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Table of Content

Is silicon capable to affect the photosynthetic performance of green maize plants? Raquel Caroline dos Santos, Marta Donato de Souza, Giovana Michele da Cruz and Daniel Baron	1279
Genetic variability of yam based on quantitative descriptors Virgílio Carménia Cossa, Elaine Costa Cerqueira-Pereira, Darcilúcia Oliveira do Carmo de Almeida, Carlos Alberto da Silva Ledo and Ricardo Franco Cunha Moreira	1288
Physiological responses of operators to handle vibration of diesel-fueled single-axle tractor Siraj K. Busse, Anthony N. Sinclair, Diresibachew H. Wondimu and Daniel T. Redda	1295
Physiological potential of lettuce seeds <i>crespa</i> Wesley Oliveira de Assis, João Luciano de Andrade Melo Junior, Luan Danilo Ferreira de Andrade Melo, Reinaldo de Alencar Paes, João Correia de Araújo Neto, Lucas dos Santos Medeiros, Larice Bruna Ferreira Soares, Rayanne Thalita de Almeida Souza, Sara Camylla de Moura Assis, Lívia Francyne Gomes Chaves, Ana Caroline de Melo Morais and José Jairo Florentino Cordeiro Junior	1304
Changes in livelihood strategies and animal husbandry practices of pastoralists in the sub-humid zone of West Africa Nouhoun Zampaligré, Dominique Ouedraogo, Claudious Chikozho, Louis Sawadogo and Eva Schlecht	1311
Comparison of proximate, mineral and phytochemical composition of enset (<i>Ensete ventricosum</i> (Welw.) Cheesman) landraces used for a different purpose Gizachew Woldesenbet Nuraga, Tileye Feyissa, Sebsebe Demissew, Kassahun Tesfaye and Ashagrie Zewdu Woldegiorgis	1326

Full Length Research Paper

Is silicon capable to affect the photosynthetic performance of green maize plants?

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Green maize (*Zea mays* L.) is a cultivated species of significant importance in the agricultural scene. The literature reports that Si has been used as an alternative option for sustainable agricultural systems. We examined the hypothesis that this beneficial element will improve the photosynthetic performance and biological productivity of crop plants, under field conditions, without nutritional stress. In this context, leaf gas exchanges, physiological indexes, and growth parameters were investigated in green maize, AG4051 and CATIVERDE 02, under Si availabilities at the initial stage of vegetative development. The treatments were: Via shoot; 0, 130, 260, 390 and 520 g ha⁻¹; and via roots; 0, 100, 500, 1000 and 2000 kg ha⁻¹. The experimental design was randomized blocks (RB) with 5 blocks (experimental plots). The beneficial mineral element Si did not have an improvement on gas exchanges of green maize plants and, consequently, plant development. In conclusion, we reject our initial hypothesis and we accepted the alternative hypothesis, that the beneficial element Si did not optimize the photosynthetic performance and biological productivity of green maize plants, without nutritional stress.

Key words: Leaf gas exchange, silicon, sustainability, *zea mays* l.

INTRODUCTION

Green maize (*Zea mays* L.) is a crop plant of immense importance on the agricultural scenery, primordial as a raw material for the formulation of several industrialized products (Shen et al., 2017). It is one of the most produced plants worldwide in a conventional system and

has influence in the economic development of countries, like USA, China and Brazil (Fao, 2017). However, the conventional production system, most commonly used by agriculture, has become limited in terms of sustainability, making this type of production unfeasible in natural

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ecosystems.

The major starting point to overcome this problem is the implantation of sustainable agricultural systems. It is necessary to the local rational use of non-renewable natural resources, as well as the use of appropriate technologies to the local reality (Rótolo et al., 2015). The literature shows some studies that use the mineral element silicon (Si) as an alternative source of plant nutrition on sustainable agricultural systems. Although Si is the second most abundant element after oxygen in soil, this element is little investigated by crop plants physiologists and its relevance for plant growth has been recognized, because it is beneficial for triggering physiological effects.

In the contemporary agriculture, Si currently has a very promising use perspective, by recent advances in the understanding of the ionic accumulation of this mineral element in the crop plants physiology (Hobara et al., 2016). It confers resistance to insect pests, microorganisms and environmental abiotic factors, and, consequently, reduction of the use of pesticides and environmental risks (Dallagnol et al., 2009; Filha et al., 2011; Datnoff and Rodrigues, 2015; He et al., 2015; Dudley et al., 2017). In addition, it improves the photosynthetic performance, which favors their growth and plant reproductive development (Song et al., 2014).

The agricultural success is linked to many physiological factors, for example, leaf gas exchanges and biomass productivity, which are regulated by biochemical processes. Gas exchanges and the loss of water in the plants occur through stomatal movement, opening and closing, respectively, according to environmental stimulation that promotes the difference on the water potentials in the guard cells. Light is a major environmental factor that stimulates an opening of the stomata, controlling the rate of CO₂ assimilation, fundamental to the photosynthetic process. In this physiological aspect, some reasearches studied the effect of Si in the gas exchanges under biotic and abiotic stress (Paula et al., 2015).

The penetration of Si into the roots is in the form of monosilicic acid (H₄SiO₄), through the process of diffusion or mass flow. This element is transported to the interior of the plant by carrier proteins (uptake) of the channel type, which are expressed by the *ZmLsi1* gene, previously described in maize plants (Imtiaz et al., 2016). The uptake of Si, via leaf, was also reported through the expression of the *ZmSi6* gene (Mitani et al., 2009) and this absorption pathway has been an alternative with development and characteristics benefits in several crops (Ma and Yamaji, 2015).

Thus, our objective in this study was to evaluate the influence of Si availabilities via soil and foliar biomass production of the green maize plants under field conditions without abiotic stress. We examined the hypothesis that this beneficial element will improve the

photosynthetic performance and biological productivity of crop plants under field conditions without nutritional stress.

MATERIALS AND METHODS

Experimental conditions and soil fertilization

The study was conducted in an experimental area belonging to the Federal University of São Carlos (UFSCar), Lagoa do Sino campus, located in the Buri municipality, State of São Paulo, Brazil (average altitude of 596 m, geographical coordinates 23° 47' 57" S latitude and 48° 35' 15" W longitude). The water application was made by using center-pivot (CP) irrigation, low-pressure spray sprinklers systems, with necessary and sufficient water volume according to Pereira-Filho (2002), the soil is labeled as Red Latosol Eutroferic as indicated in Table 1, and fertilization was performed according to Rajj et al. (1997). We used 446.28 kg ha⁻¹ of the macronutrients chemical formulation 8-28-16 of N, P and K, respectively, and plus 1% micronutrient Zn. The authors of this study emphasized that the conditions of field cultivation were rigorous, especially giving great attention to the protection of plants (phytosanitary management) and irrigation performance.

Plant materials and treatments

The treatments used were different availabilities of the silicon (Si) mineral element, applied via leaf (shoot) and soil (root), under different levels on green maize plants, *AG4051* and *CATIVERDE 02*, transgenic and non-transgenic maize plants, respectively, at 17 days after sowing (DAS), when the plants had a V2 phenological stage (2 pairs of fully expanded and photosynthetically active leaves).

The application of Si, via leaf (shoot), was performed using a backpack sprayer equipped with a manometer and a flat-fan nozzle (*Teejet® 110 02 XR*). The Si resource used was K silicate (K₂SiO₃, 12% K₂O and 10% Si) were; 0, 130, 260, 390 and 520 g ha⁻¹, according to Freitas et al. (2011). The Si resource used via soil was calcium-magnesium silicate granulated (9% Si, 9% Ca and 2% Mg) were; 0, 100, 500, 1000 and 2000 kg ha⁻¹, according to Sandim et al. (2010).

Leaf gas exchange measurements

Foliar gas exchange was measured with the aid of the portable complete system equipment with microclimate control for measurement of photosynthesis and transpiration, ADC BioScientific Ltd (model LCpro-SD). The variables measured by the equipment were net carbon assimilation rate (*A*, μmol m⁻² s⁻¹), internal carbon concentration in the substomatal chamber (*C_i*, μmol CO₂ mol air⁻¹), stomatal conductance (mol m⁻² s⁻¹), transpiration (*E*, mmol water vapor m⁻² s⁻¹), instantaneous carboxylation efficiency (*A/C_i*, μmol m⁻² s⁻¹ Pa⁻¹) and water use efficiency (*WUE*, μmol CO₂ (mmol H₂O) s⁻¹) at 124 days after sowing. The reference CO₂ concentration that was used during the evaluation was 380 μmol mol⁻¹ of air.

To ensure that the experimental conditions were consisted, the photosynthetically photons flux density (PPFD) was standardized through the use of a light-emitting diode coupled to a photosynthesis chamber, and the light-emitting diode emitted 1700 μmol m⁻² s⁻¹, as this saturating luminosity, according to light curves previously performed (Yarami and Sepaskhah, 2015).

Table 1. Chemical attributes of the Eutrophic Red Latosol of the experimental area in the 0-20 cm depth layer.

Layer (cm)	pH CaCl ₂	P mg dm ⁻³	M.O. g m ⁻³	H+Al	Al ³⁺	K ⁺	Ca ²⁺ mmol _c dm ⁻³	Mg ²⁺	CTC	SB	V %
0-20	5.2	48	31	36	2	4.1	50	15	104.7	68.7	66

Laboratory of Chemical Analysis of Soil and Plant belong to Federal University of São Carlos (Araras campus), Araras, State of São Paulo, Brazil.

Plant growth measurements

The evaluations were performed at phenological growth stages V4 and R5 (22 and 124 days after sowing DAS, respectively) with five replicates of five plants collected for each treatment for the maize cultivars. The variables measured were stem diameter (collar diameter) (mm), plant height (cm), number of leaves per plant (leaves units) and leaf area (dm²), and was performed according to Lopes et al. (2009).

Physiological indexes measurements

Through the determination of leaf area, shoot dry matter mass and total dry matter mass, physiological indexes were calculated among 22 and 124 DAS, such as leaf area ratio (LAR, dm⁻² dia⁻¹), specific leaf area (SLA, dm⁻² dia⁻¹), net assimilation rate (NAR, g dm⁻² dia⁻¹), leaf weight ratio (LWR, g/g) and relative growth rate (RGR, g g⁻¹ dia⁻¹) (Baron et al., 2018).

Experimental design and statistical analysis

The experimental design was randomized blocks (RB) with five blocks (experimental plots). Each experimental plot was composed of four sowing rows of 5.0 m in length, spacing 0.5 m between rows, totaling a population of 50,000 plants per hectare, the data were subjected to the homogeneity test (Levene test), the statistical assumptions (homoscedasticity among the variances) were analyzed statistically by analysis of variance (ANOVA), and averages compared by the Tukey test at 5% of probability using SAS 9.0 statistical analysis software.

RESULTS

The Levene's test showed homogeneity of variances between treatments. This study aimed to check if Si is capable to improve the photosynthetic performance of green maize plants cultivated in field conditions without nutritional stress. The results obtained ensure only effects of Si treatments supplied to the green maize plants.

From the measurements of leaf gas exchange, we report that there were no statistical differences in the evaluated parameters of gas exchange of green maize plants between the different levels of the mineral element Si as indicated in Table 2, and the data of vegetative growth of green maize plants with different Si availabilities, applied shoot and root, did not present

differences, except for the number of leaves at 22 DAS, for cultivar *AG4051*, and stem diameter for cultivar *CATIVERDE 02* (both applied via root) as presented in Table 2.

However, we report that for the cultivar *CATIVERDE 02*, there were no differences between the treatments except for the number of leaves (22 DAS) with Si applied via shoot as described in Table 3. In the physiological indexes in both cultivars, no differences were observed between the different availabilities of Si (shoot and root) as indicated in Table 4.

DISCUSSION

Our strategy of Si application occurred in the vegetative phenological growth stage (V2) of green maize plants (*Zea mays* L.). The Si physiological effects under photosynthetic performance using maize plants in early phenological growth stages are not elucidated in the literature, but it can be stated that the Si uptake is sufficient to trigger biochemical effects in photosynthetic processes. In addition, we can speculate that the shoot application of Si from potassium silicate (K₂SiO₃), has allowed the biopolymers formations, which would impair Si uptake by the leaves (Xie et al., 2014).

In general, the monocots species, such as rice (*Oryza sativa* L.) and sugarcane (*Saccharum officinarum* L.), uptake large quantities of Si; however, eudicots species, such as tomato and potato, appear to be impervious to the Si (Deshmukh et al., 2015). The improvements of leaf gas exchange, from the use of the Si element, were not noticed in the present study. According to the literature, the silicic acid transport in the plants by xylem vessels is generally favored by leaf transpiration (Etesami and Jeong, 2018). The Si transported from the roots uptake sites to the sprout formation allow the leaves to remain semi-upright, which reduces the transpiratory area of the leaves (Tamai and Ma, 2008; Adrees et al., 2015).

The element Si plays a key role in the maintenance and cellular integrity. It is possible that the Si was not only deposited in the shoot cell wall of vegetative development (Ali et al., 2013), although, the maize plants have Si accumulation (Ma and Yamaji, 2015). Crop plants, for example, rice, show panicles increase to plants treated

Table 2. Leaf gas exchange in green maize at phenological growth stage 'R5' (124 DAS) (*AG 4051* and *CATIVERDE02*) cultivated in different Si availabilities.

Green maize plant	Availability	Treatment (kg ha ⁻¹)	A (μmol m ⁻² s ⁻¹)	C _i (μmol CO ₂ mol air ⁻¹)	g _s (mol m ⁻² s ⁻¹)	E (mmol m ⁻² s ⁻¹)	A/C _i (μmol m ⁻² s ⁻¹ Pa ⁻¹)	WUE (μmol mmol ⁻¹)
AG4051	Shoot	0	21.53 ± 2.94 ^a	174.00 ± 38.50 ^a	0.31 ± 0.02 ^a	4.20 ± 1.16 ^a	0.13 ± 0.04 ^a	5.30 ± 0.87 ^a
		0.13	19.06 ± 3.23 ^a	169.80 ± 35.31 ^a	0.27 ± 0.08 ^a	3.74 ± 0.50 ^a	0.11 ± 0.04 ^a	5.15 ± 1.06 ^a
		0.26	19.20 ± 3.10 ^a	176.80 ± 29.98 ^a	0.28 ± 0.09 ^a	3.84 ± 0.60 ^a	0.11 ± 0.03 ^a	5.10 ± 1.13 ^a
		0.39	20.12 ± 2.01 ^a	185.20 ± 25.95 ^a	0.33 ± 0.08 ^a	4.21 ± 0.73 ^a	0.11 ± 0.01 ^a	4.89 ± 0.98 ^a
		0.52	21.09 ± 2.43 ^a	174.20 ± 30.00 ^a	0.31 ± 0.02 ^a	4.23 ± 0.74 ^a	0.12 ± 0.03 ^a	5.05 ± 0.65 ^a
		F	0.78 ^{ns}	0.16 ^{ns}	0.69 ^{ns}	0.45 ^{ns}	0.33 ^{ns}	0.12 ^{ns}
	CV (%)	13.78	18.32	22.42	19.44	28.75	18.8	
	Root	0	26.27 ± 0.91 ^a	133.6 ± 13.41 ^a	0.344 ± 0.02 ^a	5.38 ± 0.46 ^a	0.230 ± 0.02 ^a	5.34 ± 0.47 ^a
		100	27.94 ± 0.63 ^a	146.8 ± 10.07 ^a	0.426 ± 0.02 ^a	5.45 ± 0.42 ^a	0.200 ± 0.01 ^a	5.58 ± 0.48 ^a
		500	25.42 ± 1.94 ^a	150.2 ± 13.98 ^a	0.394 ± 0.04 ^a	5.23 ± 0.29 ^a	0.192 ± 0.02 ^a	4.99 ± 0.47 ^a
		1000	25.38 ± 1.17 ^a	147.4 ± 6.82 ^a	0.350 ± 0.02 ^a	4.93 ± 0.43 ^a	0.181 ± 0.01 ^a	5.71 ± 0.56 ^a
		2000	25.25 ± 1.12 ^a	156.8 ± 14.19 ^a	0.380 ± 0.01 ^a	5.60 ± 0.43 ^a	0.187 ± 0.02 ^a	4.71 ± 0.23 ^a
		F	0.26 ^{ns}	0.15 ^{ns}	0.40 ^{ns}	0.12 ^{ns}	0.25 ^{ns}	0.25 ^{ns}
	CV (%)	19.01	32.78	31.41	31.18	42.99	35.10	
CATIVERDE 02	Shoot	0	16.11 ± 3.17 ^a	174.00 ± 38.50 ^a	0.31 ± 0.02 ^a	4.20 ± 1.16 ^a	0.13 ± 0.04 ^a	5.30 ± 0.87 ^a
		0.13	16.73 ± 3.89 ^a	169.80 ± 35.31 ^a	0.27 ± 0.08 ^a	3.74 ± 0.50 ^a	0.11 ± 0.04 ^a	5.15 ± 1.06 ^a
		0.26	18.68 ± 3.97 ^a	176.80 ± 29.98 ^a	0.28 ± 0.09 ^a	3.84 ± 0.60 ^a	0.11 ± 0.03 ^a	5.10 ± 1.13 ^a
		0.39	17.89 ± 3.49 ^a	185.20 ± 25.95 ^a	0.33 ± 0.08 ^a	4.21 ± 0.73 ^a	0.11 ± 0.01 ^a	4.89 ± 0.98 ^a
		0.52	17.33 ± 2.33 ^a	174.20 ± 30.00 ^a	0.31 ± 0.02 ^a	4.23 ± 0.74 ^a	0.12 ± 0.03 ^a	5.05 ± 0.65 ^a
		F	0.42 ^{ns}	0.58 ^{ns}	0.58 ^{ns}	0.73 ^{ns}	0.49 ^{ns}	0.42 ^{ns}
	CV (%)	19.77	18.44	31.91	22.92	34.37	24.38	
	Root	0	20.69 ± 1.22 ^a	164.0 ± 10.52 ^a	0.312 ± 0.03 ^a	4.972 ± 0.36 ^a	0.130 ± 0.01 ^a	4.486 ± 0.47 ^a
		100	20.17 ± 0.67 ^a	162.0 ± 10.81 ^a	0.270 ± 0.007 ^a	4.752 ± 0.34 ^a	0.134 ± 0.01 ^a	4.467 ± 0.26 ^a
		500	22.02 ± 0.85 ^a	150.6 ± 15.01 ^a	0.310 ± 0.02 ^a	4.986 ± 0.17 ^a	0.167 ± 0.01 ^a	4.432 ± 0.14 ^a
		1000	22.20 ± 0.58 ^a	163.4 ± 11.66 ^a	0.318 ± 0.01 ^a	4.708 ± 0.29 ^a	0.147 ± 0.01 ^a	4.967 ± 0.32 ^a
		2000	19.40 ± 1.34 ^a	176.6 ± 11.44 ^a	0.292 ± 0.02 ^a	4.934 ± 0.51 ^a	0.120 ± 0.01 ^a	4.266 ± 0.38 ^a
		F	0.47 ^{ns}	0.18 ^{ns}	0.24 ^{ns}	0.04 ^{ns}	0.56 ^{ns}	0.19 ^{ns}
	CV (%)	18.81	29.39	29.88	29.33	38.28	29.69	

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability. ($n = 5$, ± standard error).

with Si and the supply of Si was beneficial to the commercial grain production (Lavinsky et al.,

2016). These authors affirm that the Si plays a physiological function on photosynthesis, which is

justified by the increase in stomatal conductance and ability to atmospheric CO₂ assimilation.

Table 3. Plant growth data of green maize (CATIVERDE 02 and AG 4051) under different Si availabilities among 22 DAS ('V4') and 124 DAS ('R5').

Green plant	maize	Availability	22 days after sowing				124 days after sowing		
			Treatment (kg ha ⁻¹)	Plant height (cm)	Number of leaves (leaves units)	Stem diameter (mm)	Plant height (cm)	Number of leaves (leaves units)	Stem diameter (mm)
AG4051	shoot	0	45.2 ± 5.27 ^a	5.00 ± 0.70 ^a	9.40 ± 1.94 ^a	289.10 ± 7.10 ^a	9.4 ± 1.51 ^a	24.6 ± 2.19 ^a	
		0.13	48.90 ± 5.76 ^a	4.80 ± 0.44 ^a	9.40 ± 3.64 ^a	280.06 ± 10.46 ^a	8.8 ± 1.09 ^a	25.8 ± 3.19 ^a	
		0.26	40.62 ± 4.62 ^a	5.00 ± 0.00 ^a	8.60 ± 3.04 ^a	275.88 ± 19.59 ^a	9.4 ± 1.34 ^a	26.8 ± 0.44 ^a	
		0.39	45.44 ± 4.62 ^a	4.2 ± 0.83 ^a	7.00 ± 1.22 ^a	296.20 ± 11.98 ^a	8.6 ± 2.70 ^a	24.40 ± 3.50 ^a	
		0.52	44.90 ± 7.29 ^a	5.40 ± 1.14 ^a	9.00 ± 3.24 ^a	278.12 ± 16.43 ^a	9.8 ± 1.09 ^a	23.20 ± 2.38 ^a	
		F	1.38 ^{ns}	1.78 ^{ns}	0.65 ^{ns}	1.90 ^{ns}	0.43 ^{ns}	1.44 ^{ns}	
		CV (%)	12.44	15.05	31.92	4.87	18.05	10.32	
	root	0	42.52 ± 5.74 ^a	5.6 ± 0.54 ^a	8.6 ± 2.30 ^a	266.5 ± 24.1 ^a	10.8 ± 1.92 ^a	24.6 ± 3.57 ^a	
		100	43.22 ± 12.24 ^a	5.0 ± 0.00 ^{ab}	8.8 ± 2.48 ^a	273.2 ± 6.05 ^a	11.4 ± 1.14 ^a	26.4 ± 9.86 ^a	
		500	40.40 ± 6.36 ^a	5.2 ± 0.44 ^{ab}	7.8 ± 2.58 ^a	267.5 ± 21.69 ^a	11.4 ± 1.34 ^a	24.6 ± 4.72 ^a	
		1000	34.36 ± 4.01 ^a	4.4 ± 0.54 ^{ab}	7.8 ± 2.04 ^a	276.0 ± 7.77 ^a	11.0 ± 1.41 ^a	23.2 ± 1.91 ^a	
		2000	41.08 ± 9.28 ^a	4.6 ± 0.54 ^b	7.6 ± 3.64 ^a	271.5 ± 19.12 ^a	11.0 ± 1.41 ^a	26.6 ± 2.79 ^a	
		F	0.95 ^{ns}	5.18 ^{ns}	0.20 ^{ns}	0.27 ^{ns}	0.18 ^{ns}	0.35 ^{ns}	
		CV (%)	20.02	9.45	32.90	6.42	12.58	21.39	
CATIVERDE 02	shoot	0	41.64 ± 4.94 ^a	5.4 ± 0.54 ^{ab}	9.4 ± 2.50 ^a	286.40 ± 10.59 ^a	9.00 ± 2.44 ^a	24.6 ± 2.88 ^a	
		0.13	40.08 ± 2.43 ^a	5.40 ± 0.54 ^{ab}	8.00 ± 1.87 ^a	284.98 ± 17.25 ^a	8.60 ± 2.07 ^a	21.40 ± 4.03 ^a	
		0.26	40.52 ± 4.69 ^a	5.60 ± 0.54 ^a	8.8 ± 1.92 ^a	271.90 ± 20.08 ^a	8.60 ± 1.34 ^a	23.00 ± 5.09 ^a	
		0.39	43.44 ± 5.30 ^a	5.20 ± 0.83 ^{ab}	8.60 ± 2.60 ^a	277.94 ± 7.44 ^a	8.20 ± 1.78 ^a	24.40 ± 4.97 ^a	
		0.52	39.82 ± 7.05 ^a	4.40 ± 0.54 ^b	6.20 ± 1.30 ^a	297.50 ± 24.00 ^a	10.20 ± 1.64 ^a	23.80 ± 2.28 ^a	
		F	0.45 ^{ns}	2.89 [*]	1.70 ^{ns}	1.61 ^{ns}	0.82 ^{ns}	0.52 ^{ns}	
	CV (%)	12.04	11.85	25.58	5.99	21.27	17.12		
	root	0	47.22 ± 7.85 ^a	5.6 ± 0.54 ^a	7.8 ± 2.94 ^{ab}	297.44 ± 44.41 ^a	11 ± 1.41 ^a	24.2 ± 2.94 ^a	
		100	48.58 ± 8.73 ^a	5.4 ± 0.54 ^a	10.8 ± 1.30 ^a	277.80 ± 13.08 ^a	10 ± 0.70 ^a	20.4 ± 2.40 ^a	
		500	42.08 ± 5.71 ^a	4.8 ± 0.44 ^a	8.5 ± 1.11 ^{ab}	281.20 ± 9.23 ^a	9.8 ± 1.09 ^a	22.4 ± 4.66 ^a	
1000		43.24 ± 7.30 ^a	5.2 ± 0.44 ^a	7.4 ± 2.07 ^{ab}	275.30 ± 25.5 ^a	10.4 ± 0.54 ^a	23.4 ± 6.10 ^a		
	2000	49.01 ± 7.53 ^a	5.5 ± 0.50 ^a	7.1 ± 2.07 ^b	286.40 ± 36.33 ^a	8.4 ± 2.70 ^a	26.4 ± 2.40 ^a		
	F	0.89 ^{ns}	2.00 ^{ns}	2.93 [*]	0.46 ^{ns}	2.06 ^{ns}	1.55 ^{ns}		
	CV (%)	16.28	9.43	23.27	10.18	15.15	17.05		

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability. ($n = 5$, \pm standard error).

