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Full Length Research Paper

Performance of segregating populations of feijoa cultivated under the agroforestry systems in southern Brazil

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In the south of Brazil, the development of agroforestry systems (AFS) lacks knowledge about the adaptation of species. Feijoa [*Acca sellowiana* (O. Berg) Burret] is a fruit species, listed as being capable to use for AFS. In order to evaluate the performance of segregating populations derived from two crosses between selected genotypes (C1 and C2), established experimental plots under AFS in five farms were evaluated with traits of indicative vegetative growth, earliness and yield. The average mortality was 46% influenced by the cultivation environment with no difference between progenies of the crosses C1 and C2. The cultivation environment here is defined by soil and climate conditions of each site, as well as the structure of the AFS and the management adopted by the farmer. Plants crossing C1 flourished and produced nearly as twice as many fruits as compared to C2. C1 in general, showed greater variability and better performance for evaluated traits at Paraí location, a place that best discriminate the segregating populations. Thus, selection can be made and should be prioritized for the progeny from crossing C1 at Paraí cultivation environment that presented the best contrasts.

Key words: *Acca sellowiana*, Goiabeira-serrana, genotype × environment interactions, pineapple guava.

INTRODUCTION

The agroforestry systems (AFS) are designed in a process of "co-domestication" of tree species in a typically human activity (Wiersum, 1997). The concept of AFS was developed around 1970 and was based in the inclusion of agricultural crops in the forests or the incorporation of trees in farming systems, (Wiersum, 2004). For Nair (1993), AFS is "the cultivating purpose or the deliberate retention of trees with crops and/or animals in interactive combinations to multiple production or benefits from the same management unit".

For AFS to succeed, it must choose the tree species according to local conditions of climate, soil and topography. However, with much relevance in relation to said priority species generating income, it is necessary to have access to quality genetic resources (Dubois, 2008). For altitude areas of southern Brazil, the development of

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AFS is still dependent on knowledge of the arrangement and adaptation of species. Besides “mate” (*Ilex paraguariensis*), araucaria (*Araucaria angustifolia*) and bracatinga (*Mimosa scabrella*) (Ilany et al., 2010; Moreira et al., 2011) other few species of these regions were studied to be used in AFS.

Based in its attributes, the feijoa (*Acca sellowiana* (O. Berg) Burret) also called pineapple-guava has been reported as one of the most promising species (Della Mea et al., 2009). It is a perennial fruit belonging to the *Myrtaceae* family, from the Southern Brazilian plateau and northern Uruguay (Mattos, 1990) and found also in Argentina (Keller and Tressens, 2007). In its natural habitat in Brazil, feijoa occurs more often in woods type formations (capons) and araucaria forests at altitudes between 900 and 1100 m above the sea level; however, with good development at lower altitudes of 700 m above the sea level (Lorenzini et al., 2007). It presents distinctive flavor, sweet-acidic and aromatic fruits (Mattos, 1990) which along with adaptation to cold climates and altitudes are characteristics that makes it attractive for cultivation in these sites (Santos et al., 2005).

Since the species is in the early stage of domestication in its naturally occurring area (Santos et al., 2005), one of the needs for its use is the availability of adapted genotypes to farmers cultivation site, in addition to presenting desirable fruit features. Thus, for commercial purpose, it is important that the selected varieties meet superior characteristics to the plants in their natural state with regard to productivity, stability in production and fruit quality (Degenhardt et al., 2005). Among the fruit species, the results of the breeding programs have been advanced in several species, especially peaches, grapes, citrus, apples, figs, pears and some that are not so common, like acerola, guava, cherimoyas and passion fruit (Pommer and Barbosa, 2009).

Although, feijoa cultivars has been launched in Brazil (Ducroquet et al., 2007, 2008), little is known about its cultivation in AFS. Also, under orchard conditions distant from biodiverse reality of the ecosystem of origin, often presents biotic disturbances with insects such as fruit flies and weevils (Hickel and Ducroquet, 1992) and fungi such as anthracnose (Ducroquet and Ribeiro, 1996), these problems could be alleviated by cultivation in AFS.

Thus, the objective of this study was to evaluate the performance of segregating populations from two crosses between feijoa superior genotypes, through the establishment of experimental plots under AFS in farmers’ fields to enable the selection.

MATERIALS AND METHODS

Progenies of two crosses made by the Agricultural Research and Rural Extension Agency of Santa Catarina (Epagri – SC) were used. The three parental were chosen among the elite genotypes of feijoas from the Active Germplasm Bank of São Joaquim; SC for fruit size, pulp yield, total soluble solids content and tolerance to biotic agents). The first cross (C1) involved the genotypes ≠101 (female) and ≠458 (male) and the second cross (C2) was performed between the genotypes ≠125 (female) and ≠458 (male). The crosses were made in 2007 and the seedlings obtained in 2008, and thereafter transplanted to the field experiments in November of the same year. At that time, the seedlings presented 10 leaves between 8 and 13 cm in height for C1 and 8 leaves between 6 and 11 cm in height for the C2 without branching.

The experiments were established in five farmers’ fields under different AFS design (see description below), in five countries of Rio Grande do Sul, Brazil. The seedlings, distributed according to available area were planted by farmers. Seedling pruning or foliar spray during the evaluation period was not done to allow the full expression of the traits under AFS conditions. The handling of ruderal herbs and fertilizers were particular to each of the four AFS.

The cultivation environments were:

i. Antônio Prado – (28° 50’42.2” S; 51° 15’10.9” W; Altitude: 690 m) - Located in permanent protection area on the edge of a stream which exits the bed with some frequency, previously an annual cropping area. The soil is of low fertility. AFS has the goal of restoring riparian vegetation and fruit production to marketing. Fruit species (native and exotic) were also planted. From planting until 2010, when each plant was fertilized with 3 to 4 kg of organic compost, the management practices consisted only of weeding. AFS is exposed to the west, and the plants have direct light from the sun only in the afternoon.

ii. Nova Bassano – (28° 42’ 23” S; 51° 41’ 21” W; Altitude: 640 m) - Area intended diversified crops was ecologically transformed into AFS, taking the quince as a species of principal interest. The transplant took place in a period of severe drought. Despite several watering after planting in the second evaluation, it was verified that no seedling survived and the site was withdrawn from further evaluations.

iii. São Domingos do Sul – (28° 31’16.3” S; 51° 52’47.9” W; Altitude: 685 m) - Area with steep slope, formerly cultivated with sugar cane and abandoned. It is of low fertility, northern exposure, and it is in the initial phase of regeneration of native vegetation. Onsite lime seedlings were planted in 2007 and 2008 as species of economic interest inset to native vegetation. Regeneration of natural species is managed with mowing them so as to maintain a suitable level of shading in the view of the farmer. No fertilizer were added other than nutrient cycling. The planting was done with good soil moisture conditions. Although, there was a period of severe drought after planting, watering was not performed. The shade level estimated in the fifth evaluation was approximately 40%.

iv. Paraí – (28° 34’47.2” S; 51° 47’27.4” W; Altitude: 645 m) – A flat terrain area was used to organically crop annual species (peanuts, beans and corn, among others) for several years. In 2007 and 2008, AFS was implemented with several tree species (native and exotic) for various purposes from fruit to lumbering. The management consisted only of weeding. In the winter of 2009 a severe frost decimated much of the tree species, except feijoa, which were replaced in the next year, or held the regrowth when present. The area is lush natural fertility. The level of shading was assessed on the fifth review of full sun.

v. Sananduva – (27° 56’59” S; 51° 48’24” W; Altitude: 636 m) - Crop area with good natural fertility, clay soil and flat terrain. Native and exotic species (fruit, timber and fertilizer plants) were planted in 2007 in line to form an AFS in alleys. In the following summer and winter the area received green manure formed by a mixture of species (sunflower, corn, sun hemp, oats, vetch and radish, among others). Also, vegetable products and annual crops (cassava, sweet potatoes, beans, and cucurbits) were cultivated. The feijoa plants were inserted between the other species in the rows and received between 1 and 2 kg/seedling of organic compost and 300 g of powder of crushed rock (basalt). However, there was a period of drought after three weeks of planting, when the plants were

Table 1. Percentage of mortality of progeny plants of two crosses of Feijoa evaluated in five different locations in Rio Grande do Sul, under AFS from 3 to 39 months of transplantation.

Cross	Location	n	Age of evaluation (months)				
			3	9	17	28	39
	Paraí	09	0	0	0	0	0
	Antônio Prado	08	0	0	0	0	0
C1	Nova Bassano	08	38	100	100	100	100
	Sananduva	13	46	-	62	62	62
	São Domingos	12	58	75	75	75	75
	Paraí	08	0	0	0	0	0
	Antônio Prado	08	0	0	0	0	0
C2	Nova Bassano	09	33	100	100	100	100
	Sananduva	13	39	-	69	69	69
	São Domingos	12	0	25	25	25	25
	Paraí	17	0	0	0	0	0*
	Antônio Prado	16	0	0	0	0	0
C1 + C2 (n=100)	Nova Bassano	17	35	100	100	100	100
	Sananduva	26	42	-	65	65	65
	São Domingos	24	29	50	50	50	50
C1		50	32	-	50	50	50^{ns}
C2		50	16	-	42	42	42
Grand Total		100	24	-	46	46	46

Only data of C1+C2 from 39 months after transplantation were submitted to the χ^2 test; *, ^{ns} = Significant (P<0.05) and not significant (P<0.05) deviation by the χ^2 test in the column, only at 39 months.

irrigated three times. In 2009, each native tree received 2 kg of poultry manure as fertilizer. The estimated level of shading in the fifth review was full sun.

The feijoa plants were evaluated for: height in centimeters, measured from the ground level to the apex of the plant; crown diameter in centimeters, obtained by averaging the diameter in the longitudinal and transverse to the line of cultivation; basal area of the stem of the plant in square centimeters, obtained from the diameter of the stem of the plant at five centimeters of soil, considering the ramifications when present; death rate; number of side branches at the stem of the plant; number of plants that flowered; number of plants showing fruiting and fruit yield, according to these categories, (Degenhardt et al., 2005) (Class 1 < 10, Class 2 = 11 - 40, Class 3 = 41 - 80, Class 4 = 81 - 120, Class 5 = 121 -160 and Class 6 > 160) the last three variables were taken only on season 2010/2011 and 2011/2012.

Assessments occurred five times: 3, 9, 17, 28 and 39 months after transplanting which occurred in November, 2008 being the last assessment conducted in February, 2012 when the analysis of soil fertility was performed. The assessments at 9 months in Sananduva and at 17 months in São Domingos do Sul were not conducted.

Data were subjected to descriptive statistics and the coefficient of phenotypic correlations were estimated by the PROC CORR procedure, using the statistical package SAS® 9.1.3 (2007). Non-orthogonal contrasts were estimated between crosses and location, and pair-to-pair for the other variables. For this evaluation, all data were analyzed by the GLM procedure of the statistical system SAS® 9.1.3 (2007). The variables mortality, fruiting and flowering were assessed qualitatively and tested with χ^2 in contingency table to determine the association between the effect of location and crosses.

RESULTS AND DISCUSSION

The final rate of mortality was not affected by crossing with C1 50 % and C2 42%, and an overall average for the experiment of 46% (Table 1). However, there was an association between seedling mortality and cultivation environments defined here as the soil and climate conditions of each site, the structure of AFS and the management adopted by the farmer. In the environments of Antônio Prado and Paraí, no plants died while in Nova Bassano all died but at Sananduva and in São Domingos do Sul the mortality were up to half of the planted seedlings (Table 1). The planting in November and the severe drought that occurred in that year (2008/2009) were probably the factors that most contributed to the high mortality, while from the nine months of transplantation there was no trend in the mortality rate.

The results showed that there were significant (P<0.05) differences between the values of means, variances and amplitudes among the progeny of crosses evaluated in four cultivation environments in Rio Grande do Sul

Table 2. Means, standard deviations, maximum and minimum values, variances and amplitudes of the traits of the plant height in cm (PHE), crown diameter (DIA), number of branches (NB), basal area (BA) and fruit yield (FY) for two crosses (C1 and C2) of Feijoa at 39 months after transplantation.

Genotype	Parameters	Characteristics				
		PHE (cm)	DIA (cm)	NB (un)	BA (cm^2)	FY (un)
C1	Mean	188	164	2.1	14.9	3.4
	Standard deviation	31	51	1.5	7.3	2.0
	Minimum	140	65	1.0	4.5	1.0
	Maximum	230	290	6.0	29.1	6.0
	Variance	971	2615	2.4	52.8	3.8
	Amplitude	90.00	225	5.0	24.6	5.0
C2	Mean	179	129	1.0	10.2	1.2
	Standard deviation	51	50	0.0	7.4	0.4
	Minimum	70	20	1.0	0.6	1.0
	Maximum	280	200	1.0	32.2	2.0
	Variance	2641	2482	0.0	54.4	0.2
	Amplitude	210	180	0.0	31.5	1.0
General	Mean	183	145	1.5	12.4	2.2
	Standard deviation	43	53	1.2	7.6	1.8
	Minimum	70	20	1.0	0.6	1.0
	Maximum	280	290	6.0	32.2	6.0
	Variance	1855	2797	1.4	58.3	3.1
	Amplitude	210	270	5.0	31.5	5.0

(Table 2). The means of the progenies of the cross C1 were higher for all traits compared to the average of the progenies of the cross C2 considering the genotypes *per se*. Regarding the amplitude and variance of the data, it can be verified that both values were higher in the C1 progeny for traits crown diameter (DIA), number of branches (NB) and fruit yield (FY). However, for the other traits, they were lower than in C2. In the C2 progenies, variance values were low for the traits NB, since their plants showed no branching and FY (Table 2).

Thus, there are presence of variability among crosses, although it cannot be completely isolated from the interaction between genotype x environment. In addition, the selection within the progeny of C1 can be more effective because, in general, there is greater variation between plants in comparison with C2 cross. However, depending on the breeder or a farm demands, gains from selection on the plant height (PHE) may be bigger when selection is practiced within the progeny of C2. This will depend on the type of plant being targeted and the fining evaluation of fruit and productivity characteristics. In addition to the higher values of the variances, the data amplitude in the segregating populations of both crosses reveals that the selection of promising individuals in segregating families is possible, since there are differences between individuals within the families. Analyzing amplitude values for the trait plant height, as an example, individuals were observed between 140 and 230 cm (C1) and between 70 and 280 cm (C2), which may provide the breeder an opportunity of selection for greater or lesser stature. It is worth noting that the differences between and within the progeny may have two origins, genetics and environmental, and the plants considered as the best can be propagated vegetatively, allowing to set the desired trait by fixing the genetic gain towards clonal propagation.

