6S-SV-11: Pinhalense-SA), which carried out a further separation, predominantly pulping green-cane fruits. Next the pulped cherry coffee was submitted to mechanical demucilage in a mucilage removal machine (DFA-3: Pinhalense-SA). The green-cane coffee in parchment was not subjected to demucilage as it naturally has a

Table 2. Characterization of pulped cherry coffee beans (PC) and pulped green-cane (PGC) according to size, corresponding to retention in circular sieves, screen 16 and above 16a, 16, 17, 18, 19 and 20.

Type of grain	Sieve 16a	Sieve 16	Sieve 17	Sieve 18	Sieve 19	Sieve 20
PC	65.41[a]	10.76[b]	22.15[a]	20.70[a]	9.78[a]	2.01[a]
PGC	47.68[b]	13.23[a]	17.69[b]	11.93[b]	4.22[b]	0.64[b]
CV%	1.76	7.84	4.96	6.97	10.30	58.48

Averages followed by the same letter in the column do not differ from each other by the 5% F test.

lower quantity of mucilage, besides which, the mucilage removal of this fraction may cause unnecessary damage to the beans. 240 L of pulped coffee was used in each batch: 120 L of cherry beans and 120 L of green-cane beans in parchment.

Initially, the beans in parchment were spread out over a concrete patio in the open air during the day, for pre-drying. After this period, the coffee was transferred to a raised patio where it remained until reaching a moisture content of around 11%. During the period of pre-drying and drying, the coffee was turned every 30 min to guarantee even moisture distribution. Every day, at 16:00, the grains were gathered up and then spread out again the next morning at 08:00. The moisture of the grains was monitored using a digital grain moisture tester (Gehaka - G600).

After drying, the samples were stored in 70 × 90 cm raffia sacks, and left to rest for a period of approximately 30 days. Then, with the objective of removing the parchment, the coffee was processed in a small hulling machine, D100 model (Pinhalense S/A). The processed grains were wrapped in 50 × 70 cm polyethylene plastic bags, which were stored at a temperature close to 23°C, sheltered from light and humidity, thus preserving the quality of the product.

Standardization of the batches of coffee was carried out between 15 and 20 days after processing, with the aim of generating homogenous samples in regard to bean size, aspect and type. Initially, the grains of each batch were screen sorted by size, using a size 16 round sieve, making use of the retained coffee, classified as screen s16 and above. Beans with intrinsic defects (Blacks, Rancids, Greens, Withered, Ragged, Crushed or Bored) and extrinsic defects (Shells, Twigs, Stones, Husks or Boat Shaped) were removed to obtain samples of type 2 coffee, which allows a lower number of defects, according to the Official Table of Imperfect and Impure Bean Equivalence (Brasil, 2003).

Samples of 100 g of processed coffee without defects and previously sorted using screen 16 and above were passed through circular sieves to separate by bean size, obeying the following decreasing sequence: Screen 20, 19, 18, 17 and 16. After the separation of beans into different sizes, those retained in each sieve were weighed and the percentage of retained beans was calculated for each size, in relation to the total weight.

For each individual sieve and a control sample of screen 16 and above, the weight of dry matter of 100 beans was determined, using the gravimetric method in a kiln at 105°C for 24 h. For weighing, precise digital scales with three decimal places were used (AL500S: Marte).

Bean samples of screen 16 and above without defects were submitted to sensorial analysis. Cupping was carried out by a team of accredited tasters from the Brazilian Specialty Coffee Association (BSCA), using the Cup of Excellence (CoE) methodology, perfected by the BSCA (2010). Eight sensorial attributes were evaluated (cleanliness, sweetness, acidity, body, taste, aftertaste, balance and overall rating), which received ratings of 0 to 8 according to the intensity each factor presented in the sample. The sum of the ratings, added to 36 base points, corresponded to the final classification of the beverage. The samples that obtained a rating above 80 were classified as specialty coffee.

The experiment was installed at DBC with seven blocks, each containing four plots, source of variation being ripeness on two different levels: Cherry fruit and green-cane. Data was submitted to variance analysis (ANOVA) and the averages compared by f test at 5%. The statistical analyses were performed using SAEG 9.1 software (2007).

RESULTS AND DISCUSSION

For percentages of beans at size 16 and above, 17, 18, 19 and 20 had higher percentages of the cherry fraction, while the percentage of beans in screen 16 was greater in the green-cane fraction (Table 2).

Coffee beans are classified according to size: small (screen 13 and 14), medium (screen 15 and 16) and large (screen 17, 18, 19 and 20) (Brasil, 2003). The quantity of beans in screen 16 and above is an important characteristic in virtue of the fact that this formation has greater acceptance and is more commercially valuable, especially on the international market (Clemente et al., 2015).

Wet process production with double separation enabled the recognition and better usage of the green-cane fraction, which even with a predominance of immature fruit possesses a quota of beans with characteristics close to those of cherry beans.

According to Pezzopane et al. (2003) and Morais et al. (2008), the development of coffee tree berries passes through a series of distinct stages, whereby grain filling precedes ripening. According to these authors, the green-cane coloring marks the beginning of ripening, when the berries begin to change from green to yellow, evolving until the cherry stage. Thus green-cane berries contain grain filled beans.

The largest proportion of beans in screen 16 being in the green-cane fraction and the value inversion in larger sieves reflects the greater production potential for larger beans in cherry fruits. The expansion phase of the cherry fruits and the filling of beans may have advantages in regard to green-cane berries, due to the greater disposition of nutrients in the first months of fruitage, especially potassium, which is essential for the growth and accumulation of dry matter (Taiz and Zeiger, 2010).