In our present study, the availabilities of Si do not increase photosynthetic rates in maize plants,

because this mineral element is more relevant in plants under biotic and abiotic stress. This finding

is corroborated by other studies in higher plants which affirm that Si effect on plants may not be

Table 4. Physiological Indexes (SLA, LWR, RGR, NAR and LAR) of green maize (*AG4051* and *CATIVERDE 02*) cultivated in different Si availabilities among 22 DAS ('V4') and 124 DAS ('R5').

Green maize plant	Availability	Treatment (kg ha ⁻¹)	SLA (dm ⁻² dia ⁻¹)	LWR (g/g)	RGR (g g ⁻¹ dia ⁻¹)	NAR (g dm ⁻² dia ⁻¹)	LAR (dm ⁻² dia ⁻¹)
AG4051	Shoot	0	0.35 ± 0.06 ^a	0.95 ± 0.01 ^a	0.019 ± 0.001 ^a	0.87 ± 0.40 ^a	0.24 ± 0.15 ^a
		0.13	0.31 ± 0.02 ^a	0.95 ± 0.008 ^a	0.019 ± 0.0008 ^a	0.74 ± 0.13 ^a	0.27 ± 0.04 ^a
		0.26	0.31 ± 0.06 ^a	0.94 ± 0.01 ^a	0.021 ± 0.001 ^a	0.80 ± 0.13 ^a	0.33 ± 0.05 ^a
		0.39	0.34 ± 0.10 ^a	0.95 ± 0.01 ^a	0.019 ± 0.008 ^a	0.67 ± 0.11 ^a	0.28 ± 0.06 ^a
		0.52	0.38 ± 0.07 ^a	0.96 ± 0.005 ^a	0.019 ± 0.002 ^a	0.66 ± 0.18 ^a	0.25 ± 0.05 ^a
		F	0.84 ^{ns}	0.84 ^{ns}	2.27 ^{ns}	0.78 ^{ns}	0.95 ^{ns}
		CV (%)	20.47	1.26	6.73	29.67	30.70
	Root	0	0.4576 ± 0.05 ^a	0.9598 ± 0.02 ^a	0.021 ± 0.001 ^a	1.201 ± 0.15 ^a	0.030 ± 0.01 ^a
		100	0.5220 ± 0.07 ^a	0.9518 ± 0.12 ^a	0.019 ± 0.002 ^a	1.090 ± 0.19 ^a	0.026 ± 0.004 ^a
		500	0.4782 ± 0.11 ^a	0.9454 ± 0.03 ^a	0.020 ± 0.002 ^a	1.226 ± 0.33 ^a	0.025 ± 0.006 ^a
		1000	0.4598 ± 0.05 ^a	0.9446 ± 0.02 ^a	0.022 ± 0.001 ^a	1.415 ± 0.13 ^a	0.023 ± 0.002 ^a
		2000	0.4898 ± 0.06 ^a	0.9502 ± 0.02 ^a	0.0204 ± 0.003 ^a	1.228 ± 0.27 ^a	0.023 ± 0.004 ^a
		F	0.56 ^{ns}	0.29 ^{ns}	1.09 ^{ns}	1.28 ^{ns}	0.85 ^{ns}
		CV (%)	16.02	2.68	10.91	18.75	26.45
Shoot	0	0.40 ± 0.14 ^a	0.96 ± 0.007 ^a	0.019 ± 0.001 ^a	0.56 ± 0.06 ^a	0.30 ± 0.07 ^a	
	0.13	0.28 ± 0.08 ^a	0.95 ± 0.009 ^a	0.020 ± 0.0006 ^a	0.73 ± 0.18 ^a	0.34 ± 0.06 ^a	
	0.26	0.38 ± 0.06 ^a	0.94 ± 0.01 ^a	0.019 ± 0.002 ^a	0.57 ± 0.20 ^a	0.36 ± 0.09 ^a	
	0.39	0.26 ± 0.12 ^a	0.97 ± 0.01 ^a	0.022 ± 0.003 ^a	0.81 ± 0.06 ^a	0.56 ± 0.52 ^a	
	0.52	0.29 ± 0.08 ^a	0.95 ± 0.01 ^a	0.021 ± 0.002 ^a	0.82 ± 0.22 ^a	0.43 ± 0.19 ^a	
	F	1.78 ^{ns}	2.25 ^{ns}	1.90 ^{ns}	2.88 ^{ns}	0.79 ^{ns}	
	CV (%)	31.79	1.35	11.28	23.17	64.19	
CATIVERDE 02	Root	0	0.4705 ± 0.06 ^a	0.9650 ± 0.008 ^a	0.0194 ± 0.002 ^a	1.182 ± 0.30 ^a	0.025 ± 0.005 ^{ab}
		100	0.6663 ± 0.44 ^a	0.9520 ± 0.019 ^a	0.0181 ± 0.001 ^a	0.912 ± 0.33 ^a	0.032 ± 0.006 ^a
		500	0.4538 ± 0.06 ^a	0.9499 ± 0.22 ^a	0.0194 ± 0.001 ^a	1.195 ± 0.16 ^a	0.025 ± 0.002 ^{ab}
		1000	0.4338 ± 0.10 ^a	0.9445 ± 0.019 ^a	0.0209 ± 0.002 ^a	1.405 ± 0.45 ^a	0.025 ± 0.004 ^{ab}
		2000	0.3869 ± 0.11 ^a	0.9499 ± 0.03 ^a	0.0194 ± 0.001 ^a	1.405 ± 0.32 ^a	0.023 ± 0.004 ^b
		F	1.24 ^{ns}	0.64 ^{ns}	1.56 ^{ns}	1.89 ^{ns}	2.61 ^{ns}
		CV (%)	44.83	2.25	9.15	27.10	19.01

Means followed by the same letter in the column do not differ by Tukey's test at 5% probability. ($n = 5$, \pm standard error).

noticed in environmental conditions without stress (Tamai and Ma, 2008). Similarly, maize plants

evaluated in a stress-free field conditions obtained an increase in the photosynthetic rate and

stomatal conductance, nevertheless, a decrease in transpiration rate and internal carbon

concentration in the leaf substomatal chamber (Xie et al., 2014).

We found studies that report Si positive effects occurred in stressful field conditions to the plants, for example, salt stress, drought stress, nutrient imbalance, presence of heavy metal (Ali et al., 2013) and inoculation of the pathogenic fungus (Polanco et al., 2014; Etesami, 2018). Phytoremediation plants treated with Si also presented positive results in the leaf gas exchanges and the photosynthetic performance of higher plants results directly in biological productivity during plant development; for example, rice plants under arsenic (As) cultivation (Sanglard et al., 2016) and green maize under cadmium (Cd) cultivation (Vaculík et al., 2015). On the other hand, in non-stressing field conditions, for potato plants (*Solanum tuberosum* L.), it was verified that the transpiration rate increased in response to Si applied through soil and foliar, due to its favorable effect in the stomatal conductance with improvements in the photosynthetic processes of plants cultivated with Si availability (Pilon et al., 2013).

Considering our results, we did not report differences on plant growth, however, the effects of Si on reducing cadmium (Cd) toxicity evaluated in tobacco (*Nicotiana tabacum* L.) show that the application of Si favored the plant growth once, and that the Cd element non-essential is a limiting factor (phytotoxicity) especially about the growth of plants (Lu et al., 2017). In the angiosperms clades, most species, particularly eudicots, are unable to accumulate elevated levels of Si and the difference in Si accumulation between species has been attributed to Si uptake ability of the roots.

Currently, different Si uptake mechanisms between monocotyledons and eudicotyledons plant species are reported in the literature. The rice plants, a monocot species, *Cucumis sativus* L. (cucumber) and *Solanum lycopersicum* L. (tomato), both eudicots species, show a similar ionic carrier transporting Si from the external solution to the cortical cells, with the same K_m value; however, the different V_{max} suggests that the density of ionic carrier differs from all three species (Mitani and Ma, 2005).

In this way, it seems like the transport of Si from cortical cells to the xylem vessels shows that the Si concentration in the xylem sap is much higher in monocots species than in eudicots species; however, Si xylem loading is mediated by passive transport (without energy expenditure) or absence of carrier, to transport Si from cortical cells to the xylem vessels in eudicots species unlike in monocots species, where xylem loading of Si is mediated by membrane-specific ionic carriers (active transport or with energy expenditure) (Mitani et al., 2009). These results provide a powerful predictive tool to classify plants on the basis of their natural capability to take up Si from the soil so that a spacing of a specific length between the two NPA domains is a

necessary and selective feature for Si among all Si-transporting plants.

On the other hand, Deshmukh et al. (2015) provide an accurate and clarified molecular basis to classify eudicots plants into accumulators of Si. Plant species that possess nodulin intrinsic proteins (NIPs), a subclass of aquaporins (AQPs) with a precise distance of 108 amino acids (AA) among the asparagine–proline–alanine (NPA) domains is fundamental to absorb Si because tomato NIP gene mutated from 109 to 108 AA exhibited a rare gain of function. Scientific evidence suggests AQPs with specific characteristics will filter Si in (Si accumulator) or out (Si excluder) of plants.

Indeed, the Si fertilization used in the eudicotyledons plant species under field conditions have lower or no accumulate in plant tissues, for example, tomato (*Solanum lycopersicum* L.) and mango (*Mangifera indica* L.), respectively. However, monocotyledons plant species, such as rice and sugarcane present a significant tissue leaf Si transported from the external solution to the cortical cells (Ma and Yamaji, 2015; Helaly et al., 2017) increasing plant growth parameters such as relative growth rate and CO₂ net assimilation rate.

Plant physiologists use the analysis of growth data in the different development stages to monitor the increased organic matter by photosynthetic activity, and the growth analysis provides a plausible study of the physiological activities of the plant influenced by the edaphoclimatic conditions to which the plant is cultivated. The relative growth rate (RGR) is established by the accumulation of plant biomass in a given period due to the greater photosynthetic performance (NAR), biomass leaf (SLA) or both.

In addition, the literature conceptualizes as part of the importance for the RGR, the leaf weight ratio (LWR), emphasizing in all cases the need for nutrients and light to plants, however do not describe if this conditioning rule is equated with beneficial mineral elements, for example, Si. The NAR is dependent of individual changes or the whole on photosynthesis and cellular respiration (Li et al., 2016) which directly impacts the leaf area useful for photosynthesis (SLA) (Skidmore et al., 2015). Moreover, Si shoot and root supplied increased the photosynthetic rate per leaf unit, even in conditions without stress (Pilon et al., 2013), which is the most rational way to explain the action of the environment on the capture of light and CO₂ assimilation for the plant; however, the response mechanisms in crop plants are not elucidated.

Although in the present study we did not report differences in the plant growth and physiological indexes, differences in plant biomass were observed, mainly related to the decline in the acquisition and use of nutrients under environmental conditions (Baret et al., 2017). In addition, greater leaf area per plant unit (LAR) was obtained in all treatments with Si applied via soil to the potato crop, but the authors did not present the

biochemical mechanisms that elucidate the role of Si in the studied plant physiology (Pilon et al., 2013).

Our study offers some advances in understanding the photosynthetic performance of maize plants (*Z. mays* L.) cultivated with Si availabilities applied via shoot and root. We reject our initial hypothesis and accepted alternative hypothesis that the beneficial element Si did not optimize the photosynthetic performance and biological productivity of green maize plants without nutritional stress.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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Full Length Research Paper

Genetic variability of yam based on quantitative descriptors

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Yam is a promising income source for small producers in the Recôncavo Baiano region. However, few genetic studies have been conducted with this culture. In this sense, the aim of this study was to assess genetic variability among 89 genotypes of yam (*Dioscorea rotundata* Poir.) collected in four municipalities in Bahia State, Brazil. These were assessed based on eight quantitative descriptors. Data were subjected to descriptive statistics, Spearman's rank correlation, Singh criterion, cluster analysis, principal component analysis and dispersion graph. The tuber weight showed the highest coefficient of variation and correlated positively and significantly with width and length of tuber. The tuber length characteristics contributed most to the genetic divergence. The criterion pseudo- t^2 divided 89 genotypes into seven groups, being that groups 4, 5 and 7 showed the highest averages for the production characteristics. The first three principal components explained 69.49% variation and dispersion graph showed partly concordant with UPGMA method. This study revealed the existence of genetic variability of yam, which may be explored in future genetic improvement programs.

Key words: *Dioscorea rotundata* Poir., multivariate methods, plant genetic resources, plant breeding.

INTRODUCTION

Yam (*Dioscorea rotundata* Poir.) is one of 600 species of Dioscoreaceae family, native from Africa and distributed through tropical and subtropical world regions (Lebot, 2009). It is a diploid plant ($2n=2x=40$) (Scarcelli et al., 2005), monocotyledon, dioecious and propagated vegetative, which produces tubers rich in carbohydrates (Lebot, 2009), which confer great economic importance.

The yam production in Brazil in 2014 was about 247,000 tons cultivated on 25,000 hectares (FAO, 2016). The largest production is concentrated in Northeast, where *D. rotundata* and *Dioscorea cayenensis* occupy 90% of production (Silva et al., 2016). Bahia State is the third-largest producer and Recôncavo region represents the largest cultivated area, including Maragogipe, São

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Table 1. Code, village, municipalities, geographic coordinates and number of 89 yam (*Dioscorea rotundata*) genotypes, collected in the Recôncavo da Bahia region (Cruz das Almas, BA, Brazil, 2016).

Code	Origin			Number	
	Village	Municipality	Latitude		Longitude
1	Tua	Cruz das Almas	12°43' S	39°03' W	1
2	Cadete	Cruz das Almas	12°42' S	39°04' W	1
3	Três Boca	Cruz das Almas	12°43' S	39°04' W	1
4 - 9	Camargo	São Felipe	12°44' S	39°03' W	6
10 - 15	Jaracandá	São Felipe	12°44' S	39°03' W	6
16 - 21	Bom Gosto	São Felipe	12°45' S	39°03' W	6
22 - 27	Boa Esperança	São Felipe	12°45' S	39°03' W	6
28 - 33	Campo das Flores	São Felipe	12°46' S	39°02' W	6
34 - 39	São Bento	São Félix	12°41' S	39°02' W	6
40 - 45	Engenho São João	São Félix	12°42' S	39°02' W	6
46 - 51	Monte Alegre	São Félix	12°40' S	39°02' W	6
52 - 57	Boa Vista	São Félix	12°42' S	39°02' W	6
58 - 63	Matatauba	São Félix	12°41' S	39°02' W	6
64 - 65	Serraria	Maragogipe	12°44' S	39°00' W	2
66 - 71	Campinas	Maragogipe	12°45' S	39°02' W	6
72 - 77	Encruzilhada	Maragogipe	12°44' S	38°54' W	6
78 - 89	Batatans	Maragogipe	12°44' S	39°02' W	12
Total	-	-	-	-	89

Felipe, São Felix and Cruz das Almas municipalities.

Despite its importance to family farming, yam has suffered biotic and abiotic stresses caused by fungal diseases, lack of propagative material of excellent quality, that threaten the genetic variability of these genetic resources and consequent culture abandonment. In this sense, it is necessary to conduct studies aiming at characterizing genetic variability existing in species of *Dioscorea* species genus, to identify superior genotypes to develop improved varieties that combine resistance to diseases and pests, and high productivity with attributes appreciated by consumers (Obidiegwu et al., 2009), such that contribute in maintaining sustainability of this culture agribusiness.

The characterization and assessment of genetic variability are fundamental activities in genetic improvement programs for several species of agronomic interest. However, these depend on the use of multivariate statistical methods. The principal component analysis, canonical variable analysis and cluster analysis are the most publicized techniques among breeders (Mohammadi and Prasanna, 2003; Sudré et al., 2007). The choice of method depends on research objectives and hypothesis to be tested (Mingoti, 2007), as well as the desired accuracy by researcher, ease of analysis and the way in which data were collected (Cruz et al., 2012). Among the many available clustering methods, unweighted pair group method with arithmetic mean (UPGMA) has been the most used, due to the present higher values of cophenetic correlation coefficients, which

measure the association between dissimilarity matrix and the cluster matrix (Mohammadi and Prasanna, 2003). For this reason, this method has been used in several studies that aim to characterize and assess the genetic variability of yams, such as those by Bressan et al. (2014) and Efiue (2015).

However, in other studies of genetic variability characterization of yams, UPGMA method has been used simultaneously with principal components and both are complemented by graphic dispersion analysis (Mwirigi et al., 2009; Norman et al., 2011; Michael, 2013; Mulualem and Michael, 2013; Siadjeu et al., 2015). These techniques are used in many studies of genetic variability characterization of yams (Mwirigi et al., 2009; Norman et al., 2011; Mulualem and Michael, 2013; Bressan et al., 2014; Efiue, 2015; Siadjeu et al., 2015). Thus, this study aimed to assess the genetic variability among genotypes of yam (*D. rotundata*), collected in four municipalities from the Recôncavo Baiano region, based on quantitative descriptors through multivariate methods.

MATERIALS AND METHODS

A total of 89 genotypes of yam were collected in 18 rural properties of villages belonging to the municipalities of Cruz das Almas, São Félix, São Felipe and Maragogipe, which are located in the region of Recôncavo Baiano. Collection points were georeferenced through global positioning system (GPS) (Table 1).

Genotype characterization was divided in two stages. First step took place in May and June 2015, in which four expeditions were conducted to villages in municipalities mentioned earlier (Table 1).

Table 2. Descriptive statistics and normality test of variables analyzed in 89 yam genotypes, Cruz das Almas, BA, Brazil, 2016.

Variable	Minimum	Maximum	Average	Standard deviation	CV (%)	Normality test
PL (cm)	3.00	7.60	4.92	1.03	20.92	0.97 ^{ns}
TL (cm)	14.10	39.30	25.19	5.74	22.80	0.97*
SD (mm)	0.20	0.60	0.43	0.10	23.62	0.91**
F1 (cm)	4.00	11.30	6.41	1.60	24.98	0.95**
TW (cm)	4.40	12.00	7.06	1.54	21.84	0.97*
P2 (cm)	9.30	19.80	14.34	1.93	13.46	0.99 ^{ns}
P3 (cm)	1.90	8.00	3.93	1.24	31.56	0.96**
WT (kg)	0.32	3.16	0.81	0.42	51.56	0.77**

**Significant 1% probability. *Significant 5% probability. ^{ns}Not significant at 5% probability by Shapiro-Wilk test.

In each rural property, six genotypes were selected at random, except in properties from Tua, Cadete and Três Boca, where only one genotype was selected and Serraria village, with two genotypes (Table 2). The genotypes were marked with a tape, which represented their identification (1 to 6), shoot (leaves and stems) were characterized using measuring tape and caliper. The second stage was made in December 2015 (two expeditions), where tubers were collected from genotypes, previously marked, bagged and transported to Food Technology Laboratory of Federal University of Recôncavo da Bahia (UFRB), and characterized with measuring tape and a scale.

The genotypes were characterized based on eight quantitative descriptors established by International Plant Genetic Resources Institute and International Institute of Tropical Agriculture (IPGRI/IITA, 1997): stem diameter (SD) in mm measured at 15 cm from ground, petiole length (PL) in cm, leaf width at the widest portion in cm (F1), distance among the petiole insertion in the leaf, the upper end (P3) and lower end of the leaf (P2) in cm, tuber length in cm (TL), tuber width in cm (TW) and tuber weight in kg (WT). Data of descriptors SD, PL, F1, P3 and P2 were collected at 90 days after planting (DAP) during vegetative growth of the plants, whereas TL, TW and WT were collected at 270 DAP when tubers were harvested.

Data values were introduced in a database and descriptive statistics: minimum and maximum values, average, standard deviation and coefficient of variation were calculated. Shapiro-Wilk test for normality was used at 5% of probability.

Genetic variability was estimated by means of cluster analysis and principal component analysis. For cluster analysis, the average Euclidean distance was used as a measure of dissimilarity from standardized data. The relative contribution of characters was determined by the method proposed by Singh (1981). The hierarchical clusters from the distance matrix were obtained by UPGMA method (unweighted pair-group method using arithmetic averages) (Sneath and Sokal, 1973). Validation of clusters was determined through the cophenetic correlation coefficient (r) (Sokal and Rohlf, 1962). The significance of the cophenetic correlation coefficient was calculated by the Mantel (1967) test with 1,000 permutations. The criterion for defining the cluster number was based on pseudo-t² index from package "NbClust" of R software (Charrad et al., 2014). Statistical analyses were made using GENES version 5.1 (Cruz, 2013) and R program version 2.2-6 software (R Core Team, 2014).

RESULTS AND DISCUSSION

Descriptive statistics for eight descriptors of 89 yam genotypes under study are shown in Table 2.

F1, Leaf width at widest portion; P2 and P3, distance among petiole insertion in leaf, the lower and upper leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter; WT, tuber weight.

The variation coefficient (CV) varied between 13.46 and 51.56%. The variables with the highest variation coefficients were WT and P3 with 51.56 and 31.56%, respectively. On the other hand, P2 presented the lowest variation coefficients with 13.46%. TL ranged from 14.1 to 39.3 cm and TW ranged 4.4 to 12.0 cm with an average of 7.06 cm. These results agree with those obtained by Anokye et al. (2014), in *Dioscorea alata* for TW, F1 and WT. In general, it was observed that production variables (weight, width and length of tuber) showed variability, expressed by their variation coefficient (CV). This variability can be exploited in improvement programs, since these features constitute the main objective in commercial exploitation of yams (Domingos et al., 2014).

The normality test revealed that SD, F1, P3 and WT were ($p < 0.01$) by Shapiro-Wilk test. On the other hand, TL and TW were ($p < 0.05$) by the same test, indicating that these variables do not follow normal distribution; therefore, Spearman's rank correlation was determined among variables.

Association among variables gives an indication to carry out an effective breeding program (Tena et al., 2016) being a useful tool for indirect selection. Table 3 shows Spearman's rank correlation coefficients among variables. The PL showed positive correlation ($p < 0.01$) with the F1 ($r_s = 0.64$), with P2 ($r_s = 0.51$) and P3 ($r_s = 0.30$). It was also observed that WT was positively correlated ($p < 0.01$) with the TW ($r_s = 0.70$) and TL ($r_s = 0.45$), indicating that increase of WT leads to increase of TW and TL consequently. Highly significant correlation among characters indicates the possibility of simultaneous genetic improvement among them (Osuagwu et al., 2014).

F1, leaf width at the widest portion; P2 and P3, distance among petiole insertion in leaf, the lower and upper leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter, WT, tuber weight.

According to Singh (1981) method, TL with 74.24%

Table 3. Spearman's rank correlation coefficient, with the respective significance of the analyzed variables (Cruz das Almas, BA, Brazil, 2016).