In order to verify the differences between the average of the crosses and growing environments, orthogonal contrasts were estimated (Tables 3 and 4). The progenies of C1 showed statistical differences ($P<0.05$) for all characters except plant height (Table 3). This result may be showing the superiority of progenies from this cross, considering the evaluated variables. Regarding four cultivation environments, significant differences were observed for almost all characters. It may be noted that, the Antônio Prado and Sananduva environments were differentiated from each other, and Paraí and São Domingos do Sul presented the greatest discrepancy. It is also important to highlight that the plants from Paraí environment had the highest averages for all evaluated traits with significant contrasts in relation to other cultivation environments, except for height (PHE) with the Sananduva environment. This result indicated that the cultivation environment is critical to the superiority or

Table 3. Contrasts pair-to-pair between two crossings and four environments for the characters plant height in cm (PHE), crown diameter (DIA), number of branches (NB), basal area (BA) and fruit yield (FY) for two crossings of Feijoa at 39 months after transplantation.

Crosses × Locals	Means				
	PHE (cm)	DIA (cm)	NB (un)	BA (cm^2)	FY (un)
Cross. 1	188	164	2.1	14.9	3.4
Cross. 2	179	129	1.0	10.2	1.2
Antônio Prado	162	136	1.4	10.3	1.7
Paraí	221	199	2.2	19.6	3.7
São Domingos	138	79	1.0	3.9	1.0
Sananduva	210	148	1.1	13.6	2.1
Contrasts			Pr>F*		
C1: Cross. 1 x Cross.2	ns	0.0154	0.0003	0.0226	<000.1
C2: A.Prado x Paraí	<000.1	<000.1	0.0282	<000.1	0.0004
C3: A.Prado x S.Domingos	0.0204	<000.1	ns	0.0016	ns
C4: A.Prado x Sananduva	<000.1	ns	ns	ns	ns
C5: Paraí x S.Domingos	<000.1	<000.1	0.0042	<000.1	<000.1
C6: Paraí x Sananduva	ns	0.0001	0.0159	0.0047	0.0145
C7: S.Domingos x Sananduva	<000.1	<000.1	ns	<000.1	ns

*Significant at the level of 5% by F test; ns = not significant (P<0.05).

Table 4. Pairwise contrasts between two crossings in four environments for the characters plant height in cm (ALT), crown diameter (DIA), number of branches (NB), basal area (BA) and fruit yield (FY) for two crossings of Feijoa at 39 months after transplantation.

Crosses x Locations	Means				
	PHE (cm)	DIA (cm)	NB (un)	BA (cm^2)	FY (un)
Cross.1 Antônio Prado	164	138	1.2	11.2	2.3
Cross.2 Antônio Prado	160	135	1.0	9.3	1.1
Cross.1 Paraí	210	218	3.3	20.8	5.6
Cross.2 Paraí	233	179	1.0	18.4	1.5
Cross.1 São Domingos	148	88	1.0	4.8	1.0
Cross.2 São Domingos	134	76	1.0	3.6	1.0
Cross.1 Sananduva	210	154	1.2	16.1	3.0
Cross.2 Sananduva	210	140	1.0	10.3	1.0
Contrasts			Pr>F*		
C1: Cross.1 x Cross.2 A. Prado	ns	ns	ns	ns	0.0035
C2: Cross.1 x Cross.2 Paraí	ns	0.0066	<0.0001	ns	<0.0001
C3: Cross.1 x Cross.2 S. Domingos	ns	ns	ns	ns	ns
C4: Cross.1 x Cross.2 Sananduva	ns	ns	ns	ns	0.0002

*Significant at the level of 5% by F test; ns = not significant (P>0.05).

otherwise of a given genotype, and it is necessary to evaluate the progenies in different cultivation environments before any recommendation. Since the effect in years is prominent in the species (Degenhardt et al., 2003, 2002), it is also prudent to monitor over time.

Additionally, the progenies of all crosses were

Table 5. Pearson's coefficients of phenotypic correlations (above the diagonal) and respective probability values (below the diagonal) for the traits plant height (PHE), crown diameter (DIA), number of branches (NB), basal area (BA) and fruit yield (FY) for two crossings of Feijoa.

Trait	PHE (cm)	DIA (cm)	NB (un)	BA (cm^2)	FY (un)
PHE (cm)	1.00	0.73	0.13	0.72	0.36
DIA (cm)	<0.0001	1.00	0.49	0.83	0.66
NB (un)	0.3203	0.0001	1.00	0.45	0.64
BA (cm^2)	<0.0001	<0.0001	0.0005	1.00	0.58
FY (un)	0.0074	<0.0001	<0.0001	<0.0001	1.00

compared pairwise within a cultivation environment in order to provide an understanding of the effect of the environment on their average behavior (Table 4). It can be seen that the Crosses C1 and C2 in general were not disparate in the Antônio Prado, São Domingos and Sananduva cultivation environments, where plants were statistically different for only one character (fruit yield). In turn, the Paraí environment allowed the largest number of statistical differences between the crosses, since the average values of the progenies of the crosses were distinct in this environment for crown diameter (DIA), number of branches (NB) and fruit yield (FY) (Table 4).

Thus, we can say that C1 was precocious for all environments, except for São Domingos where plants did not go into the reproductive phase for both crossings. Taking into account the vegetative growth, plants of C2 do not exhibit ramifications. The opposite occurred with C1 in the three cultivation environments which also resulted in plants with larger diameter at C1 in Paraí. Thus, the overall results indicated that the selection of individuals should be prioritized in the Paraí cultivation environment because selection is more pronounced in the environment that best discriminate the variables of the evaluated genotypes. Due to the similarity of the results, it is not necessary that one can be eliminated in the simultaneous selection in the Antônio Prado and São Domingos environments.

An important evaluation for the improvement of any species is the correlation between the characters. The existence of the positive and significant phenotypic correlation coefficients between all characters can be verified, except PHE × NB (Table 5). This demonstrates that the increase in height is accompanied with an increase in DIA, BA and FY (Table 5). However, the highest values of correlation coefficients were observed between the characters DIA × BA (0.83), PHE × DIA (0.73) and PHE × BA (0.72) and, consequently, those which have practical use. However, this will depend on the desired architecture of plant. Since feijoa shortest plants are most suitable for fruit harvesting, the correlation between PHE and FY may hamper genetic progress for higher productivity.

The onset and intensity (proportion of plants) of flowering and fruiting were dependent on both the crossing and the cultivation environment (Table 6). In the season of 2010/2011, two years after transplantation, 40% of the C1 plants flowered and 36% fructified, while only 3.4% of the C2 plants fructified (Table 6). In the following season, (2011/2012) three years after transplantation, approximately 90% of the C1 plants flowered and fructified. This performance reflects the genetic effect and may be related to a greater average diameter of the crown and higher average basal area of the stem (Table 3). This indicates that this family of siblings has a higher initial rate of growth and more earliness at the same time. Because it is segregating, progeny allows selection that can be done by the farmer themselves. Overall, the earliness was affected by genotype and by environment.

Importantly, the effect of cultivation environment was prominent for the beginning and intensity of flowering and fruiting (Table 6). In Paraí, in the season of 2010/2011, all plants of the C1 crossing flowered and fructified compared with 12.5% of C2 crossing, which amounted to almost 60% of plants for this environment. In addition, except for 6.3% of the plants of Antônio Prado (all of C1), no other plant bloomed or fructified this season (Table 6). For this cultivation environment, as shown above, except for plant height in relation to Sananduva, all other evaluated variables for growth were significantly higher, possibly by better fertility condition, especially phosphorus, presented by the soil from Paraí (Table 7). In the following season, (2011/2012) in Paraí and Antônio Prado, all plants flowered and most of them fructified, and in Sananduva 100% of the plants from the C1 and 25% of the C2 flowered and fructified (Table 6). In São Domingos do Sul, no plant bloomed until the 39 months of the transplant. In this cultivation environment, all evaluated parameters for growth were significantly lower in comparison to other environments, especially crown diameter and basal area of the stem, which may have influenced or not on the early reproductive stage. The lower levels of phosphorus in the soil, considered very low by soil analysis, coupled with the fact that this environment has not received any fertilizer, which may partly explain the delay in flowering. Besides soil fertility, the highest level of shading in São Domingos do Sul (40%) may have contributed to this behavior as well,

Table 6. Percentage of plants that flowered and fructified on seasons 2010/2011 and 2011/2012 derived from progeny of two crosses of Feijoa established in 2008 under AFS in four different places in Rio Grande do Sul.

Crosses/Locations	Flowering 2010/2011	Fructification 2010/2011	Flowering 2011/2012	Fructification 2011/2012
C1	40.0**	36.0**	88.0*	84.0**
C2	3.4	3.4	58.6	37.9
Paraí	58.8**	58.8**	100.0**	94.1**
Antônio Prado	6.3	0.0	100.0	68.8
Sananduva	0.0	0.0	66.7	55.6
São Domingos	0.0	0.0	0.0	0.0
C1/Paraí	100.0**	100.0**	100.0**	100.0**
C1/Antônio Prado	12.5	0.0	100.0	100.0
C1/Sananduva	0.0	0.0	100.0	80.0
C1/São Domingos	0.0	0.0	0.0	0.0
C2/Paraí	12.5ns	12.5ns	100.0**	87.5**
C2/Antônio Prado	0.0	0.0	100.0	37.5
C2/Sananduva	0.0	0.0	25.0	25.0
C2/São Domingos	0.0	0.0	0.0	0.0

* and ** = P<0.05 e P<0.01, respectively, of the adhesion to the χ^2 test in the column; ns = not significant (P>0.05).

Table 7. Soil analysis and interpretation criteria of the soils from the AFSs in the four locations studied.

Parameter	Locations			
	Paraí	Antônio Prado	Sananduva	São Domingos do Sul
Texture (% clay)	25 (Class 3)	35 (Class 3)	67 (Class 1)	19 (Class 4)
pH	5.7 (Medium)	5.8 (Medium)	5.9 (Medium)	5.6 (Medium)
SMP	6-	6.4	6.1	6.1
P (ppm)	>50 (Very High)	13.4 (High)	23.2 (Very High)	4.1 (Very low)
K (ppm)	250	133	192	226
MO (%(m/v))	5 (Medium)	2.1 (Low)	3.5 (Medium)	3.7 (Medium)
Al (cmolc/l)	0	0	0	0
Ca (cmolc/l)	10 (High)	4.1 (High)	6.7 (High)	8.4 (High)
Mg (cmolc/l)	1.6 (High)	1.6 (High)	3.2 (High)	1.7 (High)
Na (ppm)	39	24	29	31
H + Al (cmolc/l)	4.36	2.75	3.89	3.89
Sum Base (cmolc/l)	12.42 (High)	6.15 (High)	10.52 (High)	10.82 (High)
CTC (cmolc/l)	16.789 (High)	8.9 (Medium)	14.41 (Medium)	14.71 (Medium)
Saturation Bases (%)	74.02 (Medium)	69.1 (Medium)	73 (Medium)	73.56 (Medium)

Source: Physical Chemical and Biological Laboratory of the Integrated Company of Agricultural Development of Santa Catarina.

since the plants had lower growth and did not enter into the reproductive phase, while in other places, the plants have thrived practically at full sun. It is not yet scientifically enlightened about the adaptation of the plant with shading, although rarely they do occur in areas with very high levels of shading and the plants adapt preferably in open areas or on the edge of forests.

Thus, one can postulate that the entry in the reproductive period has the influence of genotype; however the conditions of feijoa plant development, that is, the soil and climatic characteristics of the site and the structure and management of AFS (environment), are also factors preponderant in this process. Whereas the main goal of using feijoa is for fruit production, which is necessary to continue the evaluations to meet with the robustness of the reproductive phase of this perennial plant, since the AFS are long-term experiments.

Conclusion

The progenies of the cross C1 (≠101 x ≠458) showed higher variation and better performance for the analyzed growth and reproductive traits. The site (soil and climate conditions, the AFS' structure and the management practices adopted) influenced the survival, growth and precocity of the plants. Paraí is the environment that best discriminated the segregating populations. Overall, feijoa can be used in distinct AFS systems.

Conflict of Interest

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effects of packaging material and seed treatment on Weevil (*Callosobruchus maculatus* (F) Coleoptera: Bruchidae) infestation and quality of cowpea seeds

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This study was conducted to determine the effects of seed treatments using Neem leaf powder, pepper fruit powder, camphor, groundnut oil and wood ash in combination with five different packaging materials (cloth bag, paper bag, glass bottle, polythene bags and plastic container) on the viability and germination vigor of cowpea seeds. The experiment was laid out in a completely randomized block design in a 5 by 6 factorial with three replications. 1 kg of dried cowpea seeds with a uniform moisture content of 11% were stored for 6 months in different packaging materials in combination with six different seed treatments: No treatment (control), Camphor (Chemical treatment) -4 g/kg of seeds, Neem (*Azadirachta indica* L.) leaf powder (Botanical) -10 g/kg of seeds, Groundnut Oil / Palm oil - 5 ml/kg seeds, Powdered Dry pepper (Capsicum spp) -10 g/kg of seeds and wood ash 1 kg/kg of seeds. Indoor temperatures were between 25.3 and 30.7°C, outdoor temperatures between 26.1 and 31.1°C and relative humidity between 61.0 and 82%. Results obtained from the study showed that packaging material and seed treatment had high significant effect on the vigor and germination percentage of cowpea seeds. Germination of seeds treated with powdered pepper (73%) and neem leaf powder (72.5%) were significantly higher than other treatments. Seeds stored in plastic containers had the highest vigor and germination percentages (61.1 and 77.1% respectively) followed by glass bottle (60.3 and 72.2% respectively) after 6 months storage period. It seems that for better storage of cowpea seeds for a period not exceeding 6 months it is preferable to use plastic containers and glass bottles in combination with dry powdered pepper or neem leaf powder to maintain seed vigor and viability.