For fruits at the cherry stage, the dry mass of 100 beans was higher in the cherry fraction for all the evaluated variables (Table 3).

Table 3. Characterization of shelled cherry-colored type coffee (SC) and peeled green cane colored type coffee (PGC) from dry mass of 100 pre-sorted grains in circular screen sieves.

Type of grain	Sieve 16ac	Sieve 16	Sieve 17	Sieve 18	Sieve 19	Sieve 20
SC	14.51[a]	12.64[a]	14.07[a]	15.65[a]	17.10[a]	19.65[a]
PGC	13.26[b]	11.85[b]	13.53b	15.25[b]	16.51[b]	19.01[b]
CV%	1.95	2.68	2.59	2.39	2.80	1.63

Averages followed by the same letter in the column do not differ from each other by the 5% F test.

Table 4. Sensory analysis by cup test of shelled cherry-colored type coffee (SC) and peeled green cane colored type coffee (PGC).

Treatment	Clean drink	Sweetness	Acidity	Corpo	flavor	Reminiscent taste	Balance	General	Total + 36
CD	5.28[a]	5.53[a]	5.32[b]	5.21[a]	5.46[a]	5.39[a]	5.25[a]	5.39[b]	78.82[a]
VCD	5.35[a]	5.17[b]	5.64[a]	5.42[a]	5.25[a]	5.39[a]	5.14[a]	5.71[a]	79.14[a]
CV%	11.23	9.55	9.23	10.04	9.33	10.51	9.96	10.11	3.20

Weight difference between the cherry beans and the green-cane beans was more accentuated in screen 16 and above. In the other sieves, 16, 17, 18 and 19, there was a tendency towards the levelling out of these values. This is due to the cherry fruits presenting a higher percentage of beans in the bigger sieves as demonstrated in Table 2. Therefore, the cherry fraction of beans in screen 16 and above contained larger beans, which reflects in the weight difference.

Ripeness of the berries is one of the factors affecting the weight of the coffee beans. The results obtained in this study showed beans at the green-cane stage with an accumulation of dry matter above 90% of that achieved by beans from cherry fruits. In the studies of Angelico et al. (2011), differences were also found in the weight of beans originating from berries at different stages of ripeness, the highest average being obtained from the cherry stage, followed by the mixed portion and green/green-cane stages and raisin/dry.

Coffee berries at the green-cane stage have already begun the maturation process (Morais et al., 2008). The development cycle of berries from *C. arabica* cultivars normally varies between 180 and 240 days, a period determined principally by genetic constitution and climate conditions (Pezzopane et al., 2003; Livramento, 2010; Carvalho et al., 2014).

According to Pezzopane et al. (2009), the transition from green-cane to cherry stage varies from 8 to 20 days. According to Dubberstein et al. (2016), the accumulation of dry matter in fruits starts from the stage of fruit expansion and granulation-maturation, and the importance of knowledge about absorption, mobilization and accumulation of nutrients for coffee fruits in the cherry stage is an important tool to estimate the nutritional needs of the crop. In studying dry matter in fruits of conilon coffee with different ripening cycles, Partelli et al. (2014) in all cases, the period of fruit formation presented sigmoidal behaviour, an initial stage with less expressive accumulation rates, followed by a stage of rapid expansion and the highest rates, and a final stage with less expressive rates at the end of the cycle of fruit formation.

Based on the results and reports of the literature, it can be understood that green-cane coffee berries may present weight yield close to that of cherry fruits. However, it should be highlighted that the coloring of the fruit is not always a good indicator of ripeness as it may, for example, be influenced by extrinsic factors such as pests, diseases and dry spells among other things (Taiz and Zeiger, 2010).

In the sensorial analysis by cupping, the attributes of cleanliness, body, taste, aftertaste and balance did not present differences between the ripeness stages. Sweetness stood out in the cherry fruit as being more highly rated than green-cane coffee, while acidity and overall rating were more pronounced in the green-cane beans.

The final rating did not differ between the cherry and green-cane beans, whereby the achieved ratings fell into the category of “Softish”, considered a superior quality beverage (Table 4).

Lack of differentiation between the majority of the attributes, as well as final rating of the cup test for coffee beans at different stages of ripeness has also been reported by other authors. Pimenta et al. (2008), in aiming to verify the quality of coffee at different stages of ripeness, analyzed coffees harvested at seven different times, obtaining a classification of all the treatments as “Hard” beverage, demonstrating that, in general, the harvest time does not affect the quality of the beverage. Angelico et al. (2011) analyzed the influence of the ripeness stage of the beans (dry, cherry and green) on the quality of the beverage through consumer preference, obtaining a similar beverage classification for batches

made up of 100% cherry beans and 60% cherry 40% green. Simões (2009), studying the quality of coffee produced through dry process in batches with different ripeness levels, obtained similar quality and concluded that the proper handling and the elimination of defects enabled batches of coffee with high rates of immature fruit to present good quality beverages.

Regarding attributes of sweetness, acidity and overall rating, intrinsic characteristics of the batches of coffee may be related to their levels of expression as a result of ripeness. According to Laviola et al. (2008), the accumulation of soluble sugars occurs in the final half of the grain filling stage and during the ripening of the coffee tree berries, the climate being a factor that can influence this characteristic.

Beans originating from coffee berries at the green-cane stage presented lower screen yield and dry matter in relation to cherry coffee.

The 16 and above screen fraction without defects obtained from green-cane coffee berries presented a beverage of similar quality to cherry coffee.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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