Variable	TL	SD	F1	TW	P2	P3	WT
PL	-0.24*	0.14 ^{ns}	0.64**	0.02 ^{ns}	0.51**	0.30**	-0.06 ^{ns}
TL		0.02 ^{ns}	-0.20 ^{ns}	-0.02 ^{ns}	-0.21 ^{ns}	-0.03 ^{ns}	0.45**
SD			0.05 ^{ns}	0.17 ^{ns}	0.10 ^{ns}	-0.20 ^{ns}	0.06 ^{ns}
F1				-0.16 ^{ns}	0.48**	0.37**	-0.20 ^{ns}
TW					-0.09 ^{ns}	-0.19 ^{ns}	0.70**
P2						0.21*	-0.25*
P3							-0.18 ^{ns}

**Significant 1% probability. *Significant 5% probability. ^{ns}Not significant at 5% probability by t test.

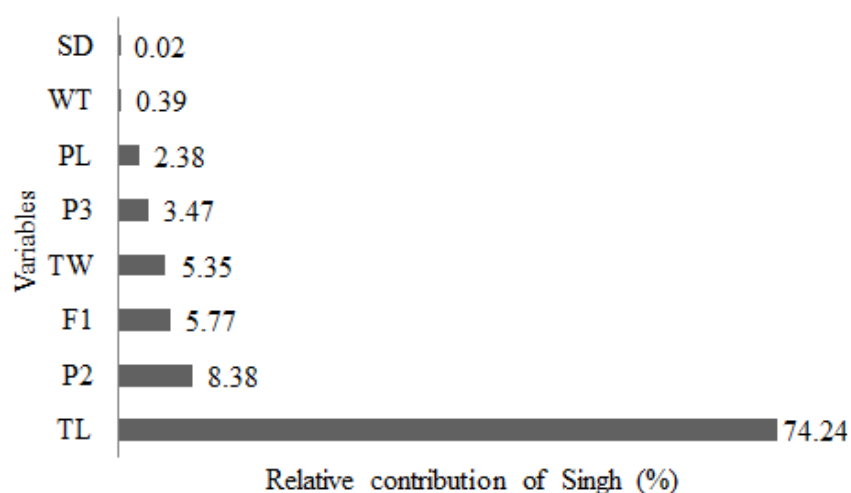


Figure 1. Relative contribution of the characters to diversity according to Singh (1981). F1, Leaf width at widest portion; P2 and P3, distance among petiole insertion in leaf, the lower and upper of the leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter; WT, tuber weight.

was the variable that showed the highest contribution to genetic dissimilarity among analyzed genotypes (Figure 1). In this sense, this variable must be prioritized to efficient choice of promising materials. However, Afonso et al. (2014) evaluating 209 yam genotypes, found that tuber length had less contribution, diverging this study. This difference is probably due to conditions management practiced by the producers. On the other hand, variables with little contribution were SD and WT, with 0.02 and 0.39%, respectively.

Cophenetic correlation coefficient (CCC) was 0.72, appropriate value according to Vaz Patto et al. (2004), considered ideal ($r \geq 0.56$), reflecting a good agreement between the distance of the dissimilarity matrix and cluster matrix.

Based on pseudo- t^2 index criterion, 89 yam genotypes were divided into seven clusters, by UPGMA method (Table 4). The number of clusters obtained in this study

was superior to those found by Beyene (2013), Bressan et al. (2014) and Siadjeu et al. (2015), thus revealing genetic variability existence of *D. rotundata* in Recôncavo Baiano region.

Group 3 combined the largest number of genotypes (39), being 16 from São Félix, 14 from São Felipe and 9 from Maragogipe. Group 2 were allocated 26 genotypes, 14 originating from Maragogipe, 7 from São Félix, 3 from São Felipe and two from Cruz das Almas. In turn, the group 1 consisted of 12 genotypes, 8 from São Felipe, 3 from São Félix and 1 from Cruz das Almas. Group 6 encompassed 4 genotypes, 3 collected in Maragogipe and the other in São Felipe. The groups 4 and 5 were composed of 3 genotypes, respectively, being that the group 4 included genotypes from São Felipe, while the group 5 was formed by two, which were obtained from São Félix and the other from São Felipe. The group 7 consisted of only two genotypes from São Félix.

Table 4. Cluster of 89 genotypes of yam (*Dioscorea rotundata* Poir.) collected in four municipalities of Recôncavo Baiano, in accordance with UPGMA method, based on eight quantitative descriptors, Cruz das Almas, BA, Brazil, 2016.

Cluster	Number of genotypes	Genotypes
1	12	1, 4, 5, 9, 10, 12, 15, 16, 17, 37, 40, 47
2	26	2, 3, 25, 27, 33, 35, 42, 44, 45, 51, 57, 62, 65, 67, 68, 69, 71, 74, 77, 78, 79, 83, 85, 86, 88
3	39	6, 7, 8, 18, 19, 20, 21, 22, 23, 24, 28, 29, 30, 32, 34, 36, 38, 39, 41, 46, 48, 49, 50, 55, 56, 58, 59, 60, 61, 63, 64, 66, 72, 73, 75, 76, 81, 84, 87
4	3	11, 13, 14
5	3	26, 53, 54
6	4	31, 70, 80, 89
7	2	43, 52

Table 5. Averages of eight quantitative variables from seven clusters formed by 89 yam genotypes. Cruz das Almas, BA, Brazil, 2016.

Variable	Groups						
	G1	G2	G3	G4	G5	G6	G7
SD (mm)	0.34	0.48	0.42	0.33	0.47	0.38	0.55
PL (cm)	3.93	5.80	4.51	5.37	3.67	6.85	4.50
P3 (cm)	4.53	4.20	3.33	7.00	2.53	5.90	2.80
P2 (cm)	13.62	15.39	14.11	10.97	11.80	18.33	10.90
F1 (cm)	5.72	7.74	5.73	5.30	4.23	9.20	5.60
TL (cm)	31.73	22.86	24.34	35.30	19.80	20.20	37.15
TW (cm)	6.53	7.05	6.89	6.50	9.53	7.05	10.85
WT (kg)	0.74	0.74	0.73	1.26	1.16	0.69	2.58

F1, Leaf width at widest portion; P2 and P3, distance among petiole insertion in the leaf, the lower and upper of the leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter; WT, tuber weight.

These results indicate that there was no relationship between geographic origin and genetic diversity, since genotypes from different municipalities were gathered within same group. This fact is probably due to existence of gene flow resulting from changes of propagative materials among producers of different communities within study area. Similar results were verified by Domingos et al. (2014), Bressan et al. (2014) and Nascimento et al. (2015).

The most dissimilar genotypes, with genetic distance of 0.68 were 31 and 52 (data not shown), being the first from São Félix and the second from São Felipe. Under the agronomic point of view, both genotypes showed the highest values, as for tuber length (35.10 and 39.20 cm), as well as for weight (2.01 and 3.16 kg). The most similar genotypes, with genetic distance of 0.06 were 30 and 32 (data not shown), both belonging to Campo das Flores village, in São Felipe municipality. These genotypes can be considered as clones, since they presented similar values in all analyzed variables. The presence of possible duplicates of *D. alata* L. in the same municipalities was

also observed by Bressan et al. (2011).

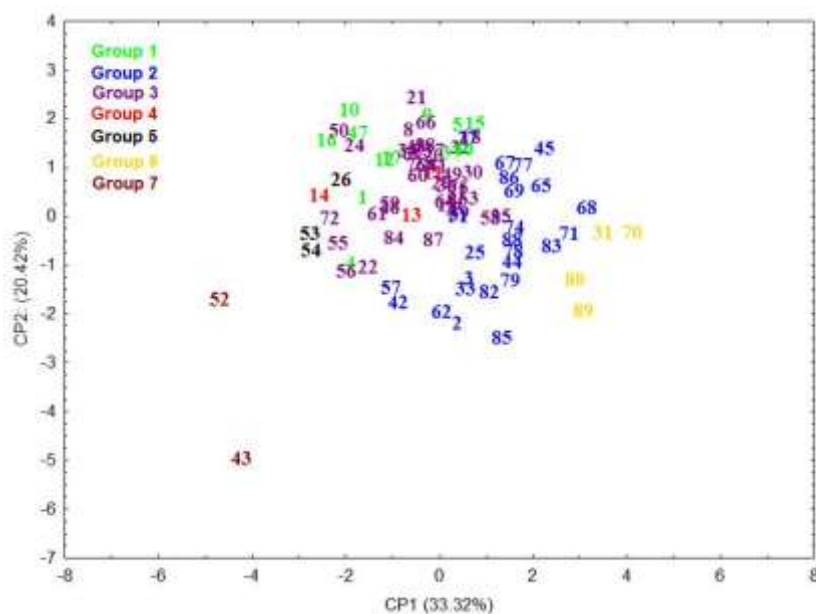
The variables averages of seven groups are shown in Table 5. It is observed that groups 4, 5, and 7, are more promising, due to higher averages for WT, whereas groups 1, 2, 3 and 6 were characterized by smallest averages for WT. Group 6 had the greatest for traits PL, P3, and F1. On the other hand, group 7 showed the greatest traits of SD, TL, TW and WT. As stated by Santos (1996), groups 1, 2, 3, 4, and 5 have tubers with export potential for United States market, while group 7 can be exported to France. Thus, this information is useful in selection of potential genotypes to be used in future genetic improvement programs of yams, aiming to develop cultivars with superior characteristics and meet market demand, as well as provide better incomes for producers in Recôncavo Baiano region, selling quality tubers.

Table 6 presents the estimates of eigenvalues associated with each variable and their total and cumulative variances. It is observed that first three principal components were able to explain only 69.49% of

Table 6. Estimates of eigenvalues associated with each variable and their total and cumulative variances, obtained in the principal component analysis of 89 yam genotypes. Cruz das Almas, BA, Brazil. 2016.

Variable	Principal components							
	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
SD	-0.066	-0.483	0.548	-0.587	-0.338	0.047	-0.030	0.020
PL	0.704	-0.508	-0.114	0.016	0.019	-0.130	0.455	-0.093
P3	0.449	-0.004	-0.711	-0.006	-0.498	0.193	-0.080	0.029
P2	0.749	-0.257	0.137	-0.014	0.331	0.481	-0.083	0.078
F1	0.753	-0.326	-0.142	-0.109	0.143	-0.391	-0.348	-0.010
TL	-0.527	-0.090	-0.560	-0.533	0.287	0.091	0.004	-0.163
TW	-0.438	-0.724	0.070	0.429	-0.083	0.112	-0.155	-0.227
WT	-0.605	-0.662	-0.292	0.078	0.088	-0.065	0.046	0.301
Total variance (%)	33.32	20.41	15.75	10.39	7.37	5.83	4.60	2.31
Cumulative variance (%)	33.32	53.73	69.49	79.89	87.26	93.08	97.69	100.00

F1, Leaf width at widest portion; P2 and P3, distance among petiole insertion in the leaf, the lower and upper of the leaf; PL, petiole length; TL, tuber length; TW, tuber width; SD, stem diameter; WT, tuber weight.

**Figure 2.** Dispersion graph of 89 yam genotypes based on the first two principal components (CP1 and CP2), according to the groups formed by the UPGMA method.

total variation. The first principal component explained 33.32% of the total variance and was associated with the PL, P2 and F1.

The second and third principal components, with explanation of 20.41 and 15.75% of the total variation, were associated with TW and P3, respectively. These results corroborate with those by Norman et al. (2011) and Muluaem and Michael (2013), in which the first three principal components explained 58 and 65% of total variation, respectively.

According to Cruz et al. (2012), dispersion graph provides satisfactory results when first two principal

components explain 80% of total variation. For this reason, dispersion graph representation of 89 yam genotypes does not only explain variability satisfactorily, since the two first principal components explained only 53.73% of the total variation (Figure 2). The groups formed by dispersion graph were partially consistent with those formed by UPGMA method. Figure 2 shows that there has not been a clear separation of clusters. However, group 7, with only two genotypes, was the most distant from others, corroborating with results obtained in cluster analysis.

The information obtained in this study can contribute to

conservation and genetic improvement of yams in edaphoclimatic conditions from Recôncavo Baiano region. However, molecular characterization of these genotypes has become necessary, to supplement information generated by morpho agronomic descriptors.

Conclusion

There is genetic variability of yams liable to exploitation in programs of conservation and genetic improvement. The tuber length was the variable with most contribution for the genetic divergence.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physiological responses of operators to handle vibration of diesel-fueled single-axle tractor

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Hand-arm transmitted vibration originating from a 15 horsepower single-axle tractor can be very strong and cause operator fatigue plus various physiological disorders in vascular, neurological, and musculoskeletal systems. The objective of this work was to measure the characteristics of such hand-transmitted vibrations and its effect on the health of the single-axle tractor operator, in a research program conducted at the Melkasa Agricultural Research Center of Ethiopia. During the study, the measurement of anthropometric data and the heart rate of the operators and vibration at the handle of single axle tractor were conducted. From measured data, the requirement of expenditure energy, physical workload, and operators' daily vibration exposure was calculated. The average measured resting heart rate, working heart rate and heart rate after the operation was 71 ± 7 , 162 ± 4 and 126 ± 24 beats/min, respectively. The calculated values of HR reserve (%HRR) or physiological workload and the ratio of working to resting heart rate were $74\pm 10\%$ and 2.3 ± 0.4 ($p < 0.0001$), respectively. The total energy expenditure of single-axle tractor operators was calculated to be 35 ± 1.2 kJ/min. The daily exposure m/s^2A (8) and total exposure points which were calculated indirectly from measured vibrations at the tractor handle and duration of operation were $12.6 m/s^2$ and 2520 points, respectively. From measurements of operators' heart rates, it was concluded that the physical workload fell into the category of extremely heavy work, for which the working heart rate more than doubled the resting heart rate ($p < 0.0001$).

Key words: Tractor vibration, hand-transmitted vibration, single-axle tractor, heart rate, physical workload, vibration exposure.

INTRODUCTION

Currently, the government of Ethiopia supports both agricultural machinery importers and domestic

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agricultural machinery manufacturers, to promote food security by increasing agricultural productivity. The percentage of a country's population employed in agriculture has a tendency to decrease with the advancement of national economic development, due to the increase in secondary and tertiary industries. This progression is supported by increased utilization of large-, medium- and small-scale farm machinery that promotes increased agricultural productivity (Sakai, 1990). Therefore, single-axle tractors are very important for small-scale farmers in the drive to shift from animal-powered farming to engine-powered farming systems. Single-axle tractors are multipurpose machines designed primarily for rotary tilling and other farm operations. A single-axle tractor is also known as a hand-tractor, walking-tractor, walk-behind tractor, etc., (Sakai, 1999). When a tillage implement is attached to a single axle tractor, the system is called a power-tiller. Mechanical vibration arises from a wide variety of processes and operations performed in industry, mining, construction, forestry, agriculture, and public utilities. Whole-body vibration occurs when the human body is supported on a surface which is vibrating, e.g. in most forms of transport and in close proximity to some types of industrial machinery. Hand-transmitted vibration occurs when the vibration enters the body through the hands, typically in various work processes where rotating or percussive power tools or vibrating workpieces are held by the hands. The human response to vibration depends mainly on the magnitude, frequency, and direction of the vibration signal (Griffin, 1990).

Mechanical vibration is the oscillatory motion of an elastic solid (or liquid) body about a reference position. All bodies with mass elements and elasticity are capable of vibration; hence, most machines and structures including the human body can experience vibration. According to Griffin (1990), human response vibration depends mainly on the magnitude, frequency, and direction of the excitation source. In the ISO Standard (ISO-5349, 2001) for measurement and evaluation of human exposure to hand-transmitted vibration, the most important quantity used to describe the magnitude of the vibration transmitted to the operator's hands is the root-mean-square (RMS) frequency-weighted acceleration expressed in m/s^2 . The RMS magnitude is correlated with the vibration energy, and hence the vibration injury potential or destructive capability over a period of time. The hand-transmitted vibration of a single-axle tractor is very strong because the handle grip is a cantilever beam, with the excitation power obtained from the one-cylinder diesel engine (Ying et al., 1998). The low-frequency vibrations to which the operator is subjected result from both linear displacements of the single-axle tractor and rotational oscillations of the pitch and roll modes (Mehta et al., 1997). The four principal effects of ride vibrations

are considered to be degraded health, impaired activities, impaired comfort and motion sickness. Daily exposure to hand-arm vibrations over a number of years can cause permanent physical damage known as 'white finger syndrome' or can damage the joints and muscles of the wrist and the elbow. White finger syndrome in its advanced stages is characterized by blanching of the extremities of the fingers, which is caused by damage to the arteries and nerves in the soft tissues of the hand (Shen and Ronald, 2017). Griffin et al. (2003) measured hand-transmitted vibration on representative samples of the vibratory tools used by the subjects in several study populations. Raw vibration magnitudes were expressed as root-mean-square acceleration values over the frequency range of 6.3 to 1250 Hz. Amplitude data were also presented using the W_h frequency weighting system that places a larger weight on frequencies that pose a higher biological risk (ISO-5349, 2001). For each tool used in the study, vibration measurements were made at each hand position in three orthogonal directions: x, y, and z as shown in Figure 1. In each case, the greatest of the three magnitudes was selected to represent the vibration magnitude of the tool. According to ISO 5349 (2001) for most power tools, the vibration entering the hand contains contributions from all three orthogonal axes of motion.

The frequency-weighted RMS acceleration values for the x-, y- and z-axes, a_{hwx} , a_{hwy} , and a_{hwz} respectively, are reported separately using the frequency weighting graph. The one-third-octave band frequencies from 6.3 to 1250 Hz constitute the primary frequency range and the calculated a_{hw} includes all one-third-octave bands within this range, where:

$$a_{hv} = \sqrt{a_{hwx}^2 + a_{hwy}^2 + a_{hwz}^2} \quad (1)$$

Frequencies outside the primary range do not generally make an important contribution to the value of a_{hw} and may be excluded from the calculation (ISO-5349, 2001). The evaluation of daily vibration exposure is based on the vibration magnitude a_{hv} and the daily exposure duration. In order to facilitate comparisons between daily exposures of different durations, the daily vibration exposures are expressed in term of 8-h energy-equivalent frequency-weighted vibration total value, $a_{hv(eq,8h)}$, as shown in Equation 2. For convenience $a_{hv(eq,8h)}$ is given the shorthand notation of $A(8)$ (ISO-5349, 2001):

$$A(8) = a_{hv} \sqrt{\frac{T}{T_0}} \quad (2)$$

where T = the total daily duration of exposure to the

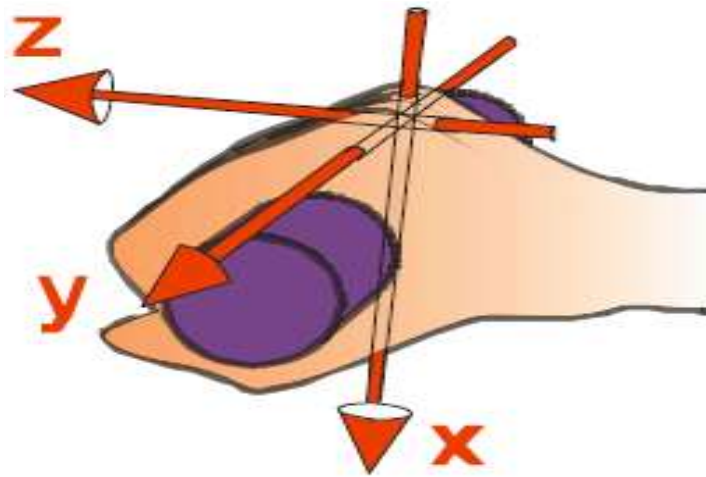


Figure 1. Three orthogonal directions of vibrational motion (ISO-5349, 2001).

vibration, called “trigger time”; T_0 = the reference duration of duration 8 h.

Studies by Muhammed et al (2003) have shown that the heart is a great information processor. Resting heart rate is measured under the condition of no physical exertion. It is best measured in the morning before any stress, caffeine or much movement. Maximum heart rate (HR_{max}), on the other hand, is an estimate of heart rate that someone could (not should) achieve during maximum physical exertion. A rough estimate of a person’s HR_{max} can be obtained by subtracting the person’s age from 220 (Vonda and Ruth, 2009).

$$HR_{max} = 220 - \text{age (in years)} \tag{3}$$

Several studies have been conducted to determine the physical workload on an operator imposed by various machines (Muzammil et al., 2004; Çaliskan and Çağlar, 2010; Eroglu et al., 2015). Sarzynski et al. (2013) suggested that the maximum workload is roughly indicated by achieving 85% of age-predicted maximum heart. Percentage Relative Heart Rate (%HRR) can be determined by applying the formula (Çaliskan and Çağlar, 2010; Vitalis, 1987):

$$\%HRR = \frac{HR_w - HR_r}{HR_{max} - HR_r} * 100 \tag{4}$$

where HR_w = average working heart rate, bpm; HR_r = resting heart rate, bpm; HR_{max} = maximum heart rate, bpm.

According to several studies (Çaliskan and Çağlar, 2010; Eroglu et al., 2015; Lammert, 1972), the half of

heart rate reserve (50% level) of the working heart rate was determined by using the formula:

$$50\%level = HR_r + \frac{HR_{max} - HR_r}{2} \tag{5}$$

Last, the ratio of working heart rate (HR_w) to resting heart rate (HR_r) is obtained from (Çaliskan and Çağlar, 2010; Eroglu et al., 2015):

$$Ratio = \frac{HR_w}{HR_r} \tag{6}$$

Basal metabolic rate (BMR) is the minimal rate of energy expenditure compatible with life. It is measured in the supine position under standard conditions of rest, fasting, immobility, thermoneutrality and mental relaxation. Depending on its use, the rate is usually expressed per min/h or per 24 h (FAO et al., 2001). Several studies have been conducted by different researchers on regarding the amount of energy required by an operator using various types of machines (Pawar and Pathak, 1980; Tiwari and Gite, 2002; Katherivel et al., 1991), the BMR was then evaluated by several researchers for assorted population samples and activities (Kroemer and Grandjean, 2000). Borah (2015) has evaluated the physiological workload of hill farm women of Meghalaya and determined heart and energy expenditure. Çaliskan and Çağlar (2010) have classified the grade of physical work as resting, very light work, moderate work, heavy work, very heavy work and extremely heavy work based on the data of energy expenditure, heart rate, and physical workload. Food and Agriculture Organization of

the United Nations (FAO), World Health Organization (WHO) and United Nations University (UNU) (FAO/WHO/UNU) expert consultation (WHO, 1985) estimated the requirement of energy expended by adults as multiples of BMR (WHO, 1985). This was later called "physical activity level" (PAL) in a manual commissioned by FAO for the calculation of human energy expenditure (FAO, 2001). PAL is defined as the total energy required over 24 h divided by the basal metabolic rate over 24 h (James and Schofield, 1990). Schofield estimated the basal metabolic rate as $0.063 W + 2.896$ in ± 0.641 MJ/day or $15.057 W + 692.2 \pm 153$ kcal/day, where W is the weight (kg) of adults in the age range of 18 to 30 years old (FAO, 2001; Schofield, 1985). Total energy expenditure is given by $BMR \times PAL$, where the value of PAL for different physical activities is given in the report of FAO (2001).

The hand-arm vibration exposure calculator enables a quick determination of the number of exposure points for every hour of exposure time for any given individual process; this then yields the time to reach the vibration Exposure Action Value (EAV) (equal to 2.5 m/s^2 of A (8) or 100 points) and time to reach the vibration Exposure Limit Value (ELV) of 5 m/s^2 of A (8) or 400 points. The calculator also calculates the Partial Exposure, which is the vibration exposure (shown in both A (8) in m/s^2 and exposure points) for the process and is calculated from the vibration magnitude and the exposure duration. The daily exposure of A (8) in m/s^2 and total exposure points can be calculated by summing the Partial Exposures (HSE, 2005). This document also offers suggestions with respect to reducing risks posed by high vibration amplitudes.

A word of explanation on notation: The action value was set at a vibration magnitude of 2.5 m/s^2 and the limit value to 5 m/s^2 . Both of these figures are A(8) values, meaning they are average vibration magnitudes over the course of an 8-h workday. According to the European Commission Directives (2002) when the EAV is reached, a program of measures to eliminate or reduce exposures to hand-arm vibration must be implemented. When the ELV is reached, immediate action to prevent exposure above the limit value must be taken, and the reasons for exceeding the ELV must be identified (Commission, 2002). According to the European Directive 2002/44/EC (Commission, 2002) on the minimum health and safety requirements regarding the exposure of workers to vibration, hand-arm vibration exposure management can be simplified by using an exposure "points" system. Exposure points are simply added together, so the maximum number of exposure points can be set for a person in one day. The exposure scores corresponding to the exposure action and limit values are (Commission, 2002) exposure action value ($2.5 \text{ [m/s}^2\text{]}) = 100$ points (maximum points for exposure action value (EAV)) and

exposure limit value ($5 \text{ [m/s}^2\text{]}) = 400$ points (maximum points for exposure limit value (ELV)).