Key words: Cowpea, germination, packaging material, seed treatment, vigor.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L) Walp) is the second most important grain legume grown in Sierra Leone after groundnut and is used mainly as a food grain. It contains 23% protein by weight and plays an important role in

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providing the protein requirements of Sierra Leoneans especially those living in the rural areas (Crop Production Guidelines for Sierra Leone, 2005). Cowpea also supplements the income of many small-scale farmers and contributes to the maintenance of soil fertility by fixing nitrogen in the soil. The use of quality seeds in cultivation is one of the most important factors that can increase farm level yield. Although seed quality is governed by genetic makeup, seed storage and retention of viability are important for seed vigor (Deepa et al., 2011). As seed is considered as one of the important basic agricultural input for obtaining higher yields. Therefore production of quality seeds of high germination is of utmost importance in a seed program. Seed security is a key to the attainment of household food security among resource poor farmers in developing countries (Gari, 2004). Among the causes of seed insecurity in Africa is inadequate facilities and the use of inappropriate methods for seed storage among rural farmers. This impairs the maintenance of sufficient and safe seed sources. Successful seed storage is key to farmers' seed security and may also enable communities to generate income through collecting, storing and selling seeds. Seed storage problems are partly responsible for farmer's failure to save seeds of traditional crops (Delouche, 1982).

Several factors affect the longevity of seeds in storage and these include the variety of seed, initial seed quality, storage conditions, moisture content, insects pests, bacteria and fungi. The poor storage of cowpea seeds is a problem because of cowpea weevil (*Callosobruchus maculatus*) infestation. The infestation starts in the mature standing crop, and then at the threshing floor and eventually carried into store house, leading to seed deterioration under ambient storage conditions. There is the growing awareness of the deleterious consequences resulting from the widespread use of many chemical pesticides. The safe and feasible approach is the treatment of seeds with botanicals, which are safe, economical, cheap, eco-friendly in nature and non-toxic to man and animals. Among the botanicals, using castor, neem powder, neem oils, lemon grass are known to be effective protectants against storage insects; they can reduce infestation and maintain the quality of the seed in terms of viability and vigour for longer period in storage. Study by Basavegowda et al. (2013) found that Chickpea (*Cicer arietinum*L.) had higher seed germination and vigour index but lower insect damage and seed infection when commercial cold storage structures are used for maintaining seed quality. Duruigbo (2010) studied the treatments of seeds using edible, non edible oils, fungicides, insecticides and plant products and its influence on seed viability and insect infestation. Maintenance of seed quality during the storage period is important not only for successful crop production but also for maintaining the quality and integrity of the seeds that are in constant threat of genetic erosion (Barua et al., 2009). To maintain the quality of seeds during storage the standardization of suitable seed treatment and packing material is most important because seed treatment is the basic measure to assure adequately healthy crops at emergence and during further growth of plants (Wani et al., 2014).

The unavailability of quality seeds is a major problem affecting cowpea production in Sierra Leone. Information on prolonging the shelf life of cowpea seeds under ambient storage conditions is very limited in Sierra Leone. The aim of this research was to collect data on techniques for storage of cowpea seeds. The specific objectives are i) to identify a suitable packaging material for better storage of cowpea seed, ii) to determine the effect of seed treatment with chemicals and plant products on viability and vigour during storage and iii) to determine the effect of packing material on insect pest damage.

Cowpea (*V. unguiculata* (L) Walp) is the second most important grain legume after groundnut that is grown in Sierra Leone and is used mainly as a source of protein food and also supplements the income of many small-scale farmers. Although seed quality is governed by genetic makeup, seed storage and retention of viability are important for seed vigor (Deepa et al., 2011). Failure of farmers to save seeds of traditional crops could be due to inadequate facilities and the use of inappropriate methods for seed storage. The poor storage of cowpea seeds is a recipe for the cowpea weevil (*C. maculatus*) to infest and destroy seeds. Infestation may start in the mature standing crop, at the threshing floor, and eventually carried into the store house leading to seed deterioration under ambient storage conditions. Factors such as the variety of seed, initial seed quality, storage conditions, moisture content, insect pests, bacteria and fungi also affect the longevity of seeds in storage. Attempts have been made by several workers with many crops to develop methods for maintaining the viability and vigour of seeds for longer storage. Wani et al. (2014) found that seeds of maize treated with Captan (3 g/kg) and packed in Cloth bags and stored for 9 months under ambient conditions maintained maximum seed quality compared to the use of Castor oil or Vitavex in cloth bags and or polythene bags. Study by Basavegowda et al. (2013) found that Chickpea (*C. arietinum* L.) had higher seed germination and vigour index but lower insect damage and seed infection when commercial cold storage structures are used for maintaining seed quality. Duruigbo (2010) studied the treatments of seeds using edible, non edible oils, fungicides, insecticides and plant products and its influence on seed viability and insect infestation. Maintenance of seed quality during the storage period is important not only for successful crop production but also for maintaining the quality and integrity of the seeds that are in constant threat of genetic erosion (Barua et al., 2009). To maintain the quality of seeds during storage the standardization of suitable seed treatment and packing material is most important because seed treatment is the basic measure to assure adequately healthy crops at emergence and during further growth of plants (Wani et al., 2014).

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MATERIALS AND METHODS

The experiment was conducted in 2012 in the Seed laboratory of the Njala Agricultural Research Centre (NARC) in Sierra Leone. The experiment was laid out in a completely randomized block design in a 5 by 6 factorial with three replications. 1 kg of dried cowpea seeds with a uniform moisture content of 11% before packaging was put in five different packaging materials (cloth bag, paper bag, glass bottle, polythene bags and plastic container) in combination with six different seed treatments: No treatment (control), Camphor (Chemical treatment) – 4 g/kg of seeds, Neem *Azadirachta indica* L.) leaf powder (Botanical) – 10 g/kg of seeds, Groundnut Oil / Palm oil – 5 ml/kg seeds, powdered dry Pepper (Capsicum spp) -10 g/kg of seeds and wood ash 1 kg/kg of seeds. Sufficient quantities of Neem leaves and dry pepper fruits were obtained, sun-dried for 5 days and ground into powder. The ground material was sieved using 1.0 mm wire mesh sieve. The treated seeds were stored for 6 months with indoor temperatures ranging from 25.3 to 30.7°C, outdoor temperatures from 26.1 to 31.1°C and relative humidity from 61.0 to 82%.

Data was collected on seed samples drawn from each treatment on the following parameters after storage for 6 months: moisture content, indoor and outdoor temperatures, relative humidity, 100 seed weight, percentage damaged seeds, percentage germination and percentage vigor. The indoor temperatures, outdoor temperatures and relative humidity were measured for 6 months using a Thermohygrometer at 9:00 am and 3:00 pm each day and monthly means recorded. The percentage of damaged seeds in each treatment were determined by dividing the numbers of seeds from 100 randomly selected seeds with holes/perforations by beetles by the total randomly collected seeds and expressed as a percentage. Percentage germination was calculated by dividing the number of germinated seeds by the total number of seeds sown after 8 days and expressed as a percentage. Percentage vigor was calculated by dividing the number of seeds that germinated at first count after 5 days by the total number of seeds sown. Data were subjected to analysis of variance (ANOVA) using Genstat Release version 7.2DE. Duncan's multiple range test (DMRT) was used for mean separation. Regression and correlation analysis were explored to examine relationships among various parameters.

RESULTS AND DISCUSSION

Results showed August had the highest indoor and outdoor relative humidity (79.2 and 82% respectively) whilst the lowest relative humidity was recorded in the month of April (61.0%) (Table 1). The high indoor temperature during the initial storage period and the high relative humidity (RH) especially at the 3 month storage period must have provided favourable conditions for the growth and multiplication of cowpea storage pests. McCormack (2004) also found that increasing the temperature during storage will increase insect activity resulting to increase seed damage. There was also significant ($P < 0.05$) difference in mean 100 seed weight of the cowpea seeds among the different packaging materials. Cowpea seeds stored in the plastic container had the highest mean 100 seed weight (14.9 g) after 6 months of storage, followed by seeds stored in the Glass bottle (14.8 g). However, there was no significant difference between the seed stored in the plastic container and glass bottle (Table 2). The lowest mean 100 seed weight (10. g) was recorded for seeds stored in cloth and paper bags. Similarly, seeds stored in the glass bottle and Plastic containers had the least seed damage and weevil severity score (Table 2). Packaging material significantly ($P < 0.001$) affected the percentage of damaged seeds and weevil severity score. The lowest percent damage in cowpea seeds was obtained by seeds stored in plastic containers (4.4%) whilst the highest was recorded by seeds stored in the cloth bag (39.7%). The seeds that were stored in the glass bottle and plastic container which had the lowest percentages of damage seeds also had the lowest weevil severity score (1.4 and 1.3 respectively). A strong positive a correlation ($r = 0.96$) was also observed between the percentage of damaged seeds and weevil severity score (Table 3). However, there was a negative correlation ($r = - 0.8$) between percent damage seeds and germination percentage.

It was also observed that the type of packaging material used to store cowpea seeds had significant effect on vigor and germination capacity. Highly significant ($P < 0.001$) difference was observed in mean vigor and germination percentages among cowpea seeds stored in the different packaging materials. The seeds stored in plastic containers which had the highest vigor (61.6%) also had the highest germination percentage (77.1%), followed by seeds stored in glass bottles which had vigor percentage of 60.3% and mean germination percentage of 72.2% (Figure 1). Seeds stored in cloth bags which recorded the least vigor percentage (43.4%) also had the lowest germination percentage (53.3%). There was also significant difference in mean germination percentage between seeds stored in the paper bag, polythene, glass bottle and plastic container and those stored in cloth bags (Figure 1). The results also indicate that there was strong positive correlation ($r = 0.94$) between percentage vigor and germination percentage (Table 3). Similar findings were also reported by Gurmit and Hari (1992) who found that storing seeds in vapour proof containers maintain the desired quality of seeds for

Table 1. Mean Monthly indoor and outdoor Temperature and Relative Humidity of storage environment during storage period.

Month	Temperature (°C)		Relative humidity (%)	
	Indoor temperature	Outdoor temperature	Indoor relative humidity	Outdoor relative humidity
April	30.7	30.3	62.2	61.0
May	30.2	31.1	65.6	66.4
June	29.0	29.6	70.1	72.0
July	27.5	28.5	74.0	74.2
August	25.3	26.1	79.2	82.0
September	28.3	29.2	75.8	77.8

Table 2. Effect of packaging material on mean 100 seed weight seed damage and weevil infestation of cowpea seeds.

Packaging material	Mean 100 seed weight (g)	Percent damage seed	Weevil severity score
Glass bottle	14.6 [a]	7.1 [a]	1..4 [a]
Paper bag	10.8 [c]	35.6 [c]	2.7 [c]
Cloth bag	10.8 [c]	39.7 [c]	2.8 [c]
Polythene bag	11.7 [b]	25.8 [b]	2.2 [b]
Plastic container	14.9 [a]	4.4 [a]	1.3 [a]
LSD (0.05)	0.56	4.2	0.2
CV (%)	9.6	40	22.6

Means followed by the letter in the same column are significantly difference (p < O.05) (DMRT).

Table 3. Correlation coefficients (r) of physiological and physical traits of cowpea seeds after 6 months storage.

Percent- D	-0.8243				
Vigor	0.9428	-0.7732			
MC	-0.4079	0.1826	-0.4742		
Seed wt	0.6041	-0.7898	0.6054	- 0.0089	
Weevil severity	-0.7921	0.9650	-0.7578	0.2002	-0.7821
	Germ	Percent- D	Vigor	MC	Seed wt

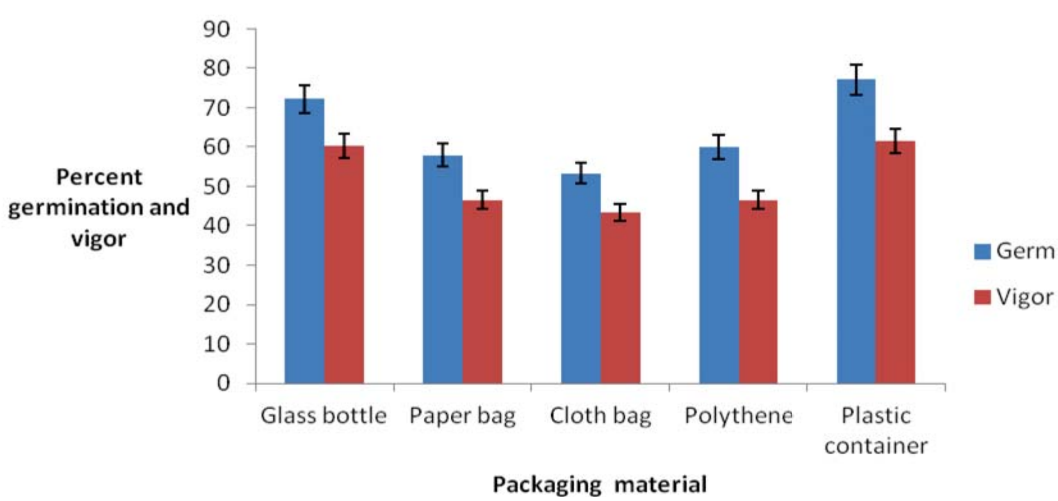

Figure 1. Effect of packaging material on vigor and germination of cowpea seeds.

Table 4. Interaction between period of storage and seed treatment on germination of cowpea seeds.

Storage period	Seed treatment					
	No treatment	Camphor	Neem leaf powder	Groundnut oil	Powdered dry pepper	Wood Ash
0	92 [a]	92 [a]	92 [a]	92 [a]	92 [a]	92 [a]
3	61.5 [d]	65.4[dc]	72.5[b]	67.8[b]	73.0 [b]	67.3[bc]
6	22.0[g]	45.5[e]	62.0[d]	29.0[f]	58.0[d]	47.5 [e]
LSD (0.05)	Storage period x Seed treatment 5.2					
CV (%)	9.2					

longer period than moisture pervious containers like cloth bag and gunny bag. This could be due to the fact that the impervious containers encouraged the activity of weevil resulting to higher percent seed damage and weevil severity and consequently low vigor and germination. Impervious containers also provided suitable environment and offered protection against contamination and serve as barriers against the escape of seed treatment chemicals than moisture pervious containers. The insects suffocate as soon as the oxygen in the container is used up. The percent seed damage caused by weevils significantly affected seed weight, vigor and germination of the seeds. The low germination could also be due to decrease in the seed weight caused by weevil damage resulting in less food reserve available in the seed to facilitate the growth of the seed.