Dong (1996), in his study on vibration transmitted by the handles of a GN-5 type model walking tractor, concluded that the main cause of vibration was the engine. Also, he found that the vibration on the handles of the GN-5 walking tractor was very strong and seriously affected the operator's health. Mehta et al. (1997) measured ride vibrations on a single axle tractor with a seating attachment under various operating conditions and compared them with the values specified under ISO-2631 (1985) in relation to working efficiency, health, and safety of the operator. They found that exposure time for the single axle tractor should not exceed 2 to 5 h per day for rototilling operations. Mamansari (1998) reported that the vibration level of a single-axle tractor increased with an increase in engine speed in both the stationary and transport modes; the vertical vibration component varied significantly in various parts of the tractor handle and was highest at the handle tip. Ying et al. (1998) reported that the major source of vibration excitation of Hand-Transmitted Vibration (HTV) of a walking tractor is the engine, and found that the highest amplitude of vibration was in the x (vertical) direction. The main objective of this paper was to measure and analyze the hand-transmitted vibration characteristics of a 15 horsepower single-axle tractor, in terms of amplitude, the orientation of motion, and safety level according to international standards.

MATERIALS AND METHODS

Experimental layout

The evaluation of vibration characteristics of hand-arm transmitted vibration originating from a single-axle tractor was conducted at the Melkasa Agricultural Research Center (MARC), located at 117 km East of Addis Ababa. The region features arid to semi-arid agro-ecological zones, at an altitude of approximately 1550 m above sea level with temperatures generally ranging from 14 to 28°C , average annual rainfall of 750 to 800 mm, Length of Growing Period (LGP) of 3 to 6 months, and sandy soil of volcanic origin with pH ranging from 7 to 8.2.

Equipment

A single-axle 15HP Dong Feng tractor with the implement, model 2b-DF-15L with a horizontal 4-stroke diesel engine with a crankshaft turning speed of 2000 rpm was used for all data collection. A model VM-6380 vibration meter Tester (M&A Instruments, Arcadia, CA) with a 3-axis piezoelectric accelerometer vibration sensor (Figure 2) was used for vibration measurements. The sensor bandwidth of 10Hz to 10 kHz includes the relevant frequency range of 6.3 to 1250 Hz for this type of assessment (ISO-5349, 2001).

The heart rate of each tractor operator was measured by a Polar T31 Coded Heart Rate Transmitter and Belt Set, using high precision bluetooth heart rate sensors connected to a Polar A370 fitness tracker manufactured by the Polar Global Company (Figure 3). The Polar heart rate receiver component (receiver wirelessly)



Figure 2. (a) VM-6380 3-Axis 3D Digital Vibration Meter Tester. (b) Field measurement of vibration characteristics.



Figure 3. Polar T31 Coded Heart Rate Transmitter and Belt Set with Polar A370



Figure 4. Finger Pulse Oximeter with digital blood pressure monitor (Contec Medical Systems Co., Ltd. China).

receives the heart rate signal from the T31 Polar coded transmitter belt, which is slim, light and waterproof. The transmitter, worn around the chest of operators transmits a pulse corresponding to

each heartbeat.

A Contec CMS-08A digital blood pressure monitor with finger pulse Oximeter shown in Figure 4 was used to measure blood

pressure and oxygen saturation of the single-axle tractor operators.

Study subjects and tasks

Melkasa Agricultural Research Center is the federal research center which is working on agricultural mechanization research to improve the living standard the farmers. For its material and research facilities this place was selected for this research work. All the operators were from this research center and they have been trained how to operate the single axle tractor and they have operated several times before the test was conducted.

The mean and standard deviation of anthropometric data of age, height, weight, and body mass index (BMI) of the seven operators of the single-axle tractor was found to be 26 ± 3 years, 170 ± 8 cm, 55 ± 4 kg and 19 ± 1 kg/m², respectively.

Seven male subjects participated as operators of the 15-HP single-axle tractor. The operators performed tilling tasks on previously tilled land with the tractor in third gear, while the vibration levels were measured at the tractor handle along the x (vertical), y (forward), and z (transverse) axes. Note that the y- and z-directions were switched from those shown in Figure 1 which corresponds to the ISO 5349 (ISO-5349, 2001) because the handle on this particular tractor model operator was oriented at 90 degrees from that shown in Figure 1. The heart rates of the single-axle tractor operators were measured and recorded before the operation, during operation at 5-min intervals for 25 min and 5 min after completion of tractor operation. The blood pressure and oxygen saturation levels of the operators were also measured at those times.

RESULTS AND DISCUSSION

Vibration data

The 3-axis piezoelectric accelerometer vibration sensor was attached at the handle of the single-axle tractor, adjacent to the hand of the operator. An RS-232C data cable link connected the sensor to a laptop computer running the XP Windows operating system, to record the vibration data. The mean and standard deviation of measured vibration values on three axes were 22.7 ± 2.85 , 11.4 ± 4.44 and 20.90 ± 2.26 m/s² in x, y and z directions, respectively. The calculated vibration total value a_{hv} and vibration daily exposure A (8) in m/s² (calculated using Equation 2) at the handle of the single axle tractor averaged 32.9 and 12.6 m/s², respectively.

Evaluation of vibration exposure

From calculated values of a_{hv} , the vibration exposure was determined according to the Control of Vibration at Work Regulations that are based on the 2002 EU Physical Agents (Vibration) Directive (Commission, 2002). Vibration total value and daily exposure of A(8) in m/s² were calculated using Equations 1 and 2. The vibration daily exposure of A(8) was also calculated from vibration total value and duration of exposure by using the Hand-Arm

Vibration Exposure Calculator designed by the Health and Safety Executive (HSE, 2005) shown in Figure 5.

Based on the exposure calculator, the vibrations picked up by the operator's hands will reach the EAV of 2.5 m/s² of A (8) after only 3 min and ELV of 5 m/s² of A (8) after only 11 min.

The calculated daily exposure of 12.6 m/s² of A (8) is well above the Action Limit Value (5 m/s²); the 2520 Total Exposure points are also well above the limit of 400 points. The data indicate that safe vibration limits are routinely exceeded in a day of normal use of a single-axle tractor, and the operators are at significant risk of developing Hand-Arm Vibration Syndrome (HAVS). Ahmadian et al. (2014) have shown that 10% of all operators of a 13-HP walking tractor with vibration total value range of 14 to 26 m/s² will be affected by Hand-Arm Vibration Syndrome within 2.32 years. Similarly, Parvin et al. (2014) determined a total vibration amplitude a_{hv} of 16.95 m/s² for roto-tilling and determined the permitted working time for such machinery was only a few seconds. They suggested a risk of musculoskeletal disorders and recommended measures to reduce hand-arm vibration transmitted to the tiller user's hands.

Physiological characteristics of the operators

The mean and standard deviation of measured diastolic blood pressures of the seven tractor operators, before and after operation of the tractor, were 73 ± 7 and 77 ± 9 mm Hg, respectively; the mean and standard deviation of measured systolic blood pressure of the seven tractor operators, before and after tractor operation was 122 ± 12 and 119 ± 7 mm Hg, respectively. The average measured oxygen saturation levels of the seven tractor operators decreased from 98 ± 1 to $96 \pm 1\%$; these levels are within normal range.

The operators' heart rates were measured 5 min before tractor operation, every 5 min during operation for 25 and 5 min after tractor operation. This yielded mean measured heart rates of operators before, during and after operation of 71 ± 7 , 162 ± 4 and 126 ± 24 bpm, respectively (Figure 6). The average measured resting heart rate for our 7 tractor operators of 71 ± 9 bpm was within the normal range of normal resting heart rates of 60 to 100 bpm (Seol et al., 2017). Similar measurements by various researchers (Çalışkan and Çağlar, 2010; Eroglu et al., 2015; Kirk and Parker, 1996; Melemez et al., 2011) yielded values from 60 to 79.6 bpm for operators of different machines. An age-dependent predicted maximum heart rate was calculated using Equation 3 and found to be 194 ± 2.6 bpm. Various researchers (Eroglu et al., 2015; Cristofolini et al., 1990; Abeli et al., 1994) obtained results for this parameter varying from 127 to 178 bpm for their particular machine

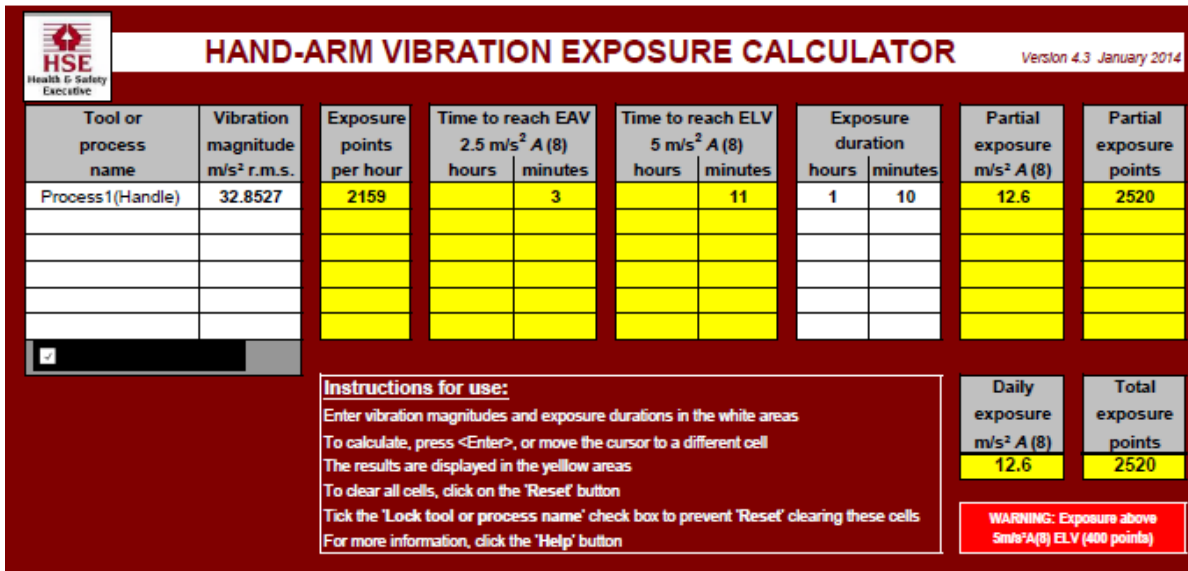


Figure 5. Hand-arm vibration exposure calculator and calculated results (HSE, 2005).

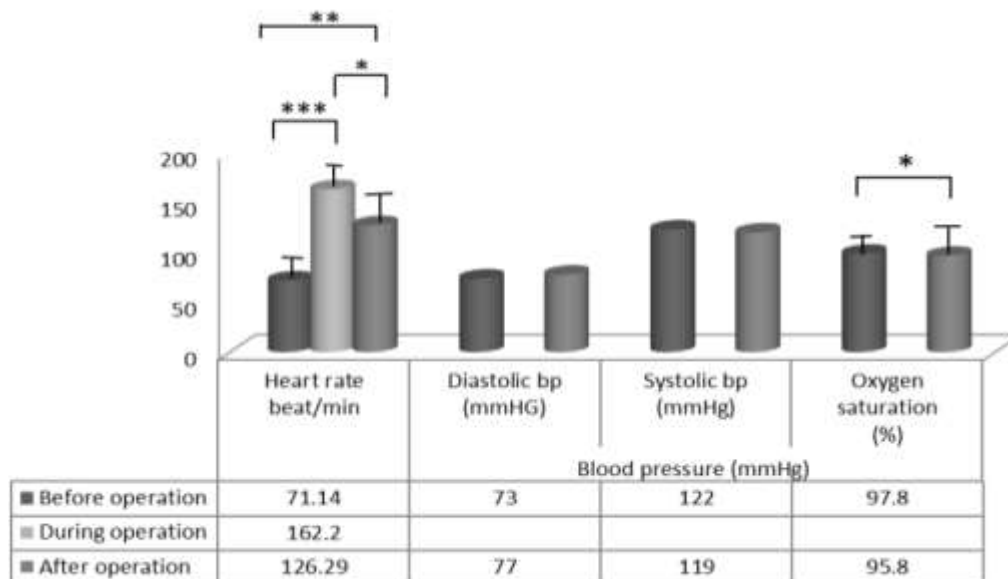


Figure 6. Physiological parameters of single axle tractor operators and their significance. *Significant difference, $p < 0.01$, **Significant difference, $p < 0.001$, ***Significant difference, $p < 0.0001$.

operators. The average measured working heart our seven single-axle tractor operators was 162 ± 4 bpm; this value is very high compared to values of 77 to 120.9 bpm reported by other researchers on a variety of machines (Abeli et al., 1994; Apud and Valdes, 1995; Kirk and Sullmn, 2001; Çalışkan and Çağlar, 2010; Eroglu et al., 2015; Muzammil et al., 2004; Shemwetta et al., 2002;

Tiwari and Gite, 2001).

According to Afshari et al. (2017) in the study of the determination of work-rest schedules based on physical workload they have categorized the workload with working heart rate above 160 bpm as “extremely heavy work”.

The average estimated percent heart rate reserve

(%HRR), which is an indication of physiological or level of physical activity of the tractor operators was 74% in the present study. Other researchers (Çalışkan and Çağlar, 2010; Eroglu et al., 2015; Abeli et al., 1994; Kirk and Sullmn, 2001) had significantly lower values in the range of 29 to 64%. The ratio of average measured working heart rate (HRw) to the calculated 50% level of heart rate reserve for the single-axle tractor operators was 1.22 ± 0.11 ; this value place the work in the category of work "continuous heavy duty" (Eroglu et al., 2015; Lammert, 1972). By comparison, this ratio was found to be only between 0.82 and 0.97 in other studies (Çalışkan and Çağlar, 2010; Eroglu et al., 2015; Lammert, 1972). The ratio of working heart rate to resting heart rate for our seven tractor operators was found to be 2.28 ± 0.39 , compared to values of 1.37 to 1.75 in other studies (Çalışkan and Çağlar, 2010; Eroglu et al., 2015; Vitalis, 1987; Afshari et al., 2017). Using the equation for basal metabolic rate (BMR) proposed by Schofield (1985), the calculated BMR was calculated to be 14.6 ± 0.5 kJ/min or 3.5 ± 0.1 kcal/min.

From these working heart rate results, it was concluded that this work falls into the category of "unduly heavy work" and the physical activity level (PAL) was 2.4 (Afshari et al., 2017). Therefore, from PAL and BMR calculations, the average energy expenditure level of the single-axle tractor operators was found as 35 ± 1 kJ/min or 8.4 ± 0.3 kcal/min. Other studies conducted with a variety of machinery types (Katherivel et al., 1991; Kroemer and Grandjean, 2000; Pawar and Pathak, 1980; Shemwetta et al., 2002; Tiwari and Gite, 2002) yielded results in the range of 7 to 18 kJ/min.

Conclusion

Vibration data were collected at the handle of a 15-HP single-axle tractor while performing routine tilling operations. Various physiological parameters were measured and analyzed. The Daily Exposure in $m/s^2 A(8)$ of vibration at the tractor handle was $12.6 m/s^2$ and total exposure points were 2520. In both cases, the $A(8)$ exposure limit value of $5 m/s^2$ and total exposure points of 400, was greatly exceeded. From measurements of operators' heart rates, it was concluded that the physical workload fell into the category of extremely heavy work, for which the working heart rate more than doubled the resting heart rate ($p < 0.0001$). This very high physical workload has a strong impact on operators of single-axle tractors over extended periods of time and may lead to the risk of different musculoskeletal disorders.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Physiological potential of lettuce seeds crespa

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The present work aimed to evaluate the germination, emergence, vigor and initial development of lettuce (*Lactuca sativa* L.) cultivars. Nine cultivars belonging to the crespa group, Cinderella, Monica SF 31, Mimosa (Salad Bowl), Veneranda, Brava, Cerbiatta (Catalonha), Itapua Super, Itaúna Prisée, Simpson and Vitória de Verão were used. The experiment was conducted to ensure the accuracy of the comparisons. The variables analyzed were: Germination, emergence, first germination and emergency counting, mean germination and emergency time, mean germination and emergence velocity, germination and emergency uncertainty index, germination and emergency synchrony, and relative frequency of germination and emergency. In order to obtain the emergency data, it was necessary to use a refrigerated room to give optimal conditions for the seeds and in the initial stage, an acclimatization for a salable development of newly emerged seedlings. For experimental design, completely randomized (CRD) was used for laboratory conditions and randomized blocks (RB) for the field, with four replicates of 25 seeds per treatment. The data were submitted for analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% probability. The analyses were carried out using SISVAR software 5.6. Cultivar Itaúna obtained the best results in physiological quality evaluations, being recommended for planting in regions of Northeastern Brazil.

Key words: *Lactuca sativa* L., germination, emergence.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) belongs to the family Asteraceae, being one of the vegetables of greater expression in the world. Brazil is a respectable producer and consumer of lettuce, both in “*in natura*” and processed salads. Every year, the interest in the consumption of this vegetable grows throughout the

country, increasing the areas of planting for different regions (Fonseca, 2016).

In Brazil, lettuce is the third most produced vegetable, occupying around 90,000 ha and moving billions of reais annually, being the hardwood vegetable of greater value, both in quantity and in commercialized price (ABCSEM,

2012). Second, according to the Brazilian Institute of Geography and Statistics (IBGE) data from 2006, the state of Alagoas, one of the most productive in the northeast region, was responsible for the production of 345 tons.

Its cultivation is usually practiced by family farming, employing a large number of labor in the field. It has been cultivated in different times and places, in different soil and climatic conditions, and during the most critical period of the culture (which is the germination and seedling emergence), the producer has not always full control of these conditions (Araujo et al., 2010).

Usually, the production is impaired in some regions of Brazil due to the occurrence of high temperatures, being one of the main problems, the fact that the high temperature interferes negatively in the process of seeds germination (Fonseca, 2016). The optimum temperature for germination of lettuce seeds is around 20°C. Several studies, in addition to the farmers' own experience, have shown that most cultivars do not germinate at higher temperatures, above 28°C. The low germination directly affects nurserymen and producers, since in addition to losing the inputs, the occupation of area for seedlings production and the work, often lose production contracts, in view of not having seedlings or plants at the time of the delivery (Fonseca, 2016).

Research shows that in addition to the environmental factor, there is an important genetic component associated with greater or lesser germination capacity, there being genetic variability for this characteristic, being important to evaluate each cultivar to be used as being different from the others, which may present different results of other goods looking visually (Argyris et al., 2008; Nascimento et al., 2012).

Due to the increase of the demand of this vegetable, it is necessary to evaluate the quality of the seeds that are commercialized in the state of Alagoas. The acquisition of quality seeds is a crucial step for producers to succeed in their activity (Marcos Filho, 2015), where seeds adapted to the region and with a minimum of satisfactory vigor allow farmers to earn enough to keep their families, enabling them to remain in the activity without the need to purchase new lands, reducing the rural exodus and fixing the man in the field.

For the state of Alagoas there is no cultivar specifically developed, it is common to use cultivars that are indicated for other regions, knowing that there is a risk of losing productivity because it is not giving the ideal conditions for the plant to express their total genetic potential. The objective of this work was to evaluate the

physiological potential of different cultivars of lettuce.

MATERIALS AND METHODS

Localization

The work was developed in the Laboratory of Plant Propagation, Center of Agricultural Sciences (CECA), Federal University of Alagoas (UFAL), Rio Largo, AL, Brazil.

Obtaining cultivars

Seeds of ten cultivars of curly lettuce were purchased from two companies: Feltrin and Isla. The used cultivars were the following: Cinderella, Mimosa (Salad Bowl), Monica SF 31 and Veneranda, coming from Feltrin and Brava, Cerbiatta (Catalonha), Itapuã Super, Itaúna Prisée, Simpson and Summer Victory, from Isla.

Water content

For the determination of the water content of the seeds, the greenhouse method was used at $105 \pm 3^\circ\text{C}$ for 24 h, as prescribed by the Rules for Seed Analysis (Brasil, 2009). This determination was made, at the time of installation of the tests, using two samples of 1 g of seeds.

Asepsis

Asepsis of the seeds was carried out by immersion in 70% alcohol for 1 min, followed by washing in running water.

Germination

The seeds were placed to germinate between two sheets of paper towel previously moistened with water volume equivalent to 2.5 times the weight of the dry paper (Brasil, 2009), in transparent plastic boxes (12.0 × 7.0 × 3.5 cm), in a germination chamber type BOD, regulated at a temperature of 20°C.

The seeds that gave normal seedlings germinated, with all their essential structures, showing in this way the potential to continue their development and produce normal plants, when grown under favorable conditions (Brasil, 2009). The daily germinated seed counts were carried out at the same time, for seven days, with the substrate being re-wetted when necessary.

Emergency

For the emergency, trays of 200 cells filled with the commercial substrate of earthworm humus were used, which were subdivided into 4 blocks of 25 (twenty five) cells for each cultivar. The sowing was done with the help of a template of 1 cm for the opening of the pit and 3 seeds were packed per cell.

At the end of the planting, the trays were conditioned in a

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refrigerated room to respond to a physiological requirement of the lettuce, where the temperature was maintained close to 20°C. With the aid of a sprayer, the substrate was maintained with humidity close to field capacity. After the initial emergence, the seedlings were submitted to acclimatization, and on the 6th post-planting day they were taken to greenhouse, where they remained until the end of the experiment. The counts were made daily after the first emergency count, on the 3rd day after sowing, for 14 days, being done at the same time.

Variables analyzed

Germination and emergency

$$g_i = (\sum_{k=1}^k n_i / N) \times 100,$$

where n_i is the number of germinated seeds/seedlings emerged at time i and N the total number of seeds placed to germinate/emerge (Labouriau, 1983; Carvalho et al., 2005).

First count germination/emergence

This was carried out in conjunction with the germination and emergency test, computing the percentage of normal seedlings obtained from the fourth day for germination and the third day for emergence after the tests were installed.

Average germination and emergency time

$t = \sum_{k=1}^k (n_i t_i) / \sum_{k=1}^k n_i$, being t_i : time from the beginning of the experiment to the i th observation (days or h); n_i : number of germinated seeds/seedlings emerged at time i (corresponding number or i th observation); k : last day of germination/emergency (Czabator, 1962).

Average speed of germination and emergency

$v = 1/t$, being t the mean time of germination/emergency (Santana and Ranal, 2004).

Relative germination frequency

$F_i = n_i / \sum_{k=1}^k n_i$, being n_i the number of germinated seeds/seedlings emerged per day and $\sum_{k=1}^k n_i$ the total number of germinated seeds/emerged seedlings (Labouriau and Valadares, 1976).

Uncertainty index

$U = -\sum_{k=1}^k F_i \log_2 F_i \approx F_i = n_i / \sum_{k=1}^k n_i$, being F_i : relative frequency of germination/emergence; n_i : number of germinated seeds/seedlings emerged at time i (corresponding number or i th observation); k : last day of germination/emergency (Labouriau and Valadares, 1976; Labouriau, 1983).

Synchronicity Index

$Z = \sum C_{n1,2} / N \approx C_{n1,2} = n_i(n_i - 1) / 2$; $N = \sum n_i(\sum n_i - 1) / 2$, being ' $C_{n1,2}$ ' the combination of germinated seeds/seedlings emerged in i the i th

time and n_i the number of germinated seeds/seedlings emerged in time i (Primack, 1980).

Statistical analyses

The experimental design was completely randomized (CRD) for laboratory conditions and randomized blocks (RB) for field, with four replicates of 25 seeds per treatment. The data were submitted for analysis of variance (ANOVA) and the means were compared by the Tukey test at 5% probability. The analyses were performed with the aid of SISVAR software 5.6 (Ferreira, 2011).

RESULTS AND DISCUSSION

In greenhouse, the mean temperature during the period of the experiment was 28°C. The maximum and minimum temperature did not exceed 30.1 and 22°C, respectively and the approximate mean relative humidity of 50.3% was maintained (Figure 1). These temperatures help the germination, emergence and development of lettuce seedlings (Brasil, 2009). The rains were not sufficient to supply the water needs of the crop, requiring three irrigation shifts.

The values of water contents of the seeds of the different cultivars ranged from 5.5 to 6.4% (Table 1), close to those reported by Barbosa et al. (2011) in different lots of lettuce seeds. It is important to note the importance of having the water content among the tested cultivars with the lowest possible variation, since when variations occur between the values of water content, there may be changes in germination and emergence that will accelerate the deterioration process, and formation of products that cause immediate damages, such as free radicals, masking the final result, as described by Marcos Filho (2015).

For all cultivars, the germination percentage was higher than the established standard for the marketing of lettuce seeds (80%), except the cultivars Itapuã (60%) and Mimosa (65%) (Table 2). The results of the germination and emergence tests indicated statistical differences between cultivars, with Itaúna being superior when compared with the others (Table 2), indicating a high physiological potential of the seeds of this plant. However, the results of these tests do not guarantee similar post-performance, even when germination and emergence are high, since seed performance depends on their physiological potential and environmental conditions (Melo Junior et al., 2018).

The optimum temperature range, for most species, is between 20 and 30°C (Marcos Filho, 2015). For the seeds in question, the temperatures of approximately 20°C provided the largest number of seeds germinated in the first count.

The physiological potential of the seeds can be evaluated by the germination test, conducted under

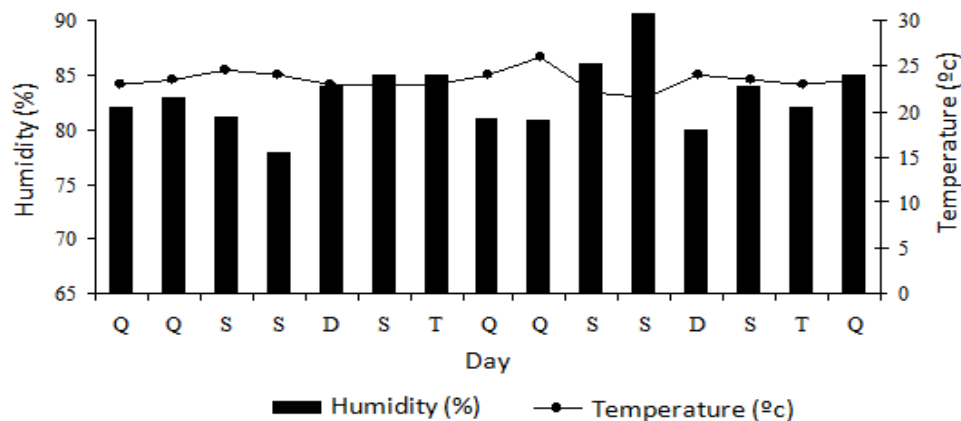


Figure 1. Daily averages of temperature and humidity occurred in Rio Largo (AL) from August 11 to 25, 2018 (CECA/UFAL, 2018).

Table 1. Water content (%) of different cultivars of crisp lettuce (CECA/UFAL, 2018).

Itapuã	Veneranda	Itaúna	Simpson	Monica	Vitória de Verão	Cerbiatta	Cinderella	Mimosa
6.4	6.3	5.5	5.8	5.9	6.2	6.1	6.0	6.2

highly favorable conditions of temperature, humidity and substrate, thus allowing the maximum potential for germination, not reflecting the behavior of the species in the field and therefore not detecting advanced stages of growth deterioration (Larré et al., 2009).

The cultivars Veneranda, Simpson, Mônica, Vitória de Verão and Cerbiatta were not differentiate statistically from each other for the first germination count, however they differed from Itaúna, which was superior (Table 2). The absolute values observed in this test were similar to the order verified in the germination test, since the two tests are conducted concomitantly; they indicate the performance of a population and under totally favorable conditions that can benefit lots of medium vigor. This test was also able to differentiate cultivars as a function of vigor in carrot (Spinola et al., 1998) and cucumber seeds (Bhering et al., 2000). In the first emergency count (PCE) (Table 2), the cultivar Itaúna also showed higher physiological potential, thus differing from the other cultivars analyzed. The PCE test often better expresses the differences in emergency velocity, but Valentini and Piña-Rodrigues (1995) emphasized that this test has a reduced efficiency in detecting small differences in force.

The first count test has generally been used as a vigor test due to its simplicity and to be conducted along with the germination test (Carvalho and Nakagawa, 2012). This test, despite being considered important for evaluating seed germination velocity, because it is not very labor intensive, and because it does not require specific equipment or infrastructure (Bhering et al., 2000),

could have its results affected by the fact that the temperature of 20°C be ideal for seed germination. Studies conducted with other species, such as cotton (Torres, 1998) and chili (Torres and Minami, 2000), also indicated low sensitivity of the first-count test to stratify seed cultivars, especially when differences in vigor are relatively narrow.

Regarding seed vigor (Table 3), indirectly measured by mean germination time (TMG), mean time of emergence (TME), mean germination velocity (VME) and mean velocity of emergence (VME), it was noted that the best results were obtained with the cultivar Itaúna, although it is not different from some cultivars in some variables. Silva et al. (2014) studying the germination of seeds stated that the speed of germination is linearly dependent on temperature, being a good index to evaluate the occupation of a species in a given environment, taking advantage of favorable environmental conditions. Oliveira et al. (2014) say that temperature is an important factor in seed germination, influencing total germination, emergence and seed vigor, as measured by the speed and the mean germination, also by emergency time.

The germination speed can be used to identify cultivars with a faster germination, indicating a higher physiological potential (Santana and Ranal, 2004). Lettuce seeds, in general, present a more homogeneous behavior around the mean time. This result corroborates the rapid germination of most seeds in the early days. According to Nascimento and Pereira (2007) the germination speed is a characteristic that can be used to establish the

Table 2. Germination (G), emergence (E), first germination (PCG) and emergency (PCE) counts of seeds and seedlings of different cultivars of crisp lettuce (CECA/UFAL, 2018).

Grow crop	G (%)	E (%)	PCG (%)	PCE (%)
Itapuã	60 ^c	62 ^d	45 ^d	6 ^{de}
Veneranda	89 ^b	60 ^d	94 ^b	4 ^e
Itaúna	100 ^a	98 ^a	100 ^a	34 ^a
Simpson	86 ^b	81 ^c	84 ^b	16 ^c
Monica	96 ^b	90 ^b	92 ^b	28 ^b
Vitória de Verão	97 ^b	88 ^b	95 ^b	17 ^c
Cerbiatta	89 ^b	57 ^d	84 ^b	0 ^f
Cinderella	85 ^b	68 ^d	66 ^c	8 ^d
Mimosa	65 ^c	41 ^e	15 ^e	8 ^d
Value of "F"	112.46**	15.35**	111.24**	253.29**
CV (%)	8.16	10.15	9.95	10.00

Means followed by the same lowercase letter in the column do not differ from each other to a 5% probability by the Tukey test. **Significant at the 1% probability level.

Table 3. Average germination time (TMG), of emergency (TME), average speed of germination (VMG) and of emergency (VME) of seeds and seedlings of different cultivars of crisp lettuce (CECA/UFAL, 2018).

Grow crop	TMG (days)	TME (days)	VMG (days ⁻¹)	VME (days ⁻¹)
Itapuã	4.4 ^a	5.4 ^d	0.22 ^b	0.31 ^{bc}
Veneranda	4.0 ^a	5.7 ^e	0.25 ^a	0.31 ^{bc}
Itaúna	4.0 ^a	3 ^a	0.25 ^a	0.15 ^a
Simpson	4.1 ^a	3.3 ^{ab}	0.24 ^a	0.22 ^a
Monica	4.0 ^a	3.3 ^{ab}	0.24 ^a	0.17 ^a
Vitória de Verão	4.0 ^a	3.3 ^{ab}	0.25 ^a	0.18 ^a
Cerbiatta	4.0 ^a	5.7 ^e	0.24 ^a	0.33 ^c
Cinderella	4.3 ^a	4.2 ^c	0.23 ^{ab}	0.25 ^{ab}
Mimosa	5.4 ^b	4.5 ^c	0.18 ^c	0.30 ^{bc}
Valor de "F"	170.78**	24.17**	322.29**	14.12**
CV (%)	5.63	10.16	4.05	13.01

Means followed by the same lowercase letter in the column do not differ from each other to a 5% probability by the Tukey test. **Significant at the 1% probability level.

emergence index in the field or greenhouse, and higher velocities would minimize the effect of possible adverse conditions after sowing. For lettuce cultivation this is very important, since high temperatures during the first hours of germination can lead to the seeds entering thermoinhibition and/or in thermodormity, affecting the establishment of the culture (Bewley and Black, 1994; Nascimento, 2003).

The analysis of the results of uncertainty (U) and synchrony of germination and emergence (Z) of lettuce seeds (Table 4) allowed the confirmation that the cultivar Itaúna was significantly superior to the others. Carvalho

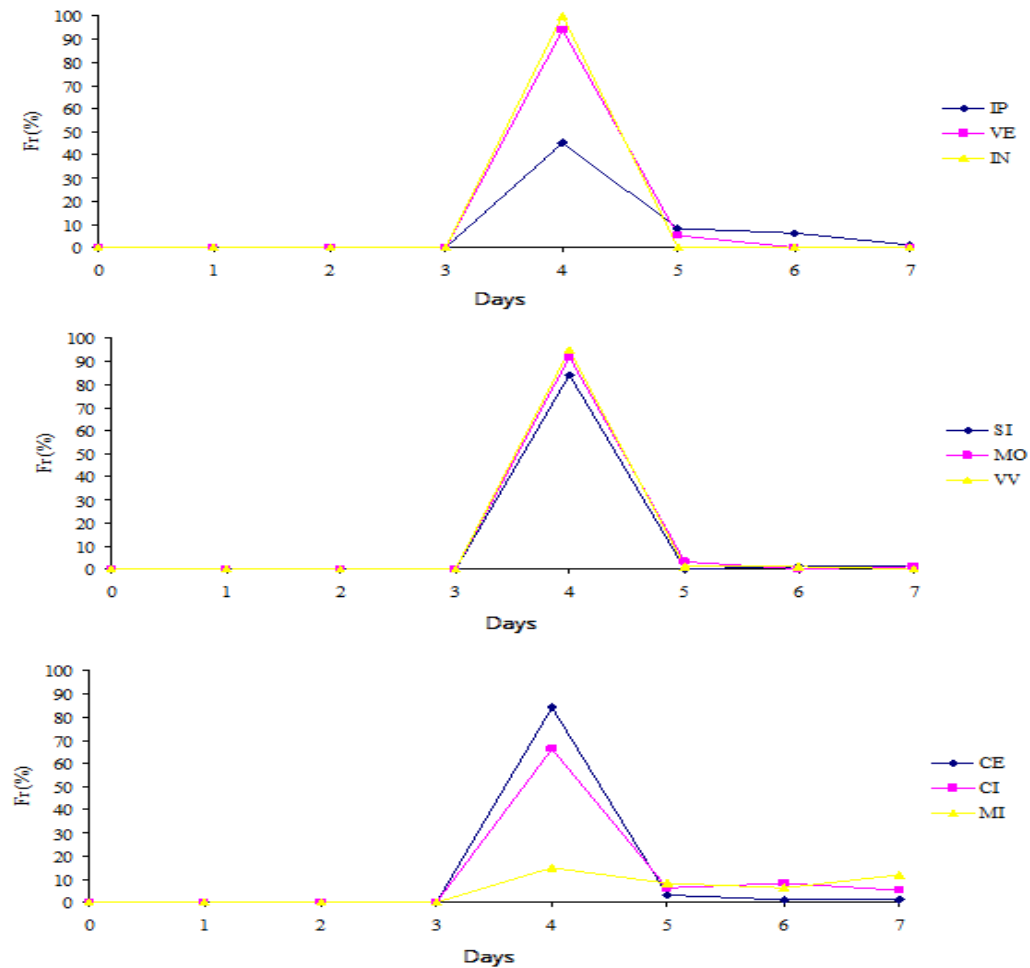
et al. (2015) working with radish seeds used the germination synchrony to evaluate different cultivars. It is emphasized that the increase of the synchrony expressed to the physiological homogeneity of the seeds at the moment of germination (Conserva, 2006), that is, the closer to the result, the more uniform the germination process.

By the values of uncertainty (informational entropy), it was observed that the cultivars caused frequencies with few peaks (Figure 2) or probable unimodal distribution of the relative frequency, that is, the most concentrated germination in time. This result shows that the higher the

Table 4. Uncertainty index (U) and synchrony index (Z) of germination and emergence of cultivars of crisp lettuce (CECA/UFAL, 2018).

Grow crop	U germination (bits)	U emergency (bits)	Z germination	Z emergency
Itapuã	1.1763 ^c	2.3451 ^c	0.5771 ^b	0.2045 ^{de}
Veneranda	0.2813 ^b	2.4377 ^c	0.9608 ^a	0.1504 ^{ef}
Itaúna	0.0001 ^a	1.6060 ^a	1.0000 ^a	0.5483 ^a
Simpson	0.4822 ^b	1.7391 ^{ab}	0.8932 ^a	0.3545 ^b
Monica	0.4011 ^b	1.6237 ^a	0.9181 ^a	0.3618 ^b
Vitória de Verão	0.3063 ^b	1.6675 ^a	0.9512 ^a	0.3599 ^b
Cerbiatta	0.4421 ^b	2.6691 ^c	0.9092 ^a	0.1313 ^f
Cinderella	1.1348 ^c	2.2019 ^{bc}	0.6249 ^b	0.2658 ^c
Mimosa	1.5082 ^d	2.3238 ^c	0.3098 ^c	0.2571 ^{cd}
Value of "F"	127.33**	16.15**	31.61**	100.97**
CV (%)	16.04	9.61	10.27	8.27

Means followed by the same lowercase letter in the column do not differ from each other to a 5% probability by the Tukey test. ** Significant at the 1% probability level.

**Figure 2.** Relative frequencies of seed germination of different cultivars of crisp lettuce. (IP: Itapuã, VE: Veneranda, IN: Itaúna, SI: Simpson, MO: Monica, VV: Vitória de verão, CE: Cerbiatta, CI: Cinderella e MI: Mimosa) (CECA/UFAL, 2018).

level of organization of the process, the greater the germination speed (Table 4).

The accumulated percentages of germination of the lettuce seeds, during the 7 days of experiment, indicate that the germination of the cultivars Itaúna, Simpson, Monica, Vitória de Verão and Cerbiatta began on the fourth day after the installation of the experiment, while in the other the germinative process started after the fourth day (Figure 2). It can be observed that the species under study have a short period of germination, with the distribution of the germination process in a few days and that the frequency of germination is associated with the synchronization index of germination, as reported by Bufalo et al. (2012).

Conclusion

The cultivars Itaúna, Simpson, Monica and Vitória de Verão obtained the best physiological potentials, being indicated for regions of Northeast Brazilian.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Changes in livelihood strategies and animal husbandry practices of pastoralists in the sub-humid zone of West Africa

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Migration to and sedentarization in the sub-humid zone of Burkina Faso has become a major adaptation measure of Sahelian pastoralists to the severe droughts and in response to a rapidly changing socio-economic environment. This study is aimed at assessing livelihoods and livestock husbandry practices as a consequence of pastoralist sedentarization. The study sites were located in the Sudano-Sahelian and Sudanian zone of Burkina Faso. Findings revealed that severe Sahelian droughts of 1973/4 and 1983/4 led to massive migration and sedentarization of Sahelian pastoralists to their current locations in the sub humid zones. Since then, the sedentarized pastoralists have diversified their livelihood strategies in which livestock keeping and small-scale crop production play a central role. As the study sites are close to urban areas, a market-oriented local dairy production has also been developing. Overall, the total annual revenue of the sedentarized households and the size of their cattle herd have increased in tandem with the time they have been sedentarized. However, there are still some constraints and challenges such as restricted access to valuable pasture areas during the rainy season and recurrent herder-farmer conflicts that need to be addressed to strengthen the livelihoods of the sedentarized pastoralists.

Key words: Pastoralism, livelihood diversification, sedentarization, sub-humid zone, West Africa.

INTRODUCTION

In many West African countries, livestock production contributes significantly to the national economy, providing food security, income, employment, and substantially contributing to the gross domestic product

(GDP) (OCDE/SWAC, 2009). Across Sahelian countries such as Mali, Burkina Faso and Niger, the total number of domestic animals is estimated at about 60 million cattle and 160 million small ruminants, which together

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contribute to about 20 to 30% of the agricultural component of GDP (CILSS, 2010). Among this huge number of livestock, transhumant pastoralism involves 70 to 90% of the cattle and 30 to 40% of the goats and sheep in the Sahelian zone, supplying about 65% of cattle and 40% of small ruminant meat (OCDE/SWAC, 2009). Historically, Sahelian pastoralists and their livestock were able to move freely across the landscapes, benefiting from the resources available in arid, semi-arid and sometimes also sub-humid environments. But today their ability to adapt to a changing environment is constrained by increasing climate variability and socio-economic transformation processes that occur in the region. The severe droughts of the 1970s and 1980s significantly reduced the Sahelian livestock population and threatened pastoral communities' livelihoods (Nelen et al., 2004). High human population growth across West Africa has profoundly altered land-use patterns and intensity, as well as livestock numbers, species composition of national herds and livestock management (Turner et al., 2005; Dicko et al., 2006). As a result, the mobility of pastoral livestock herds has been drastically reduced and human and livestock population growth have also led to increased competition between livestock keepers and crop farmers for the use of the available natural resources (Moritz, 2006; Bidima, 2011; Sanfo et al., 2015). These changes increasingly challenge the adequacy of nutrition for extensively managed ruminant stocks in the pastoral and agro-pastoral Sahelian zones of West Africa (Le Houérou, 2006) and today, these communities are suffering from the subtle and negative impacts of climate change and variability on their animals, access to drinking water and other pastoral resources and health (Brooks, 2006; Yoshioka et al., 2007; Sarr, 2012). At the same time, the ongoing changes negatively affect the vegetation of the grazing areas because confining livestock grazing to shrinking grazing areas leads to increased local grazing pressure and reduced livestock productivity (Turner et al., 2005, 2008; Bonnet and Herault, 2011). As an adaptation strategy, many pastoral livestock keepers have migrated and settled in the Sudanian zone. In fact, this strategy has been supported by the governments of some West African countries (Burkina Faso, Côte d'Ivoire, Mali, Niger and Senegal), and projects funded by the World Bank have created grazing areas or pastoral zones where, during the dry season, forage and water resources are relatively abundant (Nelen et al., 2004; Gonin and Gautier, 2015). Many of these zones are either around big cities or forested areas, including national parks and protected areas, where ranching systems are encouraged and livestock markets are also available (Basset and Turner, 2007). Immigration and sedentarization of Sahelian pastoralist in the sub-humid zone has induced some changes in their life style, livelihoods and production systems (Greenough et al., 2016). Basset and Turner (2007) reported the adoption of

small-scale crop cultivation by pastoralist immigrants, and numerous studies investigated changes in the access to and governance of common pool resources such as pastures, water and agricultural land in the wake of immigration and sedentarization of Sahelian pastoralists into the sub-humid zone - particularly in Burkina Faso (Benoit, 1977; Bonnet and Herault, 2011; Nelen et al., 2004; Gonin and Gauthier, 2015; Gonin, 2016; Greenough et al., 2016).

But some knowledge gaps still exist regarding the current livestock wealth, and implications of household settlement in the vicinity of protected areas on the livelihoods and livestock management strategies of these sedentarized pastoralists. At the same time, such information is useful in furthering understanding of the potential ecological and socio-economic developments in the region, where human and livestock motilities are dynamic and often shift in line with global changes that are taking place at any point in time (climate change, rapid population growth and urbanisation). Therefore, this study sought to analyse the changes in sedentarized pastoralists' livelihood strategies and their livestock husbandry systems over time since their sedentarization in the sub-humid zone of Burkina Faso in the 1970s and 1980s. Specifically, the study objectives are to characterise the current livelihoods and livestock management practices of sedentarized pastoralists, and in other hand identify the determinants of their livelihood strategies as they settled. The following research questions were addressed: (i) What are the current livelihood strategies and livestock management practices of sedentarized pastoralists? (ii) What are the determinants of present livelihood strategies? (iii) Does the duration of sedentarization affect the pastoralists' livestock wealth?

MATERIALS AND METHODS

Study area

The study was conducted in 2014 at two sites, namely Nobere (Latitude: 11°12' and 11°50' N, and Longitude: 0°50' and 1°40' W) and Dinderesso (Latitude: 11°11'5" and 11°18'1" N and Longitude: 4°18'46" and 4°26'40" O), which are, respectively, located in southern and western Burkina Faso (Figure 1). The first site is located near Kabore Tambi National Park, the second near the classified forest of Dinderesso. The pastoral zone of Nobere is mainly occupied by sedentarized Fulani pastoralists, while the silvopastoral zone of Dinderesso is used by sedentarized Fulani pastoralists and peri-urban non-Fulani livestock keepers originating from Bobo Dioulasso, the second largest city of Burkina Faso. Both sites also serve as dry season grazing areas and transhumance corridors for pastoralists from other regions of Burkina Faso during their yearly transit to Ghana and Ivory Coast.

The Nobere pastoral zone (the "Zone Sud Ouest"), is located next to the national park. It comprises 32,000 ha of land and was delineated in 2005. It may be used for settlement, grazing, and small-scale crop cultivation by the sedentarized pastoralists. In Nobere's pastoral zone, there is a shortage of basic infrastructure. For instance, the community reported that they only have two

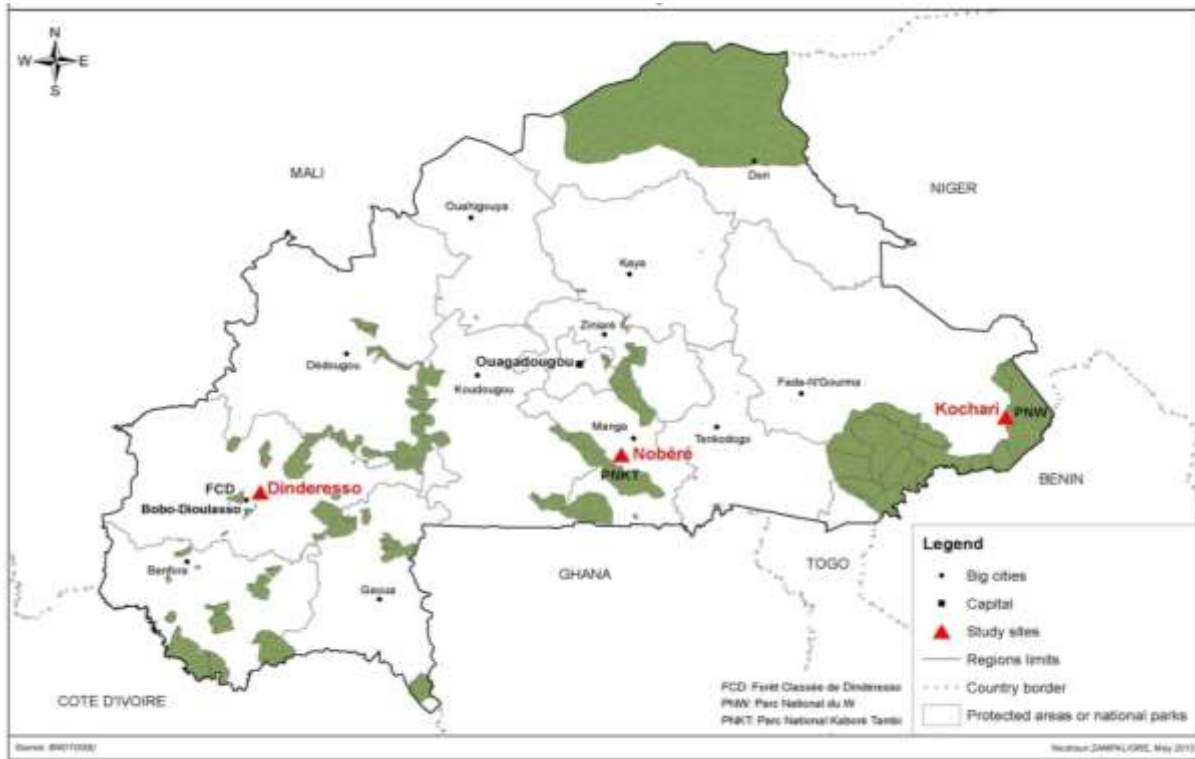


Figure 1. Study sites location in Burkina Faso.

manual pumps for fetching drinking water, one well, one rural bilingual school and one livestock vaccination park. A dam was constructed in the year 2000 for livestock herds, but their access to this watering point is very difficult especially during the rainy season due to crop fields that are located very close to it.