There was a significant interaction between storage period and seed treatment of cowpea seeds. Generally, germination percentage of cowpea seeds decreased with months after storage. At 3 months after storage cowpea seeds treated with powdered pepper had the highest germination (73.0 %) followed by seeds treated with Neem leaf powder (72.5.5%) whilst the untreated cowpea seeds recorded lowest germination percentage (61.5%). This resulted in 33% reduction in germination of untreated seeds compared to 20.6% and 21.2% reduction in germination of seeds stored in powdered pepper and Neem leaf powder respectively.

At both 3 and 6 months after storage, significant ($P < 0.05$) differences were observed between the treated cowpea seeds and the untreated. Similar trend was also observed . after 6 months storage The cowpea seeds treated with neem leaf powder and dry pepper powder had the highest germination percentage (62 and 58% respectively) followed by the seeds treated with wood ash (47.5%) and camphor (45.5%) respectively. The lowest germination percent (22.0%) was again recorded by the untreated cowpea seeds (Table 4).

The results of the study clearly show that neem leaf powder, powdered pepper, wood ash and groundnut oil were effective in protecting cowpea seeds up to 3 months in storage. However, seed quality deteriorated rapidly resulting in low vigor and germination after 6 months. These results do not agree with the findings of Duruigbo (2010) who reported that seeds treated with neem leaf powder gave higher mean germination percentage for cowpea than seeds treated with black peeper, pepper fruit and soya bean oil and palm oil. This could be attributed to ovicidal and insecticidal action of azadiractin contained in neem leaf. It also has the ability to penetrate the cuticle of the insects, kills insects by flooding their spiracles thus causing asphyxiation. Similar results were also reported by Maraddi (2002) who observed that cowpea seeds treated with neem leaf powder at 5 g per kg of seeds recorded higher germination (39.5%) and vigour index (10.72) compared to control (34.2 and 8.64%) at the end of 10 months of storage period. The results of the current study contradict earlier report by Osekre and Ayertey (2002) who suggested the use of palm oil and coconut oils to control cowpea beetles. Although the groundnut oil reduced seed damage due to weevils, it adversely affected germination after 3 months of storage. The low germination percentage of seeds treated with groundnut oil could be attributed to impairment of respiration by the seed at the time of the test.

Conclusion

Results obtained from the present study suggest that plastic containers and glass bottles are better packaging materials for cowpea storage than polythene bags, paper bag and cloth bag under ambient storage. Application of neem leaf powder, powdered pepper and wood ash were most effective in protecting seeds from weevil damage and maintaining good seed quality and viability up to six month. The combination of neem leaf powder, powdered pepper wood ash and airtight containers in plastic containers significantly reduced seed deterioration. For short term storage it is best to package cowpea seeds in moisture proof containers than cloth or paper bags to prolong shelf life and maintain good seed quality. The use of this technology by resource poor farmers is low cost and will allow them to save enough seeds of high quality for future planting. However, farmers should not store their cowpea seeds in cloth bags or paper bags.

Declaration of conflict of interest

Ernest G. Kamara hereby declare that there is no financial or any other interest behind the development and submission of this manuscript and that it is purely based on self aspiration and desire to build up my academic career as a researcher.

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Full Length Research Paper

Map of potential areas of groundwater by the multi-criteria analysis for the needs for water of the Baya's catchment basin (East of Côte d'Ivoire)

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The watershed located Baya is facing difficulties of drinking water supply. The objective of this study is to develop a model for preferential zone mapping to the implementation of water points. To achieve this objective, a combined approach of Geographic Information System and Multicriteria Analysis has been adopted for the mapping of these preferential. Multi-criteria analysis methods including Boolean method and the linear combination of weights based on pairwise comparisons were used. Two types of indicators were used: The productivity indicator which combines the accessibility, exploitability and availability of groundwater and control indicator, which is represented by the vulnerability to pollution in groundwater. It appears that the preferential zone of future water points implantation covers 84% of the catchment area. When making water productivity map, the calculation of uncertainties parameters show that the most sensitive parameters are: The slope, recharge, induced permeability and total depth. However, the topography, vadose zone and hydraulic conductivity are the most sensitive to the development of the map of vulnerability to pollution. The errors determined on different maps are respectively 12, 20 and 12% for productive zone, vulnerability and preferential zone. Preferential areas cover almost all of the study area and are influenced by the topography, vadose zone and hydraulic conductivity; the margin of error is low.

Key words: Geographic information system, multi-criteria analysis, groundwater, vulnerability, catchment of Baya.

INTRODUCTION

Groundwater is one of the largest worldwide resources. In most countries, it provides drinking water to more than half of the population and is the only source of drinking water for many rural communities and some large cities (Hamza et al., 2007). Groundwater is also the source of much of the water used for irrigation and is a major

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contributor to flow in many streams and rivers, and has a strong influence on the river and wetland habitats for plants and animals (Solley et al., 1998). However, access to this valuable resource becomes very difficult precisely in hard rock where groundwater is in fissures. The term “hard rock” commonly applies to hard and dense rocks with the main part of the groundwater flowing in secondary structures, mainly fractures. Groundwater in hard rock aquifers is essentially confined to fractured and/or weathered horizons. Therefore, extensive hydrogeological investigations are required to thoroughly understand groundwater conditions. Modern technologies such as remote sensing and geographic information systems (GIS) have proved to be useful for studying geological, structural and geomorphological conditions together with conventional surveys. Integration of the two technologies has proven to be an efficient tool in groundwater studies (Solomon and Quiel, 2006; Elewa and Qaddah, 2011; Machiwal et al., 2011). In Côte d’Ivoire, crystalline rocks and cristallophyllienne represent 97.5% of geologic formations (Jourda et al., 2006, Doumouya et al., 2012).

In addition to the difficulties related to the availability, accessibility and exploitability of these resources, these groundwater could be exposed to pollution phenomena due to the existence of significant anthropogenic sources. Any pollution of these resources is only possible if the conditions for protection are threatened. To determine the degree of protection, vulnerability to pollution was assessed. Many methods have been proposed for mapping aquifer vulnerability: DRASTIC (Aller et al., 1987), GOD (Foster, 1987), AVI (Van Stempvoort, 1993), SINTACS (Civita, 1994), EPIK (Doerfliger and Zwahlen, 1998), PI (Goldscheider et al., 2000) and COP (Vias et al., 2006). DRASTIC is one of the most common methods used internationally to evaluate the intrinsic vulnerability. The application of this method has given interesting results in Côte d'Ivoire (Antonakos and Lambrakis, 2007; Gomezdelcampo and Dickerson, 2007; Panagopoulos and al., 2006). However, the combination of quantitative and qualitative aspects including different parameters to map preferential areas is virtually non-existent, especially in the eastern part of Côte d'Ivoire. Also, the diagnostic assessment of rural water supply programs for the period 1973 to 2000, show that the proportion of households having access to safe drinking water in the Baya’s catchment, site of this study, is only 37.6% (Mangoua, 2013). Besides, in some areas, the lack of water increases during dry periods. This increase conduct forcing people to make long distances to obtain water from permanent points of water (surface water) generally of dubious-looking quality and medium of many diseases such as dysentery, cholera, etc. Faced to this situation, the improvement of access to safe drinking water to people appears paramount. It is in this context that this study was conducted. This study aims to contribute to a better knowledge of groundwater resources of the basin by multi-criteria analysis to facilitate the supplying of people with drinking water.

Study area

The watershed of the Baya is located to the East of Côte d'Ivoire between longitudes 2° and 3° 50’50W and latitude 6°90’ and 8°20N on an area of 6,324.041 km^2 (Figure 1). The geomorphic landscape is monotonous peneplain occupied by cash crops and export (coffee, cocoa, cashew, rubber) and food crops. We meet in place hills and mountains rising to 700 m on average (Siméon et al., 1995). The climate is tropical and humid. The catchment is drained mainly by Baya River. Geology of this area, which is in the Baoule-Mossi, consists of Birimian and tarkwaiennes formations. These courses are a volcano sedimentary package in which appear intrusion volcanics and granitoids eburnean (Touré, 2007). Several phases of deformation affected the area and led to the establishment of a developed fracturing. In hydrogeological terms, are found in the basin aquifers weathering and cracking. The supply of drinking water to the people is through drilling capturing most often fractured aquifers. Alteration zones when they are thick, can contain significant water circulation which are sometimes exploited by wells.

MATERIALS AND METHODS

Several types of data were used in this study. It concerned technical data collected from 150 boreholes in the Territorial Directorate of Water at Bondoukou, geological and topographical maps of the square degrees of Abengourou of Agnibilékrou and Bondoukou scale 1/200000 respectively performed by the Department of Geology and the Centre of Cartography and Remote Sensing (CCRS) of Côte d’Ivoire. The hydro-climatic data (flows, rainfall and ETP for the period 1965 to 1983) were used for the estimation of infiltration from the hydrological model GR2M, which is a model of rural engineering with 2 parameters and Monthly step. For more details on this hydrological model, we refer the reader to the work of Ardoin (2004). Satellite images ETM$^+$ Landsat 7 are used to produce lineament map. These images concerned the scenes 195-54 and 195-55 acquired in February 2000. The processing of these data required the use of Envi 4.5 software for satellite images exploitation, MapInfo 7.5 and ArcView 3.2 for the treatment of the fracture network and the implementation of GIS.

Identification of criteria

The map preferential zonations for borehole are obtained by combining criteria of power and vulnerability from the Boolean method. Several criteria characterizing the quality and quantity of groundwater will be used. According to Elewa and Qaddah (2011), the higher the number of GIS parameters used in groundwater potentiality mapping, the higher the accuracy gained.

Quantitative indicators

The parameters used for assessment the quantity of groundwater

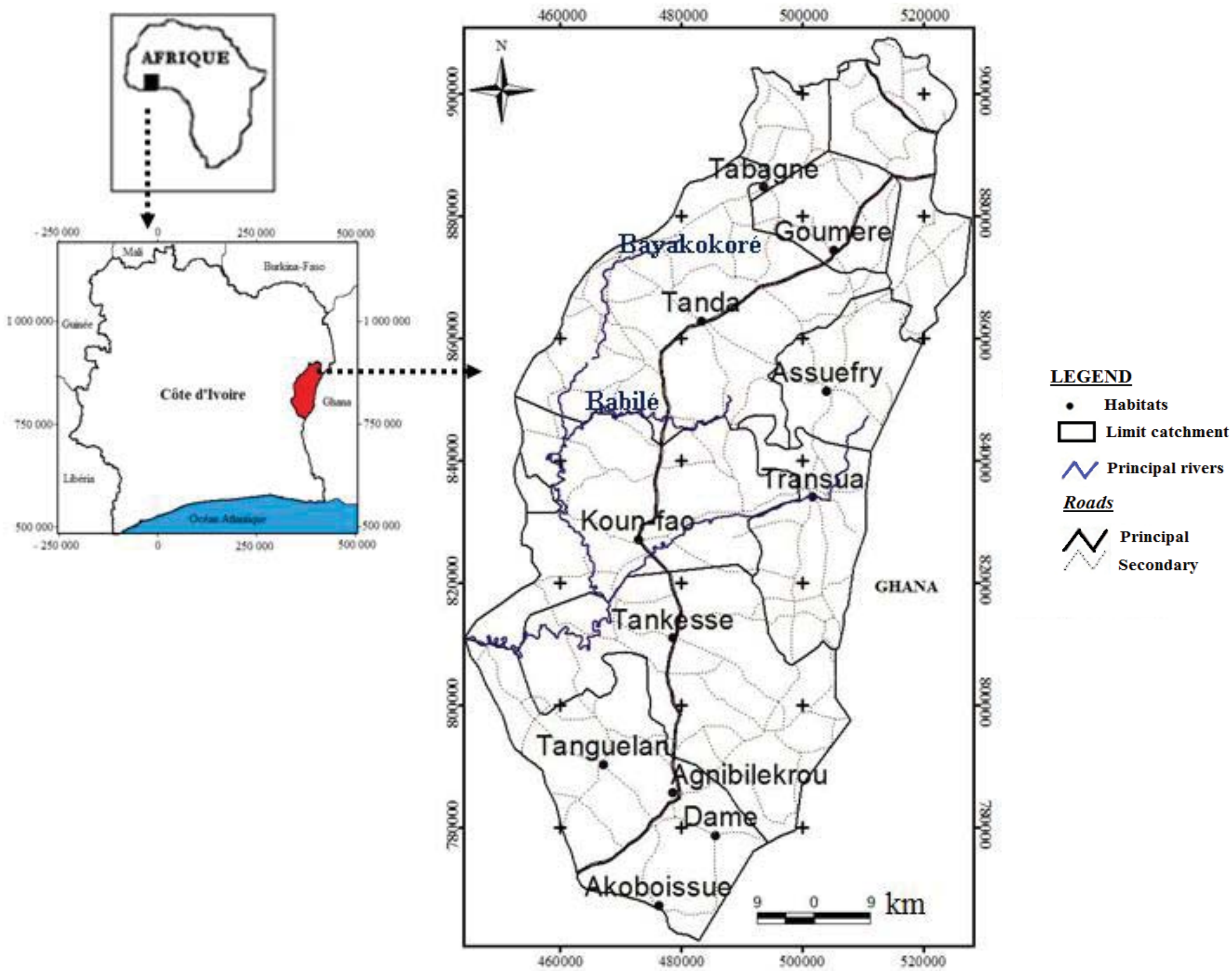

Figure 1. Study area map.

are composed of the aquifer characteristics and other external factors which are grouped into three quantitative indicators (accessibility, exploitability and availability) (Table 1).

Availability indicator

Availability provides information on the conditions of accumulation of groundwater resources. It regroups the most important parameters in the mapping of productive zone. These parameters are slope, recharge, drainage density, lineament density and the weathered zone.