The silvopastoral zone of Dinderesso (2379 ha) was established in 2005 and is reserved for livestock grazing as part of the forest participatory management plan established in 2005. The Nobere pastoral zone is managed by a farmers' association, whereas in Dinderesso access to the silvopastoral zone is regulated by two associations (one representing the sedentarized pastoralists and the other representing the peri-urban livestock keepers). These committees organize the grazing system, make sure that their members respect the set grazing rules and assure the protection of the grazing areas against bush fires and illegal users. The committees are also actively involved in the governance of the community-based forest management system of the two protected areas.

The Dinderesso site is benefiting from its proximity to Bobo Dioulasso - there are livestock vaccination parks, livestock markets and veterinary services. A dam located in the grazing zone provides water to the herds and in each settlement there is at least one manual pump for drinking water. Most of the households are located far from schools and health centres.

Climate and vegetation

The site of Nobere is located in the Sudano-Sahelian climate zone which is characterized by an alternation of a dry season (November to May) and a rainy season (June to September). The average annual precipitation ranges from 600 to 900 mm with high spatio-temporal variation. Temperature also varies depending on month and season of the year, with the average annual temperature being

28°C. The area is drained by several important rivers including the Nakambé (Red Volta) and small seasonal tributaries.

The Dinderesso site is located in the South Sudanian zone and is likewise experiencing a dry season (November to April) and a rainy season (May to October) (Figure 1). The average annual precipitation ranges from 900 to 1200 mm and again shows high spatio-temporal variation. At both sites, the vegetation is typically of Sudanian type, dominated by trees, shrubs and C4 grasses, and shaped by the combination of climatic factors and anthropogenic influences such as grazing and fire. Along rivers, gallery forests are found (Coulibaly, 2010). The soils are mostly ferruginous tropical soils and a large portion of the protected areas are fallows and agroforestry parklands (Coulibaly, 2010).

Data collection

For this study, the methodological approach developed by Ellis (2000) was applied, which is a combination of sample surveys and participatory techniques, such as semi-structured focus group discussions and key informant interviews. These served to bring out a typology of livelihood strategies and detailed analysis of the roles and importance of income, activities and assets that sustain the livelihoods and wealth of the target community. More details regarding the approach and statistical analyses are provided in the following sections.

Focus group discussions and key informant interviews

Focus group discussions were held to obtain qualitative information. Two meetings were separately held at each site, the first one for mainly women, and a second for a mixed group of young herders, adults and elders men, all belonging to the group of sedentarized

pastoralists. Each focus group consisted of 8 to 10 persons. The qualitative information gathered from the focus group discussions served as the basis for designing the questionnaire for a household survey.

Household sampling procedure

A stratified purposive sampling procedure was adopted. First, villages surrounding the Kaboré Tambi National Park and the classified forest of Dinderesso were selected for the survey. Then, in each of these villages, sedentarized pastoralist households were identified and selected based on their primary livelihood activity. The number of respondents (one per household) was selected in proportion to the total number of sedentarized pastoralist households in each area; thus a total of 108 households were interviewed. In this study a sedentarized pastoralist household is defined as a previously mobile a pastoralist household that settled in its current location for at least one decade, with livestock rearing being a primary source of livelihood while secondary sources of livelihood are subsistence cropping and other off-farm activities (supplying at least 10% of household income and food sources).

Household survey

For the household survey, a structured questionnaire was administered to the selected household head. Section 1 of the questionnaire focused on information about the respondent, the household characteristics and assets; section 2 referred to the household's economy; section 3 dealt with household access or ownership of physical assets; section 4 and 5 focused on crop and livestock production systems. In addition, the questionnaires also covered the respondent's perceptions on the protected areas' natural resource availability and users' access to these, seasonality and change in resources quality and availability, as well as governance of the natural resources (section 6). The questionnaire was designed in French and administered in local languages by trained enumerators. The questionnaire was pre-tested with 10 household heads who were not part of the final set of interviewed households.

Statistical analyses

Principal component analysis

Principal component analysis (PCA) in SPSS 22 (SPSS Inc., 2013) was used to explore the relationships between the different variables and to reduce the large number of variables to a smaller number of components for further analysis (Dossa et al., 2011). The rule of retaining the components that contain a minimum of four variables having a loading score >0.60 (Stevens, 1992 cited in Dossa et al. 2011) was used to extract the number of reliable principal components from the PCA (Dossa et al., 2011). As indicated by Dossa et al. (2011), the higher the loading of a variable on a given principal component, the more that variable contributes to the variation accounted for by this component. But as factor loading significance depends on sample size (Stevens, 2002), for this study variables with a loadings score >0.40 was retained on one of the retained components as meaningful components (Field, 2014) (Table 3).

Cluster analyses (CA)

A two step non-hierarchical cluster analysis was used to characterize the livelihood strategies adopted by sedentarized

pastoralists in the study sites. Cluster analysis has been successfully used in several studies to characterize the livelihood strategies of pastoralists (e.g., Homewood et al., 2009). In this clustering approach, a set of procedures was followed. First, the principal component scores from the previous PCA was used as inputs for the cluster analysis in SPSS 22 (SPSS Inc., 2013). Secondly, before using the categorical variables in the cluster analysis, the continuous variables were converted into nominal variables using the visual binning procedure of SPSS 22 (SPSS Inc., 2013) because in the two-step cluster analysis, the higher weighting of categorical variables at the expense of the continuous variables in the clustering process may be an important issue (Dossa et al., 2011). Thirdly, the Bayesian Information Criterion (BIC) for retaining the number of clusters in this study was used. And finally, three clusters were obtained and a cluster name assigned to each. Once clusters were identified and names assigned, cross tabulations in SPSS 22 (SPSS Inc., 2013) was ran to show the distribution of each household livelihood activity and income contribution across the three retained clusters. Differences between clusters in household and farm characteristics, livestock wealth and financial assets were explored using the Kruskal–Wallis non-parametric test.

General linear model

A general linear model was used to examine the effect of years in residence at the study site on the households' livestock wealth and asset ownership. In this study, years in residence of the household head were defined as the total time that the household head had stayed at the site (ordinal variable). Three classes were defined based on results of the focus group discussions in which three major settlement periods of had been identified by the pastoralists themselves. Thus, class 1 depicted a residence maximum of 20 years ago (that is, recently settled); class 2 a residence ranging from 21 to 40 years (that is, settled after the severe drought of 1983/4); and class 3 a residence of more than 40 years (that is, settled after the severe drought of 1973/4).

RESULTS

Historical timeframe and trajectories of pastoralists' sedentarization

Narrative information gathered during focus group discussions and key informant interviews helped to draw historic timeframes and processes of Sahelian pastoralists' sedentarization in the sub-humid zone of Burkina Faso. Reasons and motivations behind their choice of particular sites were also determined. Here, results are first presented for the general case and then specific aspects pertaining to the study sites of Dinderesso and Nobere are highlighted.

General historical sedentarization process

Three distinct periods were identified as key periods during which pastoralists migrated from the Sahel to settle in their current sites. These periods were: (i) right after 1960 when the country got independent; (ii) just after the Sahelian drought of 1973/4; (iii) just after the Sahelian drought of 1983/4. In addition, there has also

been intermittent sedentarization of transhumant pastoralists at the study sites. Community members pointed to diverse reasons that motivated people to migrate and settle in their current locations. The first group of migrants settled due to their regular dry season transhumance to the area, as a good relationship was built with the villagers. Secondly, due to the severe impacts of droughts on the natural and cultivated vegetation in the Sahel, famine for humans and death of many animals, many pastoralists migrated and settled in the sub-humid zone. The latter situation was typical for the majority of households now living in the study sites (result of focus group discussions). Most of the sedentarized pastoralists originated from the administrative region of Sahel (Dori) and the central plateau region of Burkina Faso for Nobere, and from the northern region of Burkina Faso (Ouahigouya, Kaya administrative region) for Dinderesso. Preferred settlement places were peri-urban areas, the vicinity of protected areas and, in some cases, villages where the immigrants already had relatives.

The immediate post-drought migration and sedentarization has been supported and promoted by government, NGOs, and international development agencies through programs and projects that specifically targeted the relocation of pastoralists to the so-called pastoral regions in the sub-humid zone, which also included delineated transit and refugee areas for mobile herds.

Most of these programs aimed to promote the shift of Sahelian pastoralists from nomadism to a sedentary lifestyle, including more intensive livestock production approaches such as feedlot and ranching systems. These should replace the traditional extensive livestock production system which was identified as the main cause for land degradation and desertification in the Sahel. Except for the delineated pastoral zones, sedentarization was usually facilitated by a host in the destination sites who introduced the incoming pastoralists to community leaders and villagers. Pastoralists settling in the pastoral zones were given permission by local authorities who had agreements with traditional authorities.

In the pastoral zones, some of the key infrastructure such as dams, schools, vaccination parks, and extension services was put in place to support livestock production communities. This is the case for the first generation of delineated pastoral zones in Burkina Faso, namely CEZIET, SIDERADOUYOU, SONDRE-EST and NOUHAOU in KénéDougou, Comoé, Zounwéogo, and Boulgou provinces, respectively. The choice of specific areas to settle was guided by the relative availability of rangelands, animal feed resources and hospitality of local communities on the one hand. On the other hand, availability of job opportunities and market incentives for those who settled in peri-urban areas was a key determinant. Full sedentarization of the pastoralists was

progressively achieved through household attachment to the territory, adoption of crop production for food consumption needs, and employment of household members as shepherds, which was particularly important for those who lost all their livestock during the droughts. Gains from cropping activities, earnings from waged herding as well as being involved in small jobs helped some households to rebuild the livestock herd lost during the drought and thus engage in livestock production once again. Nowadays most of the sedentarized pastoralists possess livestock and are also involved in crop production as well as in a number of other income earning activities. About 55.5% (Dinderesso) and 75.5% (Nobere) of household heads had settled in their current location for more than 20 years.

Specificities of pastoralist communities in Nobere and Dinderesso

The majority of pastoralists who are settled in Nobere are from northern central Burkina Faso (Kaya). According to the elders, initially (around 1960) there were only three households and today there are about 50 households, distributed across six (06) settlements along the border of the Kaboré Tambi national park. The park was already created when the migrants started settling, but from 2004 to 2007, due to the park extension program (PAGREN project), the households were relocated to the Nobere site in the buffer zone of the national park. The immigrants' main livelihood activities are livestock rearing, field cropping, livestock trade, petty trade and the use of non-timber forest products (NTFP), due their proximity to the national park.

At the Dinderesso site, according to the elders, the first pastoralist community settled around 1920 before the delimitation of the classified forest in 1951. After the demarcation of the classified forest, grazing was prohibited until 1983 when the government allowed grazing in the forest through the USAID/686 – 0235 project, but only during the lifetime of that project (5 years). Later, in 2004, another project, known as PAGREN (Projet d'appui à la gestion des ressources naturelles) created grazing areas in the forest for periodic grazing by livestock herds of the settled pastoralists. Those grazing areas are still open for controlled livestock grazing from June to January every year.

Current livelihoods of sedentarized pastoralists in the sub-humid zone

Household socio-demographic characteristics

All interviewed households were male-headed and mostly Fulani migrants (100 and 91.7% in Dinderesso and Nobere, respectively). Household sizes mostly ranged

Table 1. Socio-demographic characteristics of respondent households.

Variable	Dinderesso (n=57)	Nobere (n=50)
	Respondents (%)	Respondents (%)
Age (years)		
20 -30	7.4	10.2
30 - 40	16.7	26.5
40 - 50	29.6	30.6
50 - 60	22.2	20.4
60 - 70	18.5	12.2
>70	5.6	0.0
Education		
No education	20.0	68.8
Primary school	7.3	2.1
Secondary school	5.5	0.0
Informal education	5.5	4.2
Koranic school	61.8	25.0
Household size (members)		
5-10	50.0	59.2
10-15	28.6	26.5
>15	21.4	14.3
Duration of living in the village (years)		
5 - 10	21.4	8.2
10 - 20	14.3	16.3
20 - 30	17.9	26.5
>30	37.5	49.0
Ethnic group		
Fulani	91.07	100
Mossi	3.57	0
Bobo	3.57	0
Bolon	1.79	0

from 5 to 15 persons. The age of the heads of households ranged from 30 to 60 years for the majority. Among the respondents, the level of formal education was very low; 20 and 68%, respectively, in Nobere and Dinderesso, had no education and only 7 and 2% had primary and secondary level education (Table 1). About 68 and 20% of the Muslim respondents had attended Islamic school. The main livelihood activities were livestock rearing, small-scale crop farming, livestock trade, petty trade, wage earning, receiving remittances, and use of non-timber forest products (Figure 2).

Pastoral livestock keeping as main livelihood activity

In both study sites, livestock was the mainstay of the livelihoods of almost all households, and thus, all respondents owned some animals. Main species kept were cattle, sheep and goats. Besides these, donkeys were also reared by all interviewed households, mostly for ploughing fields and as a means of transportation.

Herd sizes varied from 41 to 70 for cattle and from 14 to 23 for small ruminants. Livestock feeding practices were mainly based on daily grazing, and mobility (daily grazing movement within the village territory, small and long distances seasonal transhumances) was a key feature within the livestock feeding practices (Figure 3). About 50% of households in Dinderesso and 25% in Nobere were still practicing transhumance within the country. About 20% among these even practiced transboundary transhumance during the dry season (Figure 3). Preferred destinations for the transhumant herds included northern Ghana and northern Ivory Coast. Local grazing areas were fallowed fields, fields after crop harvest, and in most case also the vegetation of the protected areas although it is considered illegal for livestock to be grazed there except in the buffer zone for Nobere and the silvo pastoral zone for Dinderesso. Cattle herds were mainly composed of breeds of zebu cattle or cross-breeds between zebu and taurine cattle; for sheep and goats the Djalonké breed prevailed but in some cases Sahelian goats were kept. Next to the consumption of livestock

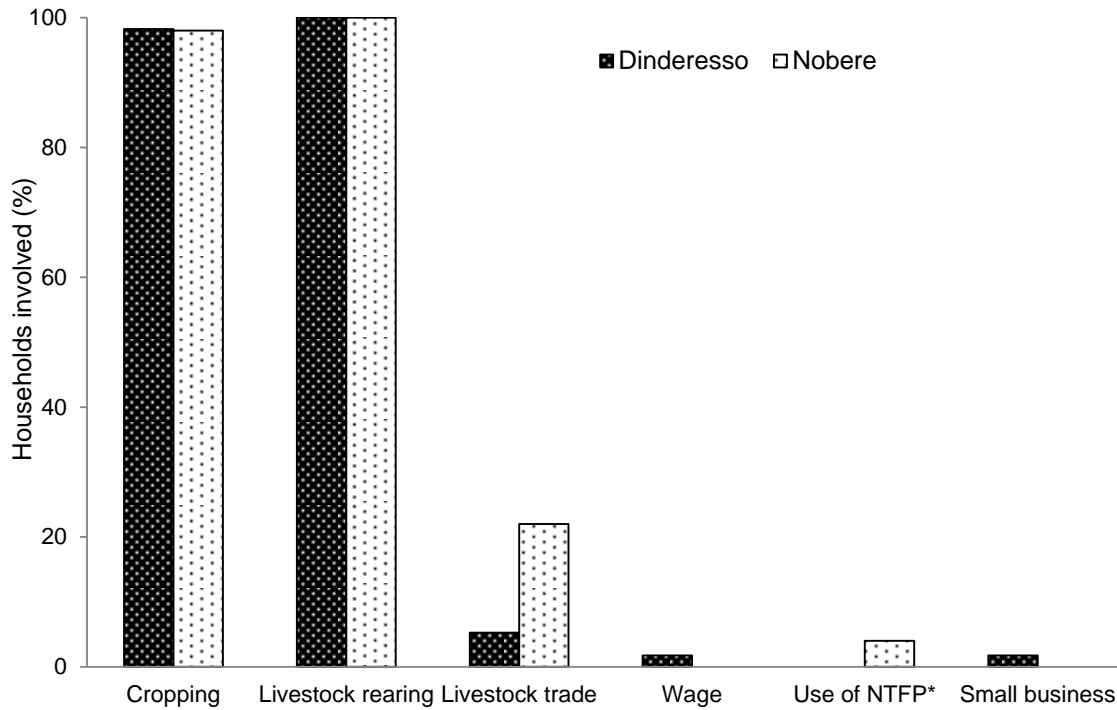


Figure 2. Proportion of households involved in different livelihood activities at the study sites. *NTFP: Non-timber forest products.

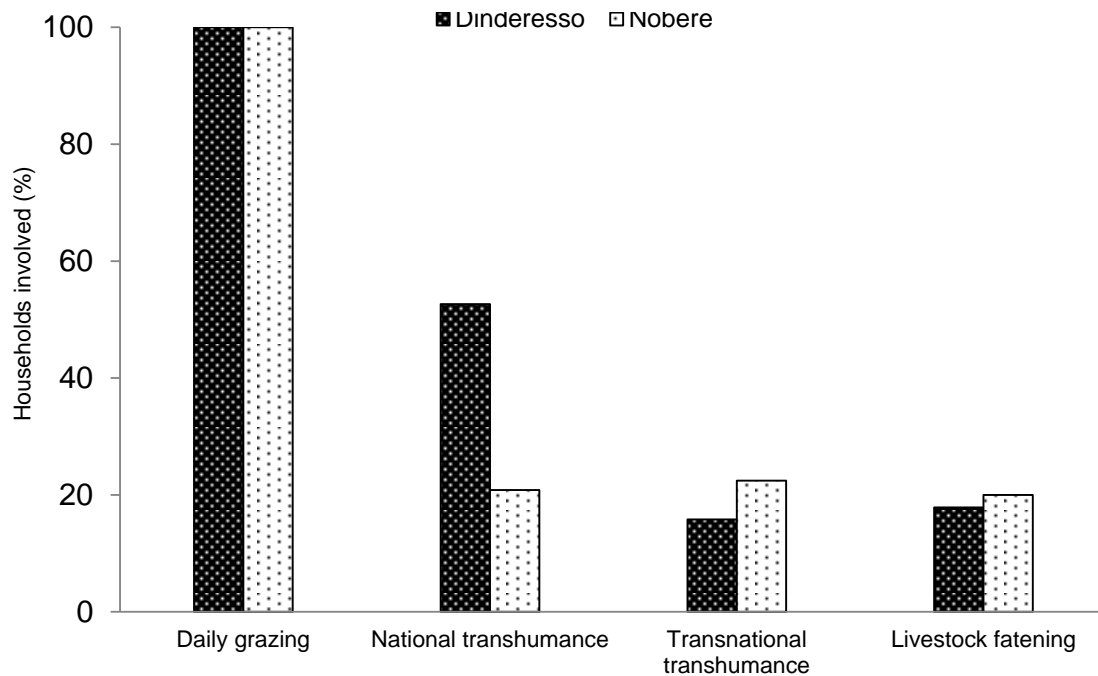


Figure 3. Current livestock management practices at the study sites.

products (milk and meat) consumption, most of the respondents derived the large share their income from the sale of live animals and cow milk.

Small-scale crop production

Results of the interviews indicated that even before

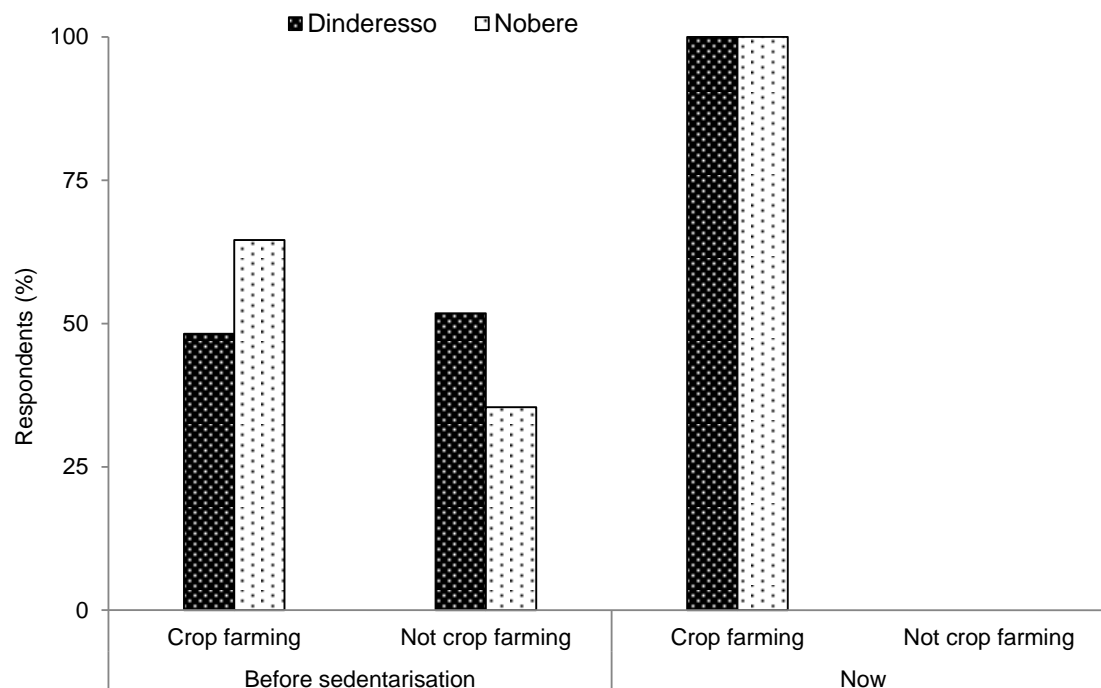


Figure 4. Changes in crop farming practice by sedentarized pastoralists.

Table 2. Species of staple (s) food and cash (c) crops cultivated by sedentarized pastoralists.

Crop (scientific names)		Households involved (%)	
		Dinderesso	Nobere
Millet (<i>Panicum glaucum</i>)	s	9.7	2.5
Sorghum (<i>Sorghum bicolor</i>)	s	27.8	33.3
Maize (<i>Zea mays</i>)	s	38.2	60.5
Rice (<i>Oryza sativa</i>)	s	2.1	0
Groundnut (<i>Arachis hypogaea</i>)	c	3.5	0
Cotton (<i>Gossypium</i> sp.)	c	3.5	0
Cow pea (<i>Vigna unguiculata</i>)	s, c	11.8	3.7
Sesame (<i>Sesamum indicum</i>)	c	3.5	0

sedentarization about half of the respondents had already been involved in small-scale crop production as another source of livelihood (48% in Dinderesso and 65% in Nobere). All the interviewees indicated that they actively cultivate crops in addition to keeping livestock (Figure 4). The crops include both cash and food crops, such as sorghum, millet and maize (Table 2). About 3.5 and 2.5% of the respondents also grow cash crops such as peanuts and cotton (mainly in Dinderesso). Cropland sizes range from 0.5 to 10 ha, with an average of 1.5 and 2.0 ha, respectively, for Nobere and Dinderesso. The interviewees reported that the adoption of cropping is a response to the disappearance of the former barter system of livestock products against field crops; it is geared at food consumption with the aim to reduce the

costs of cereal purchase.

Other livelihood activities

Other livelihood activities reported by the respondents include livestock trade, petty trade, use of NTFP and wage earnings. About 5 to 22% of the respondents were involved in livestock trade at Nobere and Dinderesso, respectively. While petty trade and wage earnings prevail at Dinderesso, wage earnings and sales of NTFPs prevail at Nobere (Figure 2). These activities are mostly income-generating and their adoption depend on the main livelihood strategy adopted by the household (Table 4). The share of income from these activities is indicated in

Table 3. Model summary and component factor loadings of variables used in the principal component analysis.

Variable description	Rotated principal component factor loadings		
	PC1	PC2	PC3
Sheep herd size (n)	0.82		
Annual income from crops sold (in FCFA)	0.78		0.10
Cropland size (ha)	0.56		-0.19
Household size (n)	0.55		
Goat herd size (n)	0.53	0.13	
Agriculture income (yes/no)	-0.52		0.42
Head of household status (native/migrant)	-0.12	-0.78	
Annual remittances (in FCFA)	0.11	0.70	-0.13
Income from livestock trade (in FCFA)	-0.30	0.57	
Livestock fattening (yes/no)	-0.29	0.57	0.28
Total annual revenue (in FCFA)	0.40	-0.46	
Primary livelihood activity (1=C, 2=LR, 3=LT, 4=WE, 4=others)	0.15		0.87
Second livelihood activity (1=C, 2=LR, 3=LT, 4=WE, 4=others)			-0.85
Number of fields cropped	0.18	-0.17	-0.44
Number of years in residence		-0.24	0.15
Age of the head of household (years)		0.17	-0.15
Transhumance (yes/no)		-0.14	
Education of the head of household (level of literacy)	-0.12		0.21
Cattle herd size (head of animals)	0.34	0.14	0.24
Income from NTFP (in FCFA)		0.13	-0.18
Income from animal sales (in FCFA)		0.18	
Income from milk sales (in FCFA)			-0.20
Income from handicrafts (in FCFA)		-0.14	
Land ownership (yes/no)	-0.32	-0.23	
Eigenvalue	3.684	2.502	2.382
% of variance explained	12.99	9.48	9.27
Cronbach's alpha	0.62	0.15	0.56

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization.

Table 5.

Livelihood clusters

The cluster analysis identified three main livelihood strategies employed by the respondents, who could be classified as: (i) Subsistence agro-pastoralists with livestock dominance; (ii) Subsistence agro-pastoralists with crop dominance; and (iii) Market oriented agro-pastoralists. The categorization reflects a household's dominant activity, its diversification, and the income level as derived from the annual sales revenues (Tables 5 and 8). The subsistence agro-pastoralists with livestock dominance are households practicing livestock rearing and small-scale crop production as their main livelihood activities, with livestock being the primary activity (Table 4). The main source of income for this group is livestock, whereby 79.7 and 84.8% of the households are involved in live animals and extra milk sales, respectively. Livestock rearing is seconded by subsistence cropping

with an average area of cropland of 1.9 ± 1.48 ha. The average herd sizes are 54, 15 and 19 heads for cattle, sheep and goats, respectively (Table 5). In addition to the two main activities, few households of this group are engaged in livestock fattening (15.3%), livestock trade (20.3%) and use of NTFP (23.7%) as other livelihood activities. Transhumance is practiced by about 37.3% of the respondents during dry season mostly, toward Ivory Coast (for Dinderesso) and Ghana (for Nobere). The average time in residence of the head of household is 26 years and household size averages 10 members.

The second group, defined as subsistence agro-pastoralists with crop dominance, is made up of households practicing livestock rearing and crop production as their main livelihood activities, with subsistence cropping being the primary livelihood activity. About 20% of the income is generated from sales of surplus crops. The mean size of a farm is 2.3 ± 1.72 ha, whereby each household owns at least two crop fields. Livestock rearing is the second most important activity, but herds are smaller than in the first cluster (Table 5),

Table 4. Frequency distribution of agro-pastoralists' (AP) involvement in livelihood activities and income earning.

Variable	Response	Subsistence AP, livestock dominance (n=59)	Market-oriented AP (n=13)	Subsistence AP, crop dominance (n=10)
Primary livelihood activity (yes)	Cropping	0.00	15.38	10
	Livestock rearing	100.00	84.62	90
Secondary livelihood activity (yes)	Cropping	100.00	76.92	10
	Livestock rearing	0.00	15.38	0
	Livestock trade	0.00	7.69	90
Livestock fattening	Yes	15.25	46.15	10
	No	84.75	53.85	10
Transhumance practice	Yes	37.29	38.46	90
	No	62.71	61.54	40
Income earning from crop sales	Yes	3.39	61.54	60
	No	96.61	38.46	20
Income earning from milk sales	Yes	84.75	69.23	80
	No	15.25	30.77	60
Income earning from live animals sales	Yes	79.66	69.23	40
	No	20.34	30.77	80
Income earning from livestock trade	Yes	20.34	53.85	20
	No	79.66	46.15	10
Income earning from NTFP sales	Yes	23.73	30.77	90
	No	76.27	69.23	10
Income earning from handicrafts sales	Yes	5.08	7.69	90
	No	94.92	92.31	0
Receiving remittances	Yes	5.08	0.00	100
	No	94.92	100.00	0

and households own on average 28 heads of cattle, 13 goats and 15 sheep. However, about 60 and 80% of the households in the second cluster also earn income from live animal and extra milk sales. Only 10% of households practice livestock

fattening, livestock trade or make use of NTFPs (Table 4) as other livelihood activities. Transhumance is practiced by about 40% of the respondents but not beyond the national borders. Average time in residence of the head of

household is 23 years and average household size is 10 members.

The third cluster comprises the market-oriented agro-pastoralists whose characteristics are similar to the subsistence agro-pastoralists with livestock

Table 5. Comparison of sedentarized pastoralists' agro-pastoralist (AP) resources across clusters (mean values and standard deviation, SD).

Variable	Subsistence AP, livestock dominance (n=59)		Market-oriented AP (n=13)		Subsistence AP, crop dominance (n=10)		Sig. ²
	Mean	SD	Mean	SD	Mean	SD	
Household size (number of persons)	10.1 ^a	5.72	17.3 ^b	10.13	10.0 ^a	7.42	*
Residence time (years)	26.6	13.50	28.8	11.66	23.6	16.79	NS
Crop land size (ha)	1.9 ^a	1.48	3.7 ^b	1.93	2.3 ^{ab}	1.72	**
Cattle herd size (head of animals)	54.6 ^{ab}	45.18	110.0 ^b	128.37	27.7 ^a	12.36	**
Sheep herd size (head of animals)	15.1 ^a	9.22	37.9 ^b	23.11	12.8 ^a	7.10	**
Goat herd size (head of animals)	18.7 ^a	14.05	37.7 ^b	27.73	14.4 ^a	9.91	*
Income from crops sold (x 1 000 FCFA) ¹	303.2 ^a	239.82	821.2 ^b	723.97	191.8 ^a	181.24	**
Income from animals sold (x 1 000 FCFA)	1177.6	1 255.8	2561.4	2830.4	871.0	978.7	NS
Income from milk sold (x 1 000 FCFA)	313.5	535.8	414.4	469.4	130.7	165.9	NS
Income from livestock trade (x 1 000 FCFA)	304.9 ^a	897.3	1819.2 ^b	3457.7	200.0 ^a	632.5	*
Income from <i>NTFP</i> sold (x 1 000 FCFA)	12.2	25.6	22.5	40.6	7.5	23.7	NS
Income from handicrafts sold (x 1 000 FCFA)	1.8	7.9	4.6	16.6	0	0	NS
Income from remittance (x 1 000 FCFA)	4.2	217.8	0	0	0	0	NS
Annual revenue (in 2014) (x 1 000 FCFA)	2117.4 ^a	2116.97	5643.3 ^b	4728.43	1401.10 ^a	1112.11	**

¹Currency in 0xFCFA (1 FCFA= 655.995 Euro); ² Kruskal-Wallis test, significance level (*= $p \leq 0.005$; **= $p \leq 0.001$); for each variable and row, respectively, mean values with different superscript letters are significantly different.

dominance. Livestock rearing, milk production, cropping and livestock trade are their main livelihood activities, whereby 46.2 and 53.9% of the households practice livestock fattening and trade. Besides, home consumption of livestock products such as milk, most of the interviewed households are also earning cash from live animal and milk sales (94 and 100% of respondents, respectively). Total annual revenue is the highest among the three groups (Table 5), due to the diversified income sources, and cluster members also hold considerable livestock wealth (Table 5).

As depicted above, crop production and livestock rearing were the main livelihood activities of most of the 108 respondents across the two sites, but households further diversified their livelihood activities and income sources as

indicated in Table 4. Results of the non-parametric Kruskal Wallis test indicated that a household's wealth (n) of cattle ($H=10.91$, $p=0.004$), goats ($H=18.89$, $p=0.001$) and sheep ($H=7.39$, $p=0.025$), its crop land area ($H=7.39$, $p=0.025$), revenues from surplus crops sold ($H=10.87$, $p=0.004$), revenues from livestock trade ($H=8.46$, $p=0.015$) and the total annual income ($H= 16.66$, $p=0.003$) were significantly affected by the cluster membership of a household (Table 5).

Analyzing the effect of years in residence showed that livestock wealth, especially ownership of cattle ($F = 3.7993$; $p = 0.26$) and the total annual revenue ($F = 5.982$; $p = 0.004$) were significantly different between the three classes of years of residence (Table 6), whereby cattle herd size and annual revenue significantly increased as

years in residence increased (Table 7).

DISCUSSION

Current livelihood strategies of settled pastoralists

Findings from this study demonstrate that Sahelian pastoralists have settled in the sub-humid zone of Burkina Faso many decades ago as a consequence of the droughts in the 1970s and 1980s. Most of these sedentarized pastoralists have diversified their livelihood strategies but livestock remains the mainstay, although the majority of them is also involved in crop production as another important livelihood

Table 6. Analysis results on effect of year in residence on pastoralists' wealth.

Dependent variable		Sum of squares	df	Mean square	F	P-value
Annual revenue (0xFCFA)	Contrast	5.83E+13	2	2.92E+13	3.793	0.026
	Error	6.53E+14	85	7.69E+12		
Cattle herd size (n)	Contrast	45264	2	22632	5.982	0.004
	Error	321572	85	3783		
Goat herd size (n)	Contrast	688	2	344	1.691	0.191
	Error	17306	85	204		
Sheep herd size (n)	Contrast	249	2	125	0.408	0.666
	Error	25971	85	306		
Cropland size (ha)	Contrast	7	2	3	1.178	0.313
	Error	242	85	3		

The F-tests on effect of year in residence are based on linear independent pairwise comparisons among the estimated marginal means. Df=degree of freedom.

Table 7. Distribution of household assets and annual revenues according to years of residence (settlement) of pastoralists.

Households assets and revenues	Year in residence		
	≤ 20 years (n=28)	[21 - 40[years (n=58)	≥ 41 years (n=9)
Cattle herd size (n)	41(12)	56 (9)	122 (21)
Goat herd size (n)	15 (3)	20 (2)	13 (5)
Sheep herd size (n)	19 (3)	22 (2)	24 (6)
Cropland size (ha)	2(0.32)	2 (0.24)	2 (0.56)
Annual revenue (in 1 000 F CFA)	1373.2(524.0)	2785.2(388.2)	3945.6(924.2)

Values depict means and (standard deviation).

activity.

Similar observations on the Sahelian pastoralist movements from the Sahel and their sedentarisation into the southern agro-climatic zones as well as adoption of crop production have been reported by Bassett and Turner (2007) and McCabe et al. (2010). The results of the present study show that size of the livestock herd and of cropland, the practice of livestock fattening, the

level of revenues from surplus crops sold, livestock trade, and total annual cash revenue characterize the type of livelihood strategy adopted by a particular household in the study area. This shows the change from specialized livestock keeping to an agro-pastoral livestock rearing system with adoption of activities that enable diversification of income sources. The prevalence of livelihood diversification among

sedentarized pastoralists suggests that this is an important feature for household wellbeing and economic transformation, especially in the context of a rapidly changing environment and rural household vulnerability (Ellis and Allison, 2004). A diversified livelihood strategy reduces vulnerability compared to a specialised strategy, and might be more sustainable over time, because it allows for rapid adaptation to changing circumstances (Ellis,

2000). Pastoralists' livelihood diversification, including adoption of crop cultivation as a consequence of restricted access to key pastoral resources and reduced possibilities for herd mobility, have also been observed in East Africa (Kenya and Tanzania; Homewood et al., 2006; McCabe et al., 2010). Indeed, most of our interviewees pointed to the complementary benefits of crop and livestock integration for their livelihood strategy, the role of crop residues for livestock feeding and of livestock manure for crop field fertilization, as also stated by Tarawali and Hiernaux (2002) and Powell et al. (2004). Other activities such as the use of non-timber forest products and involvement in livestock trade are more site-specific given the present proximity to forested areas and urban centres, respectively. Livelihood activities both for food and income contribute to alleviate poverty, build substantial wealth (in the form of assets and animals) and reduce risks associated with rural livelihoods as well as the ongoing climatic and non-climatic stresses in the study area. The impact of the number of years of residence on animal wealth shows a positive effect of sedentarization, as livestock wealth contributes substantially to yearly cash revenues. This might be explained by the fact that years in residence in the sub-humid zone allowed sedentarized pastoralists to rebuild their herd and accumulate financial resources through diversification of income sources as discussed above. In fact, availability and relative abundance of natural resources (forage and water) for livestock rearing in the designated settlement areas (Sanou et al., 2018), and the possibility for earning off-farm income near or in urban centres led to livestock wealth accumulation, since traditionally and until today, surplus cash is invested in animals. Also, as market opportunities exist in these areas especially for meat and dairy products, livestock keepers have improved health care and reproductive management of their livestock to maximize their benefits. Since traditional rangelands are scattered and encroached upon by cropland, the only possibility for herders is to graze their livestock in the rainy season in the protected areas, damage to crops outside these zones might lead to violent clashes between livestock keepers and crop farmers (Kiéma, 2007; Kiéma and Fournier, 2009). Similar grazing strategy during the rainy season is also observed (Zampaligré and Schlecht, 2017) in the Kaboré Tambi National Park where livestock herders of Nobere located in the vicinity of the park grazed their animals inside the protected area despite the grazing ban (Code forestier du Burkina Faso). However, grazing protected areas is organised and supported by governmental policy in Dinderesso, where a community controlled grazing system in the classified forest exist since 2005. This use of protected areas as rangelands by the resident former pastoralists as well as transhumant Sahelian pastoralists is a common problematic feature across (sub-humid) Sudano-Sahelian countries in West Africa who share borders with (semi-arid) Sahelian

countries (Kagoné et al., 2006; Convers et al., 2007; Fournier et al., 2009). Initiatives and policies in favour of participatory protected area management involving livestock grazing and transhumance have been developed in recent years to mitigate possible negative effects of grazing on the protected vegetation (Kagoné et al., 2006). Periodic use of the classified forests as pasture, hay-making from the vegetation of the protected areas, and opening of grazing routes for access to watering points are among these initiatives and policies. In addition, there are regional agreements on pastoralism in favour of livestock mobility across Sahelian and coastal countries through the transhumance protocol of ECOWAS/CEDEAO (La décision A/DEC.5/10/98 relative à la réglementation de la transhumance entre les Etats membres de la CEDEAO du 31 October 1998). Studies showed that periodic grazing of livestock in the classified forest of Dinderesso is appropriate in case of full adherence to rules by the key actors and strict compliance with the governance system in place (Nacro, 2007; Coulibaly, 2010, Zampaligré et al., 2018). The experience indicates the possibility of involving pastoralists and farmers in participatory sustainable management of protected areas. Similar community-based resource management strategies of forage resources and forests involving local communities and pastoralists have been reported from elsewhere in Africa and worldwide (Bedunah and Schmidt, 2004; Nelson et al., 2009; Roe et al., 2009; Reid et al., 2014). These management strategies need to be considered when developing land tenure and grazing policies (Grings et al., 2016).

Current challenges and opportunities of pastoralism in the sub humid zone

As outlined in other sections of this paper, there is recent as well as historic migration from the Sahel and sedentarization of pastoralists in the sub-humid zone of Burkina Faso. Factors such as pastoral resources availability, eradication of *Glossina* (tse-tse) flies in the sub-humid zone, as well as the multiple benefits of integrating crop and livestock production have favoured pastoralists' settlement in the area. In addition, the growing market opportunities for livestock and livestock products as a consequence of increasing urbanization and human population density, with its subsequent increase in demand for food, are fostering sedentarized agro-pastoralism in the sub-humid zones of West Africa. There are some evident constraints and challenges to the development of sustainable sedentarized agro-pastoralism in the study area. Specifically, for our study sites, the main constraints are both the restricted access to grazing areas due to the presence of the park and protected areas (where livestock grazing is at least at times prohibited) and the expansion of crop fields, which

is a problem during the rainy season (Sanfo et al., 2015). Conflicts between herders and crop farmers over access to pasture resources, restricted access to protected areas, crop land expansion, and increased insecurity are some of the challenges reported by the communities during the survey. All these constraints need to be appropriately tackled in order to ensure sustainable and peaceful land use in the sub-humid zone of Burkina Faso. At the moment, there are untapped market opportunities that can easily be exploited to improve the sedentarized pastoralists' livelihoods. The rapidly growing urban population with its high demand for animal-based food (milk and meat) is a great opportunity for those keeping livestock. For instance, about 90% of cattle, goat and sheep meat consumed in Burkina Faso originate from the country's pastoral and agro-pastoral systems, which in addition also supply live animals for export to neighbouring countries. In 2011, about 371,873, 586,082 and 744,782 heads of cattle, sheep and goats, respectively, were exported to Benin, Togo, Ghana and Nigeria (FAO/ECOWAS, 2016).

In our study sites, due to their proximity to urban areas, some of the agro-pastoralist interviewed are actively involved in the livestock trade and play central roles as livestock traders besides being livestock keepers. Their strategic involvement as producers and traders helps them negotiate good prices and generate substantial incomes for their households. Beside live animal sales, there is also a growing urban demand for (local) dairy products where the settled former pastoralists can play a central role in supplying the markets. Until now, the contribution of local milk production to cash revenues is still very low, but we observed that most of the households that were interviewed are trying to benefit from the growing urban dairy market through retaining dairy cows from their herd and supplementing them with cotton seed cake and other concentrated feed during the dry season. At the study sites, about 60 to 80% of the respondents reported earnings from surplus milk sales, regardless of their cluster affiliation. Efforts by government, projects and NGOs are supporting the local dairy production through creation of innovation platforms for dairy production, processing and marketing (APESS, 2014), but little is done to improve the actual milk output of cows during the long dry season, which is basically a matter of appropriate feeding strategies.

Conclusion

Assessment of the current livelihood strategies and animal husbandry practices of sedentarized former Sahelian pastoralists in the sub-humid zone of Burkina Faso clearly show that these former pastoralists have diversified their livelihood activities, among which pastoral livestock rearing and small-scale crop production play a central role. These changes are on one hand a consequence of their new sedentary lifestyle, the socio

economic and environmental conditions at their current locations. Thus, sedentarized pastoralists are also starting to take advantage of the growing market demand for livestock-based food in large cities, and especially market-oriented local dairy production has the potential to provide supplement incomes to settled pastoralists. But there are still a number of constraints and challenges that need to be addressed to allow livestock keepers to better benefit from those market opportunities, such as feeding strategies for the dry season, including fodder production, hay making and supplementation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Comparison of proximate, mineral and phytochemical composition of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces used for a different purpose

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Enset (*Ensete ventricosum* (Welw.) Cheesman) is one of the oldest cultivated food security crops in Ethiopia. There are a number of enset landraces used for traditional medicine. These landraces are believed to have better minerals and phytochemical compositions, which are not proved scientifically. The objective of this study was to compare the proximate, mineral and phytochemical compositions of corms of enset landraces used for treating bones illness traditionally, and two other landraces having another use value. Laboratory analysis was made by following standard methods, and the data determined were analyzed using SAS statistical software. Enset landraces, *Kibnar* and *Guarye* showed significantly ($p < 0.05$) higher protein content (4.74 and 4.06%), while *Astara* and *Guarye* were superior in phosphorus content (127.41 and 116.38 m g^{-2}) respectively. Similarly, the highest zinc and tannin contents (8.52 and 153.94 m g^{-2}) were obtained from *Astara* and *Kibnar* landraces respectively. On the other hand, *Amerat* landrace showed significantly ($p < 0.05$) higher crude fibre content. The three landraces used for traditional medicine showed better performances in protein, phosphorus, zinc and tannin contents than the other two landraces.

Key words: *Ensete ventricosum*, enset, landrace, corm, proximate composition, mineral, phytochemical.

INTRODUCTION

Enset (*Ensete ventricosum* (Welw.) Cheesman) belongs to the family Musaceae, and is one of the oldest cultivated herbaceous and monocarpic banana-like food security crops in Ethiopia. It is known to have several uses including food and non-food applications and highly

integrated with the economic, social and cultural life of enset growing societies (Admasu and Struik, 2001). It is reported that more than 20 percent of the population in Ethiopia is dependent on enset for human food, fibre, animal forage, construction materials and medicines

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(Brandt et al., 1997). The processed enset (*kocho*, *bull*a and corm) are rich in carbohydrates, and are good sources of minerals. However, the starch yield and nutrient compositions vary among enset landraces.

Some *E. ventricosum* landraces are believed to have medicinal value and are used by the enset growing community (Belehu and Endale, 1989). The use of pseudostem and seed of related species, *Ensete superbum*, for the treatment of various human ailments like debility, diabetes, kidney stone and for easy delivery was reported (Diana and George, 2013; Prashant and Bhadane, 2008). The use of boiled corm and starchy powder, *bull*a, of *Tayo* enset landrace together with milk for curing ailments such as joint displacement and swelling, broken bone and fracture, in the Bonga area was also reported (Tsehaye and Kebebew, 2006). According to Tadessa and Masayoshi (2016), there is a strong recommendation of an enset variety called *Sweete* for treating bone problems in the Areka area.

In Gurage and some other enset growing society, corms of enset landraces known by local names as *Astara*, *Guarye* and *Kibnar* are traditionally recommended for treating a bone fracture, breakage and joint displacement. The highest calcium content of the corms of *Astara* landrace, as compared to nine other landraces was reported (Ajebu et al., 2008). The selective use of some of enset landraces in traditional treatment of bone-related illness may have a relationship with their calcium, phosphorous or proximate contents, which are known to have biological importance (Marks et al., 2010) or phytochemical content, that have beneficial effects in wound healing. This hypothesis, however, should be justified with research-based investigations. Regard to this, there is only little information published so far. Therefore, the present study was carried out to compare the proximate, mineral and phytochemical contents of three enset landraces used in traditional medicine and two other landraces having another use value, growing in Gurage zone, Southern Nations, Nationalities and People Region (SNNPR), Ethiopia.

MATERIALS AND METHODS

Plant material

The study was carried out on three enset landraces known by local names *Astara*, *Guarye* and *Kibnar* which are used in traditional medicine (for treating broken bones and bone fractures) and two other landraces (*Amerat* and *Yeshrakinqe*) that have other use values. The former is known for good quality product of enset, *kocho*, in terms of colour and taste, and the later produced for its high yielding and disease resistance character as presented in Table 1. For each landrace, corm samples from three plants at the commonly used age for traditional medicine (4-5 years) were collected from farmers' fields in Gurage zone, SNNP region of Ethiopia, in August 2016. To represent the different enset growing agro-ecology of the zone, the samples were collected from three different altitudes (1900, 2100 and 2300 m) above sea level, and to reduce soil nutrient effect, each replication was collected from one farm (when possible) or from two border farms.

Sample preparation

The collected corm samples were washed, sliced into pieces (ca.5 mm) separately and dried in an oven (Binder GMBH, Model; M-115, Germany) at 70°C until constant weight was attained. The dried samples were then finely ground using laboratory miller and packed in airtight plastic cups. The samples were then stored in desiccators for further analyses at Ethiopian Public Health Institute and Addis Ababa University, Centre of Food Science and Nutrition laboratories.