Net recharge is one of the most important parameters in groundwater availability. This factor represents the amount of water per unit area that percolates to the groundwater (Sinan and Razack, 2008). The net recharge of groundwater was calculated using GR2M. The recharge as defined by the US EPA with the potential of an area to have recharge. In other words, the net recharge parameter changed to the ability of an area to act as a recharge zone relative to another area (Al-Hanbali and Kondoh, 2008). It varies from 25 to 100 mm per year.

Slope is one of the factors controlling the infiltration. Indeed, high slope will cause more run-offs and less infiltration, and thus have poor groundwater prospects compared with a low slope region. Slope plays a very significant role in determining infiltration vs. runoff. Infiltration is inversely proportional to slope, that is, the water infiltration decreases with the increase in slope steepness (Elewa and Qaddah, 2011). Slope calculation was carried out by topographic map of the study area that has been product by CCRS. It varies from 0.6 to 15%.

The drainage density characterizes the relative rainwater that could have infiltrated the subsurface. Hence, according to the common geomorphologic concept, the denser the drainage, the less recharge rate and vice versa. However, one of the major approaches to express drainage density involves the number of drainage segments per unit area, which is also called “drainage frequency” (Elewa and Qaddah, 2011). The study area was divided into grids of 5 km^2. In this study, a drainage density map of the watershed of the Baya was created manually from the topographic map. The total length of all drains in each grid evolves

Table 1. Different classes of parameters.

Classes	Parameters								
	S (%)	R (mm)	DD (Km/grid)	LD (Km/grid)	WL (m)	TD (m)	SI (%)	EF (m^3/h)	WT (m)
Very low	0-0.6	<25	<0.54	0 - 12	< 10	0 - 25	0- 20	0-1	0-5
Low	0.6-1.7	25-50	0.54-0.71	12 - 23	10 - 15	25 - 40	20 - 40	1-2	5-15
Medium	1.7-3.4	50-75	0.71-0.93	23 - 34	15 - 25	40 - 70	40-60	2-4	15-25
High	3.4-5.1	75-100	0.93-1.23	34 - 45	25 - 40	70 - 95	60-80	4-10	25-60
Very high	> 5.1	> 100	> 1.23	45 - 56	40- 70	95 -115	80-100	10-22	60-85

S, Slope; DD, drainage density; LD, lineament density; WL, weathered layer; TD, total depth; SI, success index; EF, exploitation flow; WT, water table; R, recharge.

from 2 to 41 km by 25 km^2 grid.

Lineament density map has been produce by Satellite images ETM+ Landsat 7. Lineament analysis for groundwater exploration has considerable importance, where the joints and fractures serve as conduits for movement of groundwater and have water-holding capacity. Fractures in rocks increase their secondary permeability and porosity, and thus accelerate vertical water percolation to recharge the aquifers. For this reason, identifying fractures in rocks is considered as a major factor in identifying groundwater productive areas. In this study, we use lineament density (length or number) that plays an important role in mapping of preferential location areas (Machiwal et al., 2011).

The weathered layer formed by weathering of the rock is considered a major source of aquifer recharge. It is the least important parameter in the assessment of availability. According to (Prasad et al., 2008), the availability of groundwater is classified as poor to moderate in areas with a thin weathered layer and it evolves from moderate to good in areas with a thick weathered layer. In the case of this study, the weathered layer was obtained from drilling data sheets and ranged from 2 to 92.31 m.

Exploitability indicator

The exploitability of groundwater resources regroups the parameters that are exploitation flow and the depth to water table. They are based on boreholes data sheets. The exploitation flows represent the productivity of borehole. In the Baya's watershed, the exploitation flow ranged from 0.36 to 21.6 m^3/h. The depth to water table allows for following the water level fluctuation and plays an important role in the groundwater potential zone delineation (Shankar and Mohan, 2006). In the study area, it ranges from 4 to 82.68 m. These parameters have been used in Erytrea to delineate groundwater potential zone in the central highlands of Eritrea (Solomon·and Quiel, 2006).

Accessibility indicator

Integrating accessibility parameters in the preferential zone of boreholes delineation could be explained by the important role played by this factor in drinking water. These parameters also are considered as economic and social factors because they favour access or denial to the resource. The main factors of the accessibility indicator are well total depth and the success index. The well total depth gives information on the cost of the water point depending on the number of linear meters drilled. Some authors have defined the best depth interval or the maximum depth that a water point must reach in order to obtain satisfactory productivity in crystalline rock (Youan Ta et al., 2011). Most of them have shown that, because of the closure of discontinuities by lithostatic pressure in depth, when the water point goes deeper, productivity decreases (Neves and Morales, 2007). In this study, the depths of the water points were obtained from published well data and ranged from 42 to 110 m. The success index gives the probability of having a productive water point.

Weighing the quantitative factors

The quantitative factors were weighted starting from the weights linear combination method. The linear combination allowed the production of standardized weighting coefficients whose sum is equal to 1. The procedure consisted in:

1. Comparing the relative importance of all the elements belonging to the same level of hierarchy taken two by two, compared to the element of the immediately higher level;
2. Configuring a reciprocal square matrix formed through the evaluation of the ratios of the weights (K × K), K being the number of compared elements. Thus, one obtains (Saaty, 1977): $a = a_{ij}$ with $a_{jj} = 1$ and $a_{ji} = 1/ a_{ij}$ (reciprocal value) and a, the value of each factor i (lines) and j (columns)

The Analytic Hierarchy Process (AHP) method has been used respectively by Tudes and Duygu (2009) in Turkey for land use planning in Adana–Turkey and Doumouya et al. (2012); in Côte d'Ivoire. These coefficients are given starting from a series of comparisons per pair by taking account of their importance in the productive zone determination. In order to produce weighting coefficients for each factor and each indicator (Table 2), the procedure needs the eigenvector (Vp) of the comparison matrix. The values of these vectors were obtained by calculating their geometric mean by line (Dibi et al., 2010):

$$V_{p_i} = \sqrt[n]{\prod_{i=1}^{n} Ni}$$

Where V_{pi} is the load vector of each factor, N_i is the value of each factor.

The weighting coefficient (W_{ij}) of each factor is given as follows:

$$W_{ij} = \frac{V_{p_{ij}}}{\sum_{i=1}^{n} V_{P_i}}$$

The Weighted Linear Combination (WLC) is applied according to the following equation (Eastman, 1997), where F is the favourability, W_{ij} is the weight of class j from map i and X_i is the criterion score of map i.

Table 2. Parameters weighting coefficients from pairwise comparison matrix.

Parameters	Eigen-Vector	Weighting coefficient
S (%)	2.24	0.26
R (mm)	3.70	0.43
DD (Km/grid)	0.42	0,04
LD (Km/grid)	1.10	0.13
WL (m)	0.30	0.04
Availability	3.87	0.75
TD (m)	2.24	0.83
SI (%)	0.45	0.17
Accessibility	0.26	0.05
EF (m^3/h)	2.24	0.83
WT (m)	0.45	0.17
Exploitability	1	0.20

Qualitative indicators

Groundwater vulnerability assessment is an important process for understanding the intrinsic fragility that a certain region opposes to a given threat, whether this hazard has a natural or anthropogenic origin. This indicator had proved to be an effective tool for the delineation of protection zones in areas affected by groundwater contamination due to intensive fertilizer applications (Antonakos and Lambrakis, 2007). The present evaluation of the risk is based on the DRASTIC method as described by Aller et al. (1987), which uses seven parameters to evaluate the vulnerability of groundwater: depth to water, net recharge, impact of vadose zone media, soil media, topography, aquifer media and permeability of the aquifer. After the parametric evaluation, the index of DRASTIC vulnerability (Id) is given. This index allows the characterization of the vulnerability degree of a given sector of aquifer.

$$I_d = D_n D_P + R_n R_p + A_n A_p + S_n S_p + T_n T_p + I_n I_p + C_n C_p$$

With D, R, A, S, T, I, C, parameters referred to above n is the notation granted to each parameter; p is the factor loading granted to each parameter.

Map of preferential implanting zone (MPIZ)

MPIZ involves delineation of preferential zones to allow distinguishing areas suitable for the establishment of boreholes. Quantitative and qualitative indicators were combined from the Boolean method. This method uses Boolean operators: Such as AND and OR. It has consisted in assigning a weight ‘‘0’’ to the threatened area represented by the strong vulnerabilities, and ‘‘1’’ to the non-threatened area represented by the average and weak vulnerabilities.

Uncertainty and error map

Uncertainty analysis

The first step of the analysis was to compute uncertainties on averages of various parameters of the main indicators of potentiality and vulnerability. The uncertainty is calculated using the following equation:

$$\Delta\bar{x} = \frac{\sigma}{\sqrt{m}}$$

With $\Delta\bar{x}$ uncertainty on average of the data series, σ standard deviation, m number of data.

To determine to determine the confidence interval, an expansion factor (K) is then calculated. The determination of this parameter is based on the statistical principle of computation of the expanded uncertainty. The factor k allows the definition of an interval of sufficient scope to have great confidence in the results. The expression of this factor is as follows:

$$K = \frac{|E - \bar{x}|}{\sigma}$$

Where k is the expansion factor, E is the extreme value of the statistical series, which can be the maximum or the minimum of this series.

Confidence levels of the different parameters have been deduced from different values of k. Thus, k = 1 for a confidence of 68%, k = 2 for a confidence of 95%, and k = 3 for a confidence of 99%.

Map errors

The errors of the productive zone index and the DRASTIC index on the maps were calculated by the following formula:

$$Er = \frac{\Delta\bar{x}}{I} \text{X} 100$$

With Er, the error (%) committed on the map of the productive zone or DRASTIC index; $\Delta\bar{x}$ or Inc, the uncertainty on the map of the productive zone index (Inc_{pz}) or the DRASTIC index (Inc_d); I, Productive Zone Index (I_{pz}) or DRASTIC index (I_d).

The error of the MPIZ, E_{MPIZ} was obtained by the following calculation:

$$E_{MPIZ} = \left|\frac{Incpz - Incd}{Ipz - Id}\right| \text{X}100$$

RESULTS

Quantitative map indicators

The application of the weights linear combination method allowed obtaining maps of indicators of accessibility, exploitability and availability methods. The results of the availability map (Figure 2) revealed 4 classes dominated by good availability conditions, which occupied 74.95% of the entire study area, except the northern part. The class of excellent availability (11.67%) was observed on the outlet of watershed. The medium class of availability represented 13.17% of the area and occupies the northern part with a few appearances in the departments of Assuefry and Tanda. The low class of availability that remains practically absent (0.21%) was observed on the granitic and sedimentary formations where water points are shallow.

Three unequal classes (Figure 3) characterized map of groundwater accessibility resources of the Baya’s

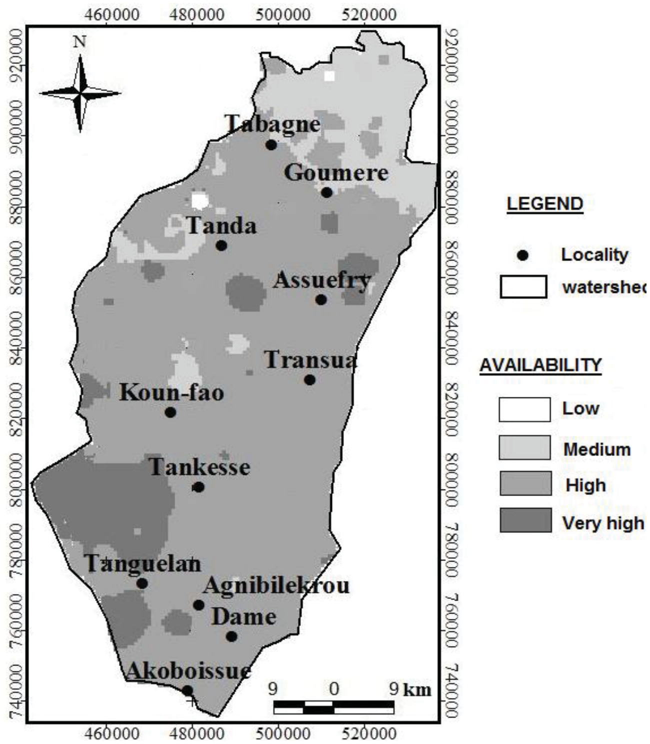

Figure 2. Availability map.

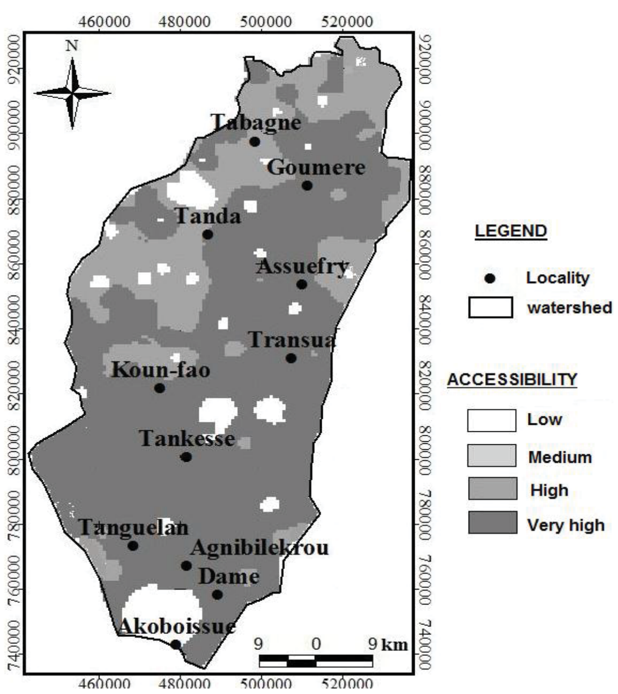

Figure 3. Accessiblity map.

watershed, the medium class was non-existent. On this map, the excellent and good classes of accessibility held

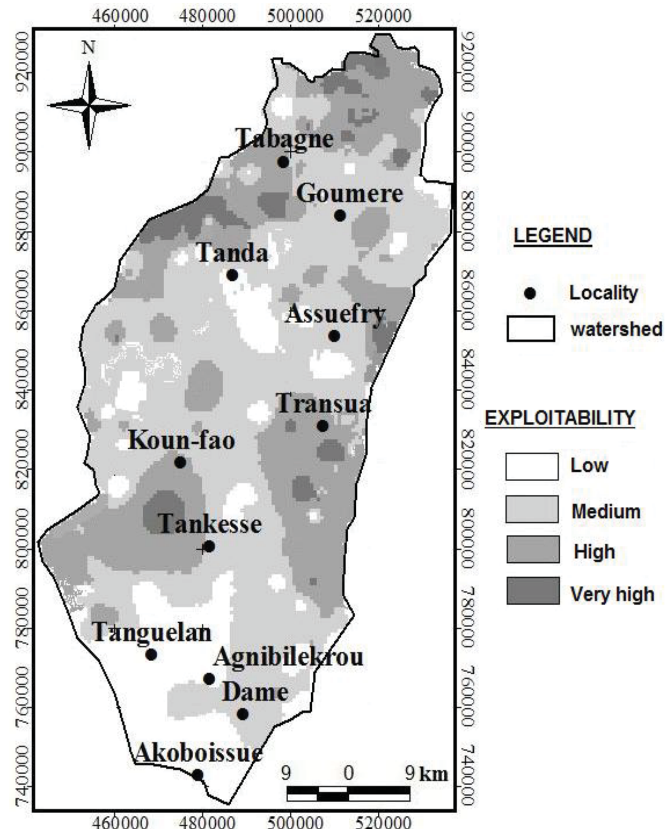

Figure 4. Exploitability map.

respectively 70 and 20% of the study area. The class of excellent accessibility was characterized by low and medium depths and indices of success very high (> 80%). The low class of accessibility represented 10% of the area and occupies the south part (Akoboissue) with few appearances in the north.

The medium class (51.09%) and the good class (28.74%) dominated the exploitability of groundwater (Figure 4). These classes are located mostly on the shale. The excellent and good classes of exploitability (33.5%) were generally present in the granitoids with exploitation flows greater than or equal to 9.5 m^3/h. These areas are the most sought for the supply of drinking water to urban centers as is the case of the city of Bondoukou.

The linear combination of all the indicators according to their respective weights has produced the map of productive zones (Figure 5). The result showed that productivity was dominated by good and excellent classes. These classes represented 84% of the watershed area and were found in almost all the study area except the north zone and departments of Tanda and Koun-Fao. For the low and medium classes, which covered the remainder of the study area (16%), they were observed in the North with a few pockets in the Central-west.

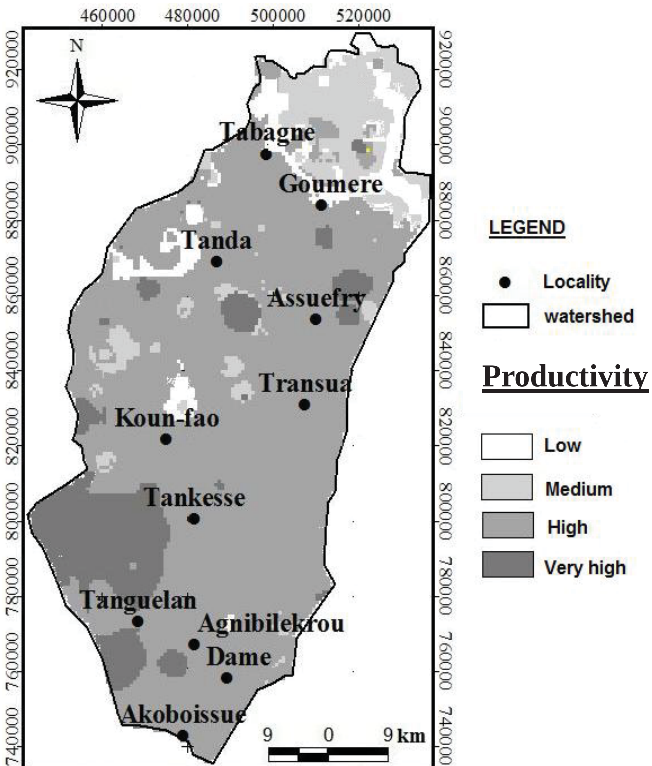

Figure 5. Map of Productive zone.

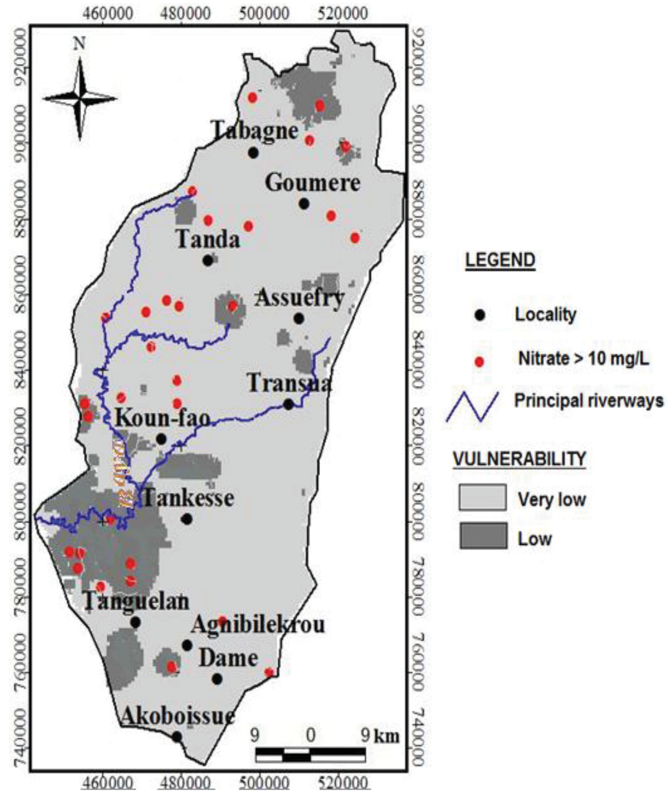

Figure 6. Vulnerability indicator map.

Qualitative map indicator

Qualitative indicator presented by vulnerability map (Figure 6) indicated two classes (very low and low). Very low vulnerability class covered 85% of the study area and was regarded as having low pollution potential. This class was usually found throughout the study area from north to south. Groundwater in this area probably does not risk contamination. The class of low vulnerability representing 15% of the watershed occupied small portions in the west and some pockets in the north.

Map of preferential implanting zone (MPIZ)

The MPIZ resulting from the combination between the quantitative indicator maps and that of the vulnerability to pollution shows the areas, which are most appropriate for implantation of well points (Figure 7). The watershed of the Baya is well protected and is not a threat to groundwater in the study area. Indeed, it does not show any influence on the map of productive zone. The MPIZ has the same features as the map of productive zone. The good classes and excellent location occupied 84% of the basin. For the low and medium classes, which covered the remainder of the study area (16 %), they were observed in the North with a few pockets.

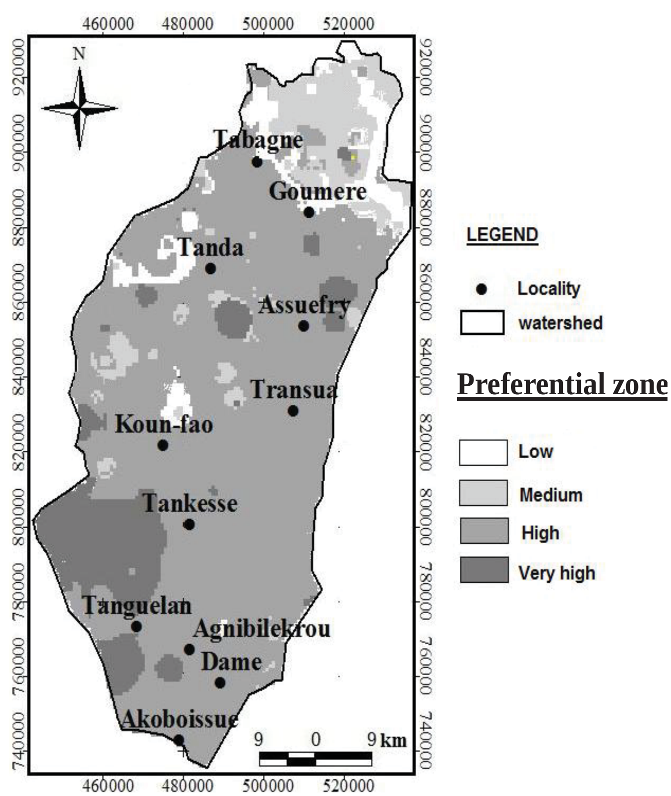

Figure 7. Map of Preferential implanting zone.

Table 3. Statistical summary of the potentiality parameters.

Parameters	Min	Max	Mean	Standard deviation	Uncertainty	k	Confidence level (%)
S	107	481	250	50	± 0.02	3	99
R	17	141	139	5	± 0.01	3	99
DD	0	5	2	1	± 0.04	2	95
LD	0	12	5	2	± 0.02	2	95
WL	0	92	40	12	± 0.12	3	99
Availability	5	9	7	1	± 0.01	3	99
TD	43	111	71	14	± 0.12	2	95
SI	9	100	61	5	± 0.13	3	99
Accessibility	0	3	2	1	± 0.01	2	95
EF	0	22	4	1	± 0.02	2	95
WT	0	83	28	11	± 0.10	2	95
Exploitability	1	10	5	2	± 0.02	2	95
Productive zone	202	526	380	55	± 0.46	3	99

Table 4. Statistical summary of the vulnerability factors.

Parameters	Min	Max	Mean	Standard deviation	Uncertainty	k	Confidence level (%)
D	10	50	22	11	± 0.09	1	68
R	12	24	18	8	± 0.07	1	68
A	12	24	16	6	± 0.05	1	68
S	8	20	14	5	± 0.04	1	68
T	1	10	5	2	± 0.01	2	95
I	20	40	30	10	± 0.08	1	68
C	0	33	5	2	± 0.02	2	95
DRASTIC index	*40*	*95*	*55*	*13*	± 0.11	*1*	*68*

Uncertainty analysis

The uncertainty analysis computed by determining confidence levels is presented in Tables 3 which gives the statistical summary of the parameters or factors of the productive zone indicator. The map of productive zone was obtained by combining maps of availability, exploitability and accessibility. The uncertainties were high on the parameters WL (±0.10) and DD (±0.04) with confidence levels 99 and 95% respectively. However, errors on the three other parameters (slope (S), recharge (R) and lineament density (LD)) were low. Their uncertainties were ± 0.02 for the lineament density and the slope and ±0.01 for the recharge. The slope and the recharge had a high confidence level of (99%). The lineament density confidence level is 95%. For the exploitability indicator, uncertainly of water table was higher (±0.10) than that of the exploitation flow (±0.02). However, the confidence level of these two parameters was 95%. According to the indicator of accessibility, the uncertainties showed that there is statistically more risk of error on the two parameters (success index (SI) (±0.13) and the total depth (TD) (±0.12)). The confidence level was therefore higher at the success index (99%) than the total depth (95%).

Uncertainties and confidence levels were also determined for factors of vulnerability (Table 4). The vulnerability map was obtained by a combination of D, R, A, S, T, I, C factors. The confidence level is 68% for the parameters D, R, A, S, I and 95% for T, and C. Also, analysis of the uncertainties allowed a classification of levels of error committed on the factors. The highest risk of error which could be committed in the conception of the vulnerability map originated from the recharge (±0.07), depth (±0.09) and the impact of the vadose zone (±0.08). The second risk of error originated from the soil (±0.06) and the type of aquifer (±0.05). The lowest level of error on the vulnerability map could be attributed to the topography (±0.01) and the hydraulic conductivity (±0.02).

Map errors

The results of calculation errors on the map of productive zone and DRASTIC map determined from the indices are shown in the following Table 5. On the map of productive

Table 5. Statistical summary of productivity and vulnerability factors.

Parameters	Min	Max	Mean	Standard deviation	Error (%)	k	Confidence level (%)
Productive zone	202	526	380	55	12	3	99
Vulnerability	40	95	55	13	20	1	68
Preferential zone	200	526	378	12	12	3	99

zone, the error was only ±12% whereas it was ±20% on the map of the DRASTIC that indicating the vulnerability to pollution of the groundwater. The final map (Map of Preferential implanting zone) representing the combination of the two previous maps gave an error percentage of ±12%. Therefore, the confidence levels of these three maps were respectively 99, 68 and 99%.

DISCUSSION

The use of GIS and multi-criteria analysis in the watershed Baya had resulted in the production of maps of availability, accessibility and exploitability of groundwater resources. The linear combination of these maps had achieved the map of productive zone. This map represented a pre-exploration phase, which avoided heavy, slow and costly research as indicated by the work of Langevin et al. (1991). The availability indicator is important for preferential zone delineation. This indicator was strongly influenced by the slope, recharge and lineament density in the area of Baya watershed. This catchment had a good availability of groundwater (87% of the watershed) that was due to a low slope and good lineament density which would lead to a good infiltration of water into the aquifer. This result is confirmed by Shankar and Mohan (2006) and Doumouya et al. (2012). According to these authors, the slope has direct control over groundwater recharging. The groundwater recharge by rivers was favored by the density of lineaments. Therefore, areas of very high lineament density corresponding to areas of high hydraulic conductivity were considered as areas of high recharge. According to Anbazhagan et al. (2005), this lineament density is closely linked to drainage density. The exploitability indicator was dominated by the class of medium (51.06%) and good (28.74%) exploitability which occured on shales and granitoids respectively. The good exploitability of water from the granitoids could be explained by the fact that they were the most productive formations in the region according to Mangoua (2013). Indeed, this good exploitability of granitoids could be linked to their strong fracture density. The medium productivity of the shale was due to the lack of intercalations of carbonate in shale beds and pegmatite dykes and quartz in these formations (Touré, 2007).

As for accessibility, the results showed that they were still dominated by the class of good accessibility condition observed in almost all the study area. The presence of that class of good accessibility could be linked to the relatively shallow depth of water points. Besides depths, the exploitation flow which allowed determination of the success index was often superior or equal to 3.5 m^3/h, corresponding to a success index higher or equal to 80% (Doumouya et al., 2012). In watershed of Baya, it was observed that the higher the water point is deeper, low the productivity is. This phenomenon could be linked to the closure of discontinuities due to lithostatic pressure in depth as noticed by Neves and Morales (2007) in Southeast Brazil or due to financial constraints which would restrict drilling not to exceed (Biémi, 1992).

The study of groundwater vulnerability to pollution showed that the study area was dominated by a class of the very low vulnerability (85%) with some pockets of low vulnerability (15%) observed in the South-west and the North. Indeed, steep sloping favours runoff accumulation in the valleys and thereafter its infiltration into underlying layers. Valley bottoms seen in the North and the outlet of the watershed are in general likely to favour contamination of groundwater by pollutant infiltration. This contamination is more likely in areas where water is shallow because the vadose zone is in general made up of a generally permeable sandy clay formation. Note that the watershed had a very low vulnerability generally with DRASTIC index of 55, so that the watershed is well protected.

The calculation of uncertainties and the confidence level allowed the organization of importance of errors made on preferential zone and vulnerability maps. The parameters which had high uncertainty were less important than those with low uncertainties. As for the preferential zone map, uncertainties were more important at the water table, the total depth, the success index and the weathered layer. The other parameters (slope, drainage density, recharge, exploitation flow and lineament density) presented relatively low uncertainty giving a high reliability to the map they allowed us to make. As for DRASTIC parameters, which determine vulnerability, the uncertainty mostly came from the topography and the hydraulic conductivity with confidence levels of 99%.

Overall, it was found that the parameters obtained from satellite images and geological maps (slope, drainage density, lineament density and recharge) had low uncertainties. These low uncertainties could be explained by the fact that these data came from sources already validated and therefore more reliable.

GIS and MCA have many advantages because they bring an undeniable contribution to the management of water resources and rational decision-making. However, they have limitations. The parameter estimation often lack of precision because of the inadequacy or total absence of data in some parts of the study area. In short, GIS shall be and remain an invaluable contribution in the management of water resources generally speaking (Putz, 2003).

Conclusion

In this research, an attempt was made to delineate the productive zone and the vulnerability of the aquifer to determine preferential zones for implantation of water points in the watershed of Baya. GIS and MCA are two complementary techniques used in the delineation of preferential areas. The results of this study indicate indicates indicated that the preferential zones represented 84% of Baya area. This class was distributed throughout the region, with an exception in the North. The determination of confidence levels showed that groundwater reserves in the watershed of Baya were governed by the slope, recharge, Exploitation flow, depth and lineament density. However, the vulnerability of the aquifer was essentially the fact of the topography, the impact of vadose zone and the conductivity. Errors determined from the uncertainties are respectively were 12, 20 and 12% for maps of productive zone, vulnerability and preferential zones respectively. Preferential areas cover almost all of the study area and are influenced by the topography, vadose zone and hydraulic conductivity. The margin of error is low and showing the quality of the results.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Impact of soil salinity on fungal vector of rhizomania virus infecting *Beta vulgaris*

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***Polymyxa betae* Keskin is the only natural transmitting vector of the Beet necrotic yellow vein virus (BNYVV) among the cultivated sugar beet. This work aims to study the impact of salt stress on fungus-virus-host relationships. The fungal infected fine roots of sugar beet plant naturally infected with BNYVV were collected and treated with different salt concentrations (0, 2000, 4000, 6000 and 8000 ppm) of NaCl for one day prior to mixing it with a sterile soil. After virus symptoms appeared on leaves, disease severity has been determined, leave and roots tissues were collected for BNYVV detection. It was found that severe symptoms on sugar beet inoculated with treated roots by low salt concentrations (2000 and 4000 ppm) while at high salt concentrations, 8000 ppm less injury approximately as control treated with H_2O. On the other hand it was found the cystosorial colonization was increased in low salt concentrations (2000 and 4000 ppm) while decrease in high salt concentrations (6000 and 8000 ppm) especially in 8000 ppm. The same trend results were observed in virus concentration in roots where as the BNYVV concentration was increased in low salt concentrations (2000 and 4000 ppm) while decrease in high salt concentrations (6000 and 8000 ppm). So the relation between capacity of fungal vector to infect the plant by virus and salinity concentration are antagonistic. Soil salinity extremes most often lead to decrease infection of BNYVV via effect on fungal zoospore.**

Key words: *Polymyxa betae*, sugar beet, rhizomania, beet necrotic yellow vein virus (BNYVV), salinity.

INTRODUCTION

Polymyxa betae Keskin is an obligate parasite of sugarbeet roots and the plasmodiophorid vector of *Beet necrotic yellow vein virus* (BNYVV), which causes rhizomania disease. *P. betae* is found in almost all soils where sugarbeet is grown, spreading from plant to plant by means of motile zoospores and survive in the soil for many years for at least 15 years in the form of thicked-wall resting spores or cystosori (Davarani et al., 2013). Despite its ubiquitous distribution and parasitic habitat, *P. betae* is generally considered to cause relatively little damage in temperate climates, although it may be pathogenic in areas of the world where sugar beet is grown in warm soils (Blunt and Gilligan, 1991). *P. betae* can cause stunting and necrosis in lateral roots

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(Filiz et al., 1998) .In contrast, rhizomania disease causes severe economic losses in many countries and is spreading into new regions (McGrann, 2009). *P. betae* is not truly considered as pathogen but as vector of sugarbeet viruses, and it plays crucial role in the epidemiology of viral disease (Ourania et al., 2011).

The virus is carried internally by *P. betae* resting spores in the soil. *P. betae* Keskin has an important role in the transmission and distribution of rhizomania (Delbianco, et al. 2013). The relationship between inoculum potential and the actual inoculum density of viruliferous *P. betae* population is currently unknown (McGrann, 2009).

P. betae Keskin is the only natural transmitting agent of the rhizomania disease caused by *Beet necrotic yellow vein virus* (BNYVV) among the cultivated sugar beet (Safarnejad et al., 2013). Chemical control methods against the vector are either too expensive (methyl bromide soil disinfection) or ineffective. The only useful control measure is the growing of tolerant varieties (Richard-Molard, 1996).

Soil extremes most often lead to problems for plant growth and thus, indirectly for virus accumulation, a saline soil condition has been shown to specifically decrease the accumulation of *Beet necrotic yellow vein virus* (BNYVV) in sugar beets (Bartsch and Brand, 1998).

Liu et al. (2009) reported that soil extremes most often lead to problems for plant growth and thus, indirectly for virus accumulation, a saline soil condition has been shown to specifically decrease the accumulation of beet necrotic yellow vein virus (BNYVV) in sugar beets. Bartsch and Brand (1998) found that saline soil condition decreases rhizomania infection of *B. vulgaris* and concluded that it is still uncertain whether soil salinity operates directly on BNYVV multiplication or causes inactivation of vector zoospores or, influences endogenous plant factors such as root physiology and morphology.

The relationship between inoculum potential and the actual inoculum density of viruliferous *P. betae* population with salinity is currently uncertain, as well as whether soil salinity causes inactivation of vector zoospores or not. So the aim of this work was to study salinity potential on fungal vector of Rhizomania virus infecting *B. vulgaris.* The research work presented in this manuscript was conducted to evaluate effect of salinity potential on capacity of *P. betae* vector to infect *Beta vulgaris* by virus under different levels of salt (NaCl) in the greenhouse.

MATERIALS AND METHODS

Detection of fungal zoospore

Heavily roots from naturally BNYVV infected sugar beet plants were harvested from infested field kafr el-sheikh government (Egypt). Samples were obtained from soil surrounding sugar beet roots exhibiting rhizomania syndrome. These roots were cleaned from soil and washed with tap water then by calcium hypochloride solution (Clorox 5%), and sieved by 50 micro sieve. The roots were examined by optical microscope for the presence of cystosori of *P. betae* and the fungal structures. The tested roots were randomly selected and stained in lacto-phenol solution after boiling and examined with light microscope at 40x magnification.

Preparation of *P. betae* inoculums is as follows:

1. Fine roots of the naturally infected plant carrying zoospore were cut into small pieces 1.5 to 2.0 cm length consisting of the tap root or/and lateral roots attached to it and divided to five equal parts into 5 g.
2. The parts were then immersed in different salt concentration (0, 2000, 4000, 6000 and 8000 ppm) of NaCl in Petri dishes for one day.
3. Preparation of sterile soil was done by using 5% formalin for 2 weeks.

Experimental design

B. vulgaris var. Samba was obtained from Sugar Crops Research Institute, Egypt. Two grams of infected root were added to each pot of sterile soil. Five seeds of sugar beet were sown in a sterile soil in each pot. The relative humidity was approximately 70%. The plants were kept in greenhouse. After virus symptoms appeared on leaves, disease severity, tissue print immuno assay and dot plot immuno assay (TPIA and DPIA) were carried out for virus detection.

Detection of virus infection

Disease severity measurement

The percentage of infected plants and the severity of symptoms were examined using the following rating scale: For leaves (0 = no symptoms; 1,2 = chlorotic and mild mosaic; 3, 4 = Vein yellowing; 5, 6 = necrotic on leaf or petiole; 7,8 = leaf malformation). For roots [0 = no symptoms; 1,2 = root cracks; 3, 4 = root darkness by cutting (oxidation); 5,6 = root atrophy (small in size); 7,8 = root proliferation (beard shape)]. Disease severity (DS) values were calculated using the following formula according to Xicai et al. (1996):

$$DS(\%) = \frac{\sum(\text{disease grade} \times \text{number of plants in each grade})}{(\text{total number of plants} \times \text{highest disease grade})} \times 100$$

Serological technique

Dot plot and tissue print immunoassay

The systemic spread of BNYVV within sugar beet leaves were analyzed using DPIA and TPIA carried out exactly as described by Kaufmann et al. (1992) onto positively charged cellulose membranes. BNYVV were detected by specific antibodies with a chromogenic substrate reaction, leading to a bluish colour development on the blot surface if virus particles were present.

Root sampling

The remaining part of the roots was fixed in lactophenol after boiling for microscopic examination for detection of cystosorial.

Assessment of cystosorial colonization and infection development

The number of cyctosorial and infection percentages were

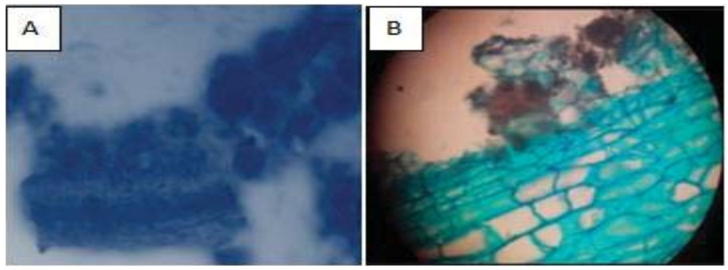


Figure 1. Cross section showing disease manifestation of sugar beet lateral roots upon infection with viruliferous *P. betae* Keskin (A) *P. betae* infected roots stained with lactophenol blue associated with cystosori (B) Transection of sugar beet root.

determined using modified method of (Duc 1989). The presence of cystosorial infection in sugar beet roots were estimated by observation of 10 cleared and stained 1 cm root segments under the microscope for every treatment. For the quantitative characterization of root cystosorial the following parameters were used: Colonization frequency (F%): The percent of roots with fungal structures (cystosori). Colonization intensity (M%): The percent of colonized cortex (cystosoria) in each root were determined according to Duc (1989).

Virus assay

Determination of BNYVV concentration in fungal infected roots. Some leaves of sugar beet plant of all treatments were taken and ground in a mortar containing 0.1 M phosphate buffer, pH 7.0 (1:2 w/v). The homogenate was filtrated through two layers of muslin. The leaves of healthy *Ch. quinoa* plants were dusted with carborundum and rubbed gently with a cotton swab previously dipped into the suspension of virus inoculum.

RESULTS

Virus and fungal incidence in naturally infected sugar beet

Naturally BNYVV infected sugar beet collected from infested field in Kafer El-Shek government exhibited disease syndrome typical to rhizomania virus where as leaves show narrow blade and long petioles. In some instances foliar proliferation of small leaves are observed in the root crown area.

Light microscopy examination cross section of infected sugarbeet roots were observed for presence of fungal cystosori associated with root cell death. Cystosori can be seen lined up on the external and internal layers (Figure 1).

Detection of disease elements in infected sugarbeets

BNYVV detection

Foliar symptoms: Infected sugar beet plants exhibited disease syndrome typical to rhizomania of sugar beet infected leaves show narrow blade and long petioles (Figure 2). In some instances foliar proliferation of small leaves are observed in the root crown area.

Root symptoms: Disease symptoms of infected roots vary greatly depending on the inoculums treatment. The invading fungus causes the killing of secondary roots and even the young tap roots lead to root proliferation and induce symptoms known as the bearded roots. This can be used as positive identification for rhizomania upon making longitudinal section in infected tap roots.

Serological detection of BNYVV: Infected sugar beet plants were confirmed serologically by DPIA and TPIA technique. The results were revealed as purplish blue color developed with specific polyclonal antibody of BNYVV compared with printing from healthy plants which remain green in the negative reactions (Figure 2).

The BNYVV infected sugar beet plants were confirmed by DPIA and TPIA technique. The results revealed different color intensity where *P. betae* infected roots without salt treatment give high density color. The color density was decreased in salt treatments by increasing level of NaCl where it revealed low color density with 8000 ppm revealed moderate color density at 4000 and 6000 ppm and revealed high color density with 2000 ppm (Figure 2).

Viral disease severity

It was observed different external symptoms. The plant treated with *P. betae* infected roots without salt treatment show severe symptoms as chlorosis, vein yellowing and vein necrosis with long petiole with disease severity 65% comparing to non sterile soil which give 10% only. The plant treated with *P. betae* infected roots treated with different concentration of salt show decrease in symptom by increasing concentration of salt Figures 2 and 3 with 58, 35, 27 and 20% for 2000, 4000, 6000 and 8000 ppm

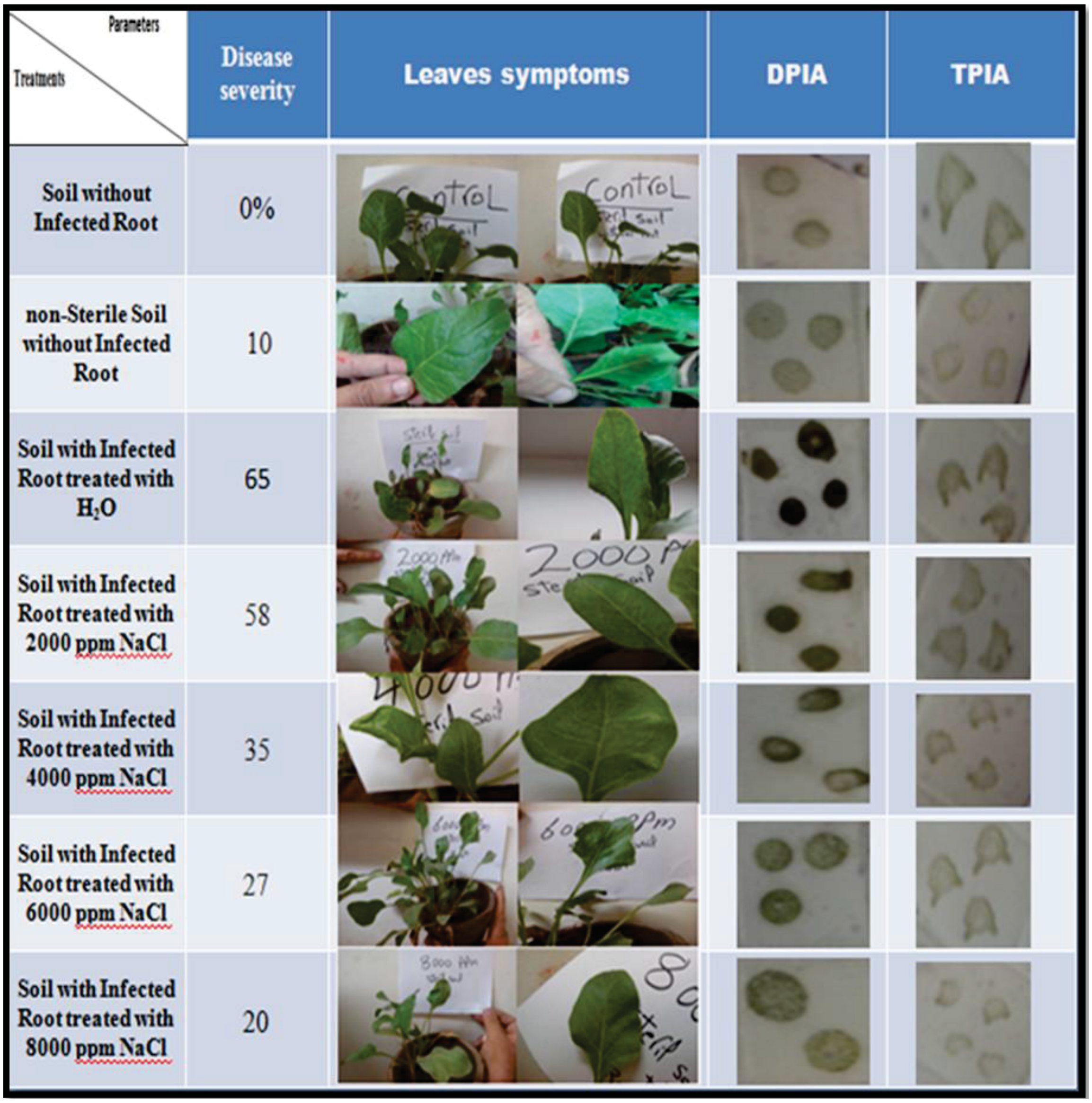


Figure 2. Disease severity percentage of BNYVV infecting sugar beet treated with different concentration of salt growing in sterile soils.

NaCl, respectively (Figures 2 and 4).

P. betae detection in roots of infected sugar beets

Microscopic examination of infected sugar beet roots from plants grown in inoculated soil with different fungal inoculums treatments revealed the presence of fungal cystosori (Table 1 and Figure 5). The degree of infection depended on the number of cystosori in the roots. The infection was greatest in the roots treated with water treatment followed by treatment of salt concentration 2000, 4000, 6000 and 8000 ppm.

The infection cystosorial frequency with *P. betae* were 65, 75, 54, 45, 35 and 8% for non-sterile soil without infected root, root treated with H_2O, root treated with 2000 ppm NaCl, root treated with 4000 ppm NaCl, root treated with 6000 ppm NaCl and root treated with 8000 ppm NaCl respectively (Table 1).

P. betae infection colonization intensity was changed with salt treatments. The P. *betae* infection was high percentage in infected root treated with H_2O with 64.6% while infected root treated with different concentrations of salt were revealed different level of infection 35.5 and 9.25% with 2000 and 8000 ppm, respectively.

The determination of the virus titer as a local lesion showed a continuous decrease in BNYVV content (by decrease in number of L.L with increase salt

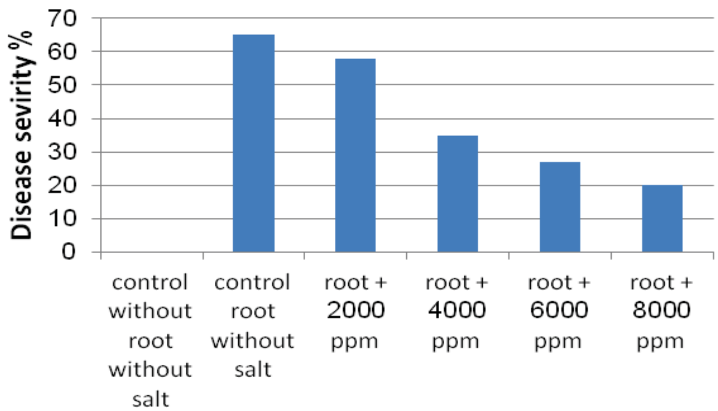


Figure 3. Histogram showing decrease in the disease severity of BNYVV infecting sugar beet with increasing NaCl concentration.

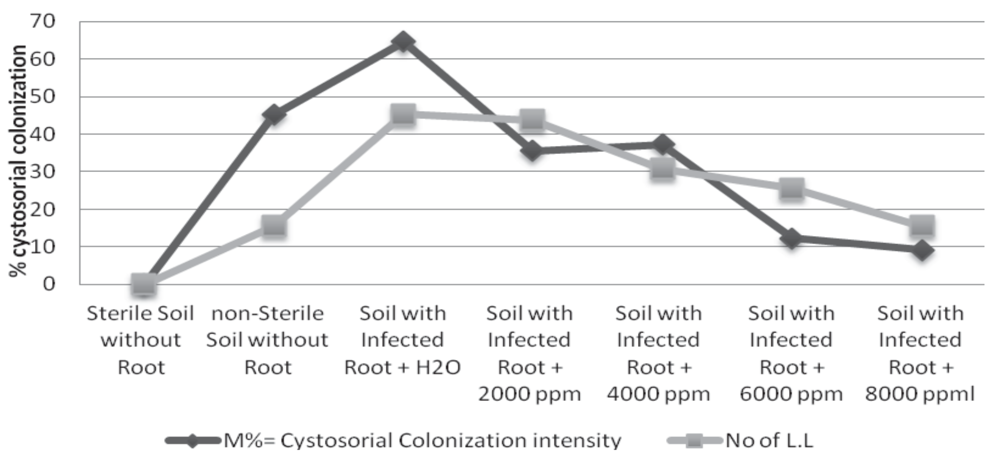


Figure 4. Relation between cystosorial colonization intensity and BNYVV concentration as a LL.

concentration) (Figures 4 and Table 2).

Relation between M%= Cystosorial Colonization intensity and BNYVV concentration as a L.L

It was found a relationship between cystosorial colonization intensity and BNYVV concentration as a L.L (Table 2 and Figure 4). The virus concentration and the fungal cystosorial density in roots treated with high salt concentration was decreased and reached the lowest level especially in case of 8000 ppm NaCl treatment. On the contrary, it was found that the cystosorial fungus density and virus L.L increase in the case of treatment of infected root without salt.

DISCUSSION

The study of the impact of soil salinity on fungal vector of rhizomania virus infecting *B. vulgaris* are very rare, despite the record of the disease in a number of Arab countries in Egypt (Mahmoud and Hashem, 2005), Syria (Mouhanna et al., 2002) and Lebanon (Choueiri et al., 2001). The study came to the importance of fungus spread in most of the land, especially governorates of Kafr el-Sheikh, Fayoum (Megahad, 2013). Naturally infected sugar beet harvested from infested field in kafer el-shek government exhibited disease syndrome typical to rhizomania of sugar beet infected leaves show narrow blade and long petioles. In some instances foliar proliferation of small leaves are observed in the root crown area.

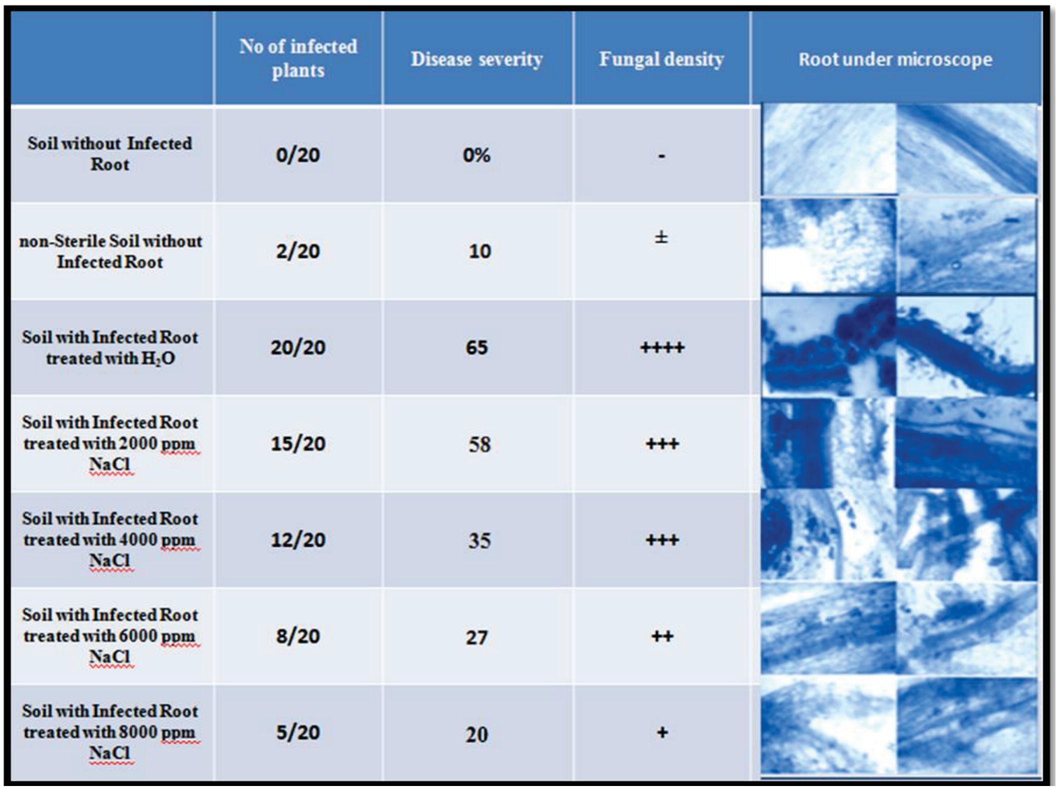
Microscopic examination showed the infection of beet plants grown in soil collected from Governorate Kafr el-Sheikh by *P. betae*. The results were confirmed by serological tests.

Examination of infected lateral roots of sugarbeet plants showed the presence of cystosori of *P. betae* Keskin outside and inside root tissues. Root necrosis and tissue degradation were associated with the presence of cystosori as described by Rush and Heidel (1995).

Table 1. Effect of different concentration levels of salinity on *P. betae* infection of sugar beet growing in sterile soils.

Treatments	Parameter	
	F%	M%
Soil without infected root	0	0
Non-sterile soil without infected root	65.72	45.2
Soil with infected root treated with H_2O	75.75	64.6
Soil with infected root treated with 2000 ppm NaCl	54.25	35.5
Soil with infected root treated with 4000 ppm NaCl	45.72	37.25
Soil with infected root treated with 6000 ppm NaCl	35.2	12.25
Soil with infected root treated with 8000 ppm NaCl	8.22	9.25

F%= Cystosorial colonization frequency M%= cystosorial colonization intensity.



	No of infected plants	Disease severity	Fungal density	Root under microscope
Soil without Infected Root	0/20	0%	-	
non-Sterile Soil without Infected Root	2/20	10	±	
Soil with Infected Root treated with H_2O	20/20	65	++++	
Soil with Infected Root treated with 2000 ppm NaCl	15/20	58	+++	
Soil with Infected Root treated with 4000 ppm NaCl	12/20	35	+++	
Soil with Infected Root treated with 6000 ppm NaCl	8/20	27	++	
Soil with Infected Root treated with 8000 ppm NaCl	5/20	20	+	

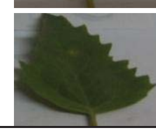
Figure 5. Cystosorial colonization and disease severity under different concentration levels of salinity on *Polymxa betae* infection of sugar beet growing in sterile soils. **-:** There was no presence of the fungal cystosori. **+/-** : Presence of fungus is very low where it was observed with difficulty (at a rate of one cystosori in many fine roots). **+**: Low infection (at a rate of 1-2 cystosori in single fine roots). **++**: Moderate infection (at a rate of 3-6 cystosori in single fine roots). **+++**: High infection (at a rate of 7-10 cystosori in single fine roots). **++++**: Severe infection (the single fine root full with cystosori).

It was recorded infection by *P. betae* with the spread of rhizomania disease either directly or indirectly, as the presence of the disease considered as definitive evidence of the existence of *P. betae* in the soil, while the presence of the *P. betae* does not necessarily mean the presence of the virus, which infects beet plants (Eckart, 1997)

Here the results in this study indicate the possibility of transmission of the fungal cystosorial from infected beet roots in the soil to the healthy roots and these results are agreed with Abe and Ui, (1986), Gerik and Duffus (1987) and Goffart et al. (1989).

Microscopic examination showed that beet root after 10 weeks age proved to be infected by *P. betae*. The

Table 2. Determination of virus titre as a local lesions:

Treatments	Parameter	
	No of L.L	Development of L.L
Soil without infected root	0	
Non-sterile soil without infected root	15.5	
Soil with infected root treated with H_2O	45.3	
Soil with infected root treated with 2000 ppm NaCl	43.7	
Soil with infected root treated with 4000 ppm NaCl	30.8	
Soil with infected root treated with 6000 ppm NaCl	25.5	
Soil with infected root treated with 8000 ppm NaCl	15.5	

Number of L.L was calculated as a mean of five replicate.

number of infected plants were varied because of the density of cystosori of *P. betae* or may