Proximate analysis

Moisture content

Moisture content was determined by the method of the Association of Official Analytical Chemists (AOAC) (2005), using the official method 930.15. After the cleaned and dried crucibles were placed in desiccators and weighed (W_1). The sample around 40 g was accurately weighed (W_2) in a previously weighed crucible and put in desiccators every step. Then the crucible with its content was put into an oven (Binder GMBH, Model; M-115, Germany) at 70°C for 42 h until constant mass was attained, and after cooling in desiccators to room temperature, they were weighted again (W_3). The moisture content was determined using the equation below:

$$\text{Moisture content in percent (\%)} = \left(\frac{W_2 - W_3}{W_2 - W_1} \right) \times 100 \quad (1)$$

Crude fibre content

Crude fibre content was determined by the method of International Organization for Standardization (ISO 5498) (2002). A two-gram sample was transferred to 600 ml beaker and boiled with 200 ml 1.25% sulphuric acid for 30 min. After digestion by 20 ml 28% NaOH for 30 min, the mixtures were filtered through a crucible filled with a layer of sea-sand using a vacuum pump and the residues were washed with hot distilled water several times. The residue left was washed three times under vacuum, each time with 30 ml of 1% sulphuric acid solution and then distilled water, 1% sodium hydroxide solution and then distilled water and acetone and dried by suction. It was then dried at 130°C for 2 h, cooled in desiccators and weighed (W_1). After incinerating the samples in a muffle furnace at 550°C for 2 h, they were cooled in desiccators and weighed again (W_2). The total crude fibre was expressed in percentage as:

$$\text{Crude fibre} = \left(\frac{W_1 - W_2}{W_3} \right) \times 100 \quad (2)$$

Where; W_3 is the weight of samples

Ash content

Ash was determined by the method of AOAC (2016), using the official method 923.03. Clean crucibles, dried at 100°C in an oven were cooled in desiccators and weighed (W_1). Then 3 g sample was weighed into a previously weighed crucible (W_2). Then crucibles with their contents were burned in a muffle furnace (Stuart SF, U.K) set at 550°C for 2 h until light gray ash resulted and then weighed (W_3) after cooling. The weight of the ash was expressed as a

Table 1. Enset (*Ensete ventricosum*) landraces used for proximate, mineral and phytochemical composition analysis collected from SNNPR.

S/N	Name of enset landrace	Major use	Predominant character	Collection zone
1	Amerat	food	good <i>kocho</i> quality	Garage
2	Astara	food and traditional medicine	sweet corm	Garage
3	Guarye	food and traditional medicine	sweet corm	Garage
4	Kibnar	food and traditional medicine	sweet corm	Garage
5	Yeshirakinqe	Food	high yielding and disease resistance	Garage

percentage of the initial weight of the sample as follows:

$$\text{Total Ash (\%)} = \left(\frac{w_2 - w_3}{w_2 - w_1} \right) \times 100 \quad (3)$$

Crude protein content

Crude protein was determined by the Kjeldahl method (AOAC, 2016), official method 2001.11. 1 g of the sample was weighed into a digestion flask and 12 ml of concentrated H_2SO_4 was carefully added to each flask and 3 ml of 30% hydrogen peroxide was added step by step after mixed carefully. As soon as a violent reaction ceased, the tubes were shaken and 3 g catalyst, a mixture of copper sulphate with potassium sulphate was added and digested by heating at 420°C . After the digestion had been completed, the digestion flasks were connected to a receiving flask containing 2% boric acid and indicators methyl red and bromocresol green. The solution in the digestion flask was then made alkaline by addition of 40% NaOH to liberate ammonia gas into receiving flask where it converts the boric acid to the borate ion. Finally, the distillate was titrated with standard 0.1 M HCl and crude protein content was calculated as total nitrogen, according to AOAC (2016).

Crude fat content

Crude fat was determined based on the Soxhlet extraction method (AOAC, 2003), using the official method 2003.06. After aluminium cups with boiling chips were dried in drying oven at $102 \pm 2^\circ\text{C}$ for at least 30 min, they were cooled in the desiccators to room temperature and weighed (W_1). Five gram of sample was weighed into thimbles and extracted with 70 ml diethyl ether. After finishing the extraction process, the cups were removed from the extractor and dried in $102 \pm 2^\circ\text{C}$ oven for 30 min to evaporate the solvent. The extraction cups were cooled in desiccators for 30 min and weighed (W_2) immediately after taken out from the desiccators. The fat obtained was expressed as a percentage of the initial weight of the sample using the formula:

$$\text{Crude fat (\%)} = \left(\frac{W_2 - W_1}{SW} \right) \times 100 \quad (4)$$

Where; SW is the weight of the sample

Total carbohydrate content

The percentage of total carbohydrate was determined by difference, which involves adding the total values of crude protein, crude fat, moisture and ash constituents of the sample and subtracting it from 100%. It is determined as follows:

$$\text{Total\% Carbohydrate} = 100 - (\%M + \%P + \%F + \%A) \quad (5)$$

Where; M = moisture, P = protein, F = fat and A= ash contents

Mineral analysis

Approximately 3 g of each powdered enset corm sample was ignited to ash at 550°C in a muffle furnace and dissolved in 20% HCl and boiled to bring the ash into solution form. The solution was cooled and filtered through a filter paper (42 mm Whatman) into 100 ml acid washed volumetric flask. The residue was dissolved and transferred to the volumetric flask and the volume was adjusted with distilled, deionised water. Blank solution was prepared in a similar way and the minerals calcium, magnesium, iron, zinc, manganese and copper were determined using atomic absorption spectrometer (Shimadzu, model AA-6800, Tokyo, Japan). Phosphorus was determined by using UV-VIS spectrophotometer (Thermoscientific model; evolution 220, USA) based on AOAC (1990), while, sodium and potassium contents were determined by employing flame photometry (Jenway model; pfp7, UK) as per (Osborne and Voogt, 1978).

Phytochemical analysis

Laboratory procedure for the determination of phytate was as outlined by Latta and Eskin (1980) and later modified by Vaintraub and Lapteva (1988). For analysis of phytate, the supernatant was extracted from 0.5 g sample with 10 ml of 0.2 N HCl and 2 ml wade reagent and then the absorbance at 500 nm was measured using spectrophotometer (Thermo Scientific model; evolution 220, USA). For analysis of tannin concentration, a supernatant was extracted from a 1 g sample and after centrifuging at 1000 g for 5 min, the solutions were mixed with 5 ml vanillin-HCl reagent. Then the absorbance was read at 500 nm on a spectrophotometer, based on Burns (1971) principle, as modified by Maxson and Rooney (1972).

Statistical analysis

The data determined on proximate, mineral and phytochemical contents of five enset landraces with three replications were subjected to analysis of variance using SAS statistical software, version 9.2 (SAS, 2002). When a significant difference existed between the landraces, comparisons of means were made using Duncan's multiple range test at 5% probability levels.

RESULTS

In order to measure the difference in proximate, mineral

Table 2. The mean proximate composition (on dry matter basis) of corms of 3 enset landraces used in traditional medicine and 2 other landraces, for 3 replications.

Enset landrace	Proximate composition (%)					
	Moisture content	Crude fibre	Ash	Crude fat	Crude protein	Total carbohydrate
Astara	68.57 ^a	2.99 ^b	2.97 ^a	0.62 ^a	3.36 ^b	77.62 ^a
Guarye	65.30 ^a	2.93 ^b	2.64 ^a	0.62 ^a	4.06 ^{ab}	79.85 ^a
Kibnar	67.06 ^a	2.38 ^b	2.88 ^a	0.52 ^a	4.74 ^a	75.84 ^a
Amerat	69.06 ^a	4.43 ^a	2.22 ^a	0.64 ^a	3.37 ^b	79.39 ^a
Yeshirakinqe	71.94 ^a	2.87 ^b	3.22 ^a	0.56 ^a	2.42 ^c	77.49 ^a

Value with different superscript in the same column is statistically different at probability $p < 0.05$.

Table 3. The mean mineral composition of corms of three enset landraces used for traditional medicine and two other landraces that have other use values, for 3 replication.

Enset landraces	Mineral composition (mg g ⁻²)								
	Ca	Mg	P	K	Na	Fe	Zn	Cu	Mn
Astara	114.27 ^a	14.31 ^a	127.41 ^a	1440.9 ^{ab}	21.06 ^a	2.83 ^a	8.52 ^a	0.80 ^a	0.92 ^a
Guarye	99.10 ^a	13.69 ^a	116.38 ^{ab}	1160.17 ^c	19.16 ^a	2.66 ^a	4.84 ^b	0.60 ^a	0.72 ^a
Kibnar	97.27 ^a	14.12 ^a	99.97 ^{dc}	1261 ^b ^c	14.58 ^a	2.05 ^a	5.06 ^b	0.53 ^a	0.62 ^a
YK*	101 ^a	12.99 ^a	111.31 ^{bc}	790.8 ^d	17.86 ^a	2.5 ^a	4.49 ^b	0.56 ^a	1.22 ^a
Amerat	114.1 ^a	15.52 ^a	94.76 ^d	1654.2 ^a	22.5 ^a	2.81 ^a	5.22 ^b	0.71 ^a	1.08 ^a

Value with different superscript in the same column are statistically different at probability $p < 0.05$, *YK = *Yeshirakinqe* landrace.

and phytochemical composition of enset corms, three landraces used in traditional treatment of bone fracture and related illness and two other purpose landraces were analyzed.

Proximate composition

Analysis of variance showed significant differences ($p < 0.05$) only in crude fibre and crude protein contents of corms of enset landraces on a dry weight basis as presented in Table 2. The moisture content of the corm samples analyzed varied from 65.3-71.94%, while the crude fibre content was from 2.38-4.43%, significantly highest ($p < 0.05$) value (4.43%) was obtained from *Amerat* enset landrace as shown in Table 2. The ash and crude fat contents of the landraces ranged from 2.22-3.22% and 0.52-0.64% respectively as presented in Table 2. Significantly highest ($p < 0.05$) crude protein contents (4.74 and 4.06%), which are statistically at par, were obtained from the two enset landraces *Kibnar* and *Guarye* respectively, whereas, the lowest (2.42) from *Yeshirakinqe*, no significant difference was observed in the total carbohydrate content of the landraces as presented in Table 2.

Mineral composition

Analysis of variance showed a significant difference ($p < 0.05$) only in phosphorus, potassium and zinc contents

among the five enset landraces analyzed, whereas, no significant difference was observed in calcium, magnesium, sodium, iron, copper and manganese contents as shown in Table 3.

The calcium content of enset corm samples analyzed varied from 97.27 to 114.27 mg g⁻² and no significant difference was observed between the landraces used for traditional medicine and other types of landraces as presented in Table 3. The magnesium content ranged from 12.99 to 15.52 mg g⁻² and there was no significant difference among the landraces, whereas a significant variation ($p < 0.05$) in phosphorus content was observed as shown in Table 3. The highest phosphorus contents (127.41 and 116.38 mg g⁻²) were found from *Astara* and *Guarye* landraces respectively, while the lowest (94.76 mg) from *Amerat* landrace.

Among the landraces, there was a significant difference ($p < 0.05$) in potassium content as shown in Table 3. The highest values (1654.2 and 1440.9 mg g⁻²), which are statistically at par, were obtained from *Amerat* and *Astara* enset landraces respectively. On the other hand, the lowest value (790.8 mg g⁻²) was obtained from the *Yeshirakinqe* enset landrace. The sodium content ranged from 14.58 to 22.5 mg g⁻² and there was no significant difference statistically, while iron content was in a range of 2.05 to 2.83 mg g⁻² as presented in Table 3.

Analysis of variance showed a significant difference ($p < 0.05$) in zinc content of enset landraces, the highest value (8.52 mg g⁻²) was obtained from *Astara* landrace, while the remaining were statistically similar as shown in

Table 4. Comparison of some of the proximate and mineral composition of enset with other root and tuber crops.

Type of food	proximate (%) and minerals (mg g ⁻²)							Reference
	Protein	Fat	Tot CHO	Calcium	Phosphorus	Iron	Zinc	
Enset corm	2.4-4.7	0.5-0.6	75.8-79.9	97.3-114.3	94.8-127.4	2.1-2.8	4.5-8.5	Present study
Sweet potato	2.1-2.8	1.3-1.5	90-91.5	20.7-25.1	5.1-5.5	5.1-10.2	2.2-3.2	Mitiku and Teka (2017).
Taro	0.9-1.7	0.1-0.2	97.6-98	55	Nd*	2.95	1.67	Alcantara et al. (2013) and Tattiyakul et al. (2006).
Yam	3.1-5.4	0.3-1.2	Nd*	31-118.8	15.1-56.5	20.3-69.7	0.5-0.8	Atnafua and Endashaw (2018).
Cassava	1.2-1.8	0.1-0.8	80.1-86.3	32-44	98-118	0.6-7.8	0.7-2.0	Rojas et al. (2007) and Charles et al. (2005).
Irish potato	4.8	1.6	73.8	Nd*	14.4-35.8	1.7-16.4	0.8-2	Abebe et al. (2012) Adegunloye and Oparinde (2017).

Nd* = not determined.

Table 5. The mean phytate and tannin composition of corms of three enset landraces used for traditional medicine and two other landraces, for 3 replications.

Enset landrace	Phytochemical composition (mg g ⁻²)	
	Phytate	Tannin
Astara	172.06 ^a	65.23 ^{bc}
Guarye	149.33 ^a	50.48 ^c
Kibnar	166.04 ^a	153.94 ^a
Amerat	152.12 ^a	103.56 ^b
Yeshirakinqe	195.15 ^a	68.50 ^{bc}

Value with different superscript in the same column are statistically different at probability $p < 0$.

Table 3. The copper and manganese contents were in a range of 0.53-0.8 and 0.62-1.22 mg g⁻² respectively, with no significant difference among the landraces as presented in Table 3.

Comparison of proximate and mineral composition of enset corm with other root and tuber crops

Some of the proximate and mineral compositions of enset corm obtained in the present study were compared with commonly used root and tuber crops (sweet potato, taro, yam, cassava and Irish potato) as reported in Table 4. The protein contents of enset (2.4-4.7%) were slightly lower than yam (3.1-5.4%) and Irish potato (4.8%), but it is better than sweet potato (2.1-2.8%), cassava (1.2-1.8%) and taro (0.9-1.7%). Total carbohydrate (75.8-79.9%) was comparable to Irish potato (73.8%) and cassava (80.1-86.3%), while it was slightly lower than taro (97.6-98%) and sweet potato (90-91.5%). The fat content of enset corm was lower than sweet potato, Irish potato and yam, while it was better than taro and cassava.

The calcium composition of enset (97.3-114.3 mg g⁻²) obtained in the present study was superior to sweet potato (20.7-25.1 mg g⁻²), taro (55 mg g⁻²), yam (31-118.8 mg g⁻²) and cassava (32-44 mg g⁻²), as reported by

different authors in Table 4. The phosphorus content (94.8-127.4 mg g⁻²) of enset was also the highest of all root and tuber crops compared, while the zinc content (4.5-8.5 mg g⁻²) of enset corm was extremely superior to all root and tuber crops. On the other hand, the enset corm showed inferiority in iron content as compared to all root and tuber crops as presented in Table 4.

Phytochemical composition

The phytate contents of the five enset landraces corm ranged from 149.33-195.15 mg g⁻² but there was no significant difference among the landraces as shown in Table 5. Analysis of variance showed a significant difference ($p < 0.05$) among enset landraces in their tannin content as presented in Table 5. The highest tannin content (153.94 mg g⁻²) was observed in *Kibnar* landrace, and it was followed by *Amerat* landrace. The remaining three landraces did not show a significant difference.

DISCUSSION

Proximate composition

The moisture contents obtained were by far lower than

the value (85.92%) reported by Mohammed et al. (2013) for unspecified enset landrace, the variation could be due to varietal, age, harvesting period or environmental difference (Alphonse et al., 2018). The crude fibre contents obtained in the current study were slightly lower than the value (5.65%) reported by Mohammed et al. (2013) for unspecified enset landrace, whereas, quite lower than 17.4% reported by Tadessa and Masayoshi (2016) for *Naqaqa* enset landrace of unspecified age. The wider variations could be related to genotype growing, environment or harvesting period differences. The ash content was similar to the value (3.2%) reported by Tadessa and Masayoshi (2016) for *Naqaqa* enset landrace. Whereas, this finding was quite lower than the value (5.2%) reported by Forsido et al. (2013) for the *Nobo* enset landrace, the variation could be due to genotype, growing environment or harvesting period differences (Alphonse et al., 2018).

The current result on crude fat content was similar to the value (0.6%) reported by Tadessa and Masayoshi (2016) for *Naqaqa* enset landrace, whereas, Mohammed et al. (2013) reported 0.4%. On the other hand, relatively higher fat content (1.24%) of the *Nobo* enset landrace was reported by Forsido et al. (2013). The wider variation could be related to the difference in genotype, age, growing environment or harvesting period (Alphonse et al., 2018). The highest protein content was obtained from the two enset landraces used in traditional treatment of bone breakage (*Kibnar* and *Guanye*). Probably this could be the reason for selective use of these landraces for the treatment of bone fracture and breakage, traditionally. Mohammed et al. (2013) reported comparable crude protein content of 3.33% to the current finding from corms of unspecified enset landrace. However, larger crude protein contents of 8.3% from *Naqaqa* and 8.23% from *Nobo* landraces were reported by Tadessa and Masayoshi (2016) and Forsido et al. (2013) respectively. In terms of total carbohydrate content, the current finding was superior to the value (64.8%) reported by Tadessa and Masayoshi (2016) for *Naqaqa* enset landrace. The variations could be related to genotype, environmental or harvesting period difference (Alphonse et al., 2018).

Mineral composition

As opposed to the hypothesis, no significant difference in calcium content was observed among enset landraces used for traditional medicine and the other landraces. Comparable calcium contents of 99.7 mg g⁻² from *Neqaqa* and 100 mg g⁻² from *Nobo* enset landraces were reported by Tadessa and Masayoshi (2016) and Forsido et al. (2013) respectively. On the other hand, Ajebu et al. (2008) reported calcium contents of ten enset landraces ranging from 50-200 mg g⁻² with an average calcium content of 130 mg g⁻². The variation could be related to the genotype or growing environment difference (Ajebu et

al., 2008; Wekesa et al., 2014). As compared to the current finding, larger magnesium contents (63.2 mg g⁻²) from *Naqaqa* and (60-260 mg g⁻²) from ten different landraces were Tadessa and Masayoshi (2016) and Ajebu et al. (2008) respectively. The variations could be related to genotype or environmental difference (Ajebu et al., 2008; Wekesa et al., 2014).

The highest phosphorus contents were obtained from *Astara* and *Guanye* landraces, which are used in the traditional treatment of bone fracture and breakage. This could be the reason for the selective use of the landraces for the mentioned traditional medicine, as the presence of phosphate is crucial for bone growth and mineralization (Penido and Alon, 2012). The current finding was in line with different findings. Forsido et al. (2013) reported 90 mg g⁻² from *Nobo* landrace and Mohammed et al. (2013) reported 110 mg g⁻² phosphorus content of unspecified enset landrace. Whereas, Ajebu et al. (2008) reported in a range of 80-140 mg g⁻² and average value of 120 mg g⁻² phosphorus content from ten enset landraces. According to Ajebu et al. (2008), the phosphorus content of corms from *Astara* landrace was 80 mg g⁻², which is quite lower than the current finding (127.41 mg g⁻²); the variation could be due to the difference in the growing environment (Wekesa et al., 2014) or it may be related to giving the same vernacular name for different enset landraces (Endale, 1997).

Similar to the current finding, a wider variation in potassium content (860-3050 mg g⁻²) of enset landraces was reported (Ajebu et al., 2008). According to Forsido et al. (2013), the potassium content was reported to be 2180 mg g⁻². In all cases, it was shown that enset corm is very rich in potassium content. The sodium content was in the same range with the values 4, 23 and 15 mg g⁻² reported by Ajebu et al. (2008) for ten enset landraces and Forsido et al. (2013) for *Nobo* landrace respectively. On the other hand, smaller value of 5.2 mg g⁻² for enset corms of *Neqaqa* and 3 mg g⁻² for unspecified enset landraces were reported by Tadessa and Masayoshi (2016) and Mohammed et al. (2013) respectively. These variations reported could be related to the difference in landrace, growing environment or age of sampled plants (Ajebu et al., 2008; Wekesa et al., 2014). The iron content of enset corm was found to be small in general, but it was higher than the value (0.7 mg g⁻²) reported by Yewelsew et al. (2007) in boiled unspecified enset landrace. However, it was smaller than the value (12.3 mg g⁻²) reported by Tadessa and Masayoshi (2016) for *Naqaqa* landrace. This variation could either be due to the genotype or growing environment difference (Ajebu et al., 2008; Wekesa et al., 2014) or boiling of the corm may result in the reduction of iron content (Bethke and Jansky, 2008).

Astara enset landrace was the best in zinc content of all other landraces. The selective use of this landrace in traditional treatment of bone fracture or breakage, probably related to its richness in Zn content, as scientific

findings on the synergetic stimulatory effect of zinc on the healing of bone, was reported by Kaya et al. (2015). Lin et al. (2018) also reviewed that zinc plays a major role in regulating every phase of the wound healing process. The highest zinc contents (12.3 and 7.3-22.6 mg g⁻²) of different landraces were reported by Tadessa and Masayoshi (2016) and Ajebu et al. (2008) respectively, while smaller value (1.33 mg g⁻²) by Yewelsew et al. (2007). The variation could be related to the difference in genotype (Ajebu et al., 2008), the environment (Wekesa et al., 2014) or processing form (Bethke and Jansky, 2008).

The copper content was within a range of the findings (0.28-1.17 mg g⁻²) reported by Ajebu et al. (2008) on ten enset landraces and it was comparable to the values (0.7 mg g⁻²) from *Naqaqa* and (0.52 mg g⁻²) from *Nobo* landraces reported by Tadessa and Masayoshi (2016) and Forsido et al. (2013) respectively. The current finding in manganese content was quite lower than the values (4.33 and 5.84 mg g⁻²) reported by Mohammed et al. (2013) and Tadessa and Masayoshi (2016) respectively, the variation could be related to genotype or environmental difference (Ajebu et al., 2008; Wekesa et al., 2014).

Comparison of proximate and mineral composition of enset corm with other root and tuber crops

As compared to other root and tuber crops, enset corm was found to be lower in protein, while comparable in total carbohydrate content as presented in Table 5. Traditional healers usually recommend the inclusion of animal products rich in protein, like milk, yogurt and meat together with enset corm for treating bone breakage; probably they have indigenous knowledge about the protein deficiency of enset products. It was also observed that enset corm showed a better performance in calcium content than most of the root and tuber crops, and the highest of all root and tuber crops compared in phosphorus contents. It was also extremely superior to all root and tuber crops in zinc content, while it showed inferiority in iron content as shown in Table 5. The zinc content of enset corm is even much higher than teff, which is known to have good zinc content (Tadessa and Masayoshi, 2016). In areas where enset serves as a staple food, therefore, strategic supplementation of foods rich in protein, fat and iron is required to alleviate the deficiencies.

Phytochemical composition

Studies on phytate and tannin contents of on enset products are very scanty. Hiwot (2015) reported a lower phytate content (84.25-112.56 mg g⁻²) of another enset product, *bulla*. Enset corm showed relatively higher phytate content than the value (115.43 mg g⁻²) reported on taro (Gulelat et al., 2013). The highest phytate content

may have an implication on human health. Although numerous studies have described the negative effect of phytate on the availability of minerals, a number of dietary components that can counteract its inhibitory effect were reviewed (Schlemmer, 2009). In addition to its calcification inhibitory effect, the author reviewed anticancer and antioxidative activities of phytate.

Recently, interest in food phenolic and tannins has increased greatly due to their antioxidant capacity and their possible beneficial implications in human health. The antimicrobial activities of tannins are well documented (de Sousa et al., 2015; Nouioua et al., 2016). The recommendation to consume *Kibnar* landrace corm at the first stage of bone illness treatment traditionally, is probably due to its highest tannin content, which would be important to protect infection. Haslam (1996) also reported that plants rich in tannins are used in traditional medicine as drugs for the treatment of various organic diseases, including the healing process of wounds, burns and inflammation.

Conclusion

This study revealed that the three enset landraces (*Astara*, *Guarye* and *Kibnar*) used for traditional medicine are significantly different from the other two landraces used for other purposes, in protein, phosphorus, zinc and tannin contents in general; and *Astara* landrace is superior in most of the proximate and mineral contents. As compared to other root and tuber crops, enset showed the highest calcium, phosphorus and zinc content and comparable in protein and carbohydrate composition, while its iron and fat content was quite smaller. The selective use of *Astara*, *Guarye* and *Kibnar* landraces, rather than other landraces, for traditional treatment of bone fracture, bone breakage and joint displacement in enset growing society is probably related to their superiority in protein, phosphorus, zinc and tannin contents. As there is only little information on the composition of corms of enset landraces used in traditional medicine, this study can be an important input for future study and management the landraces.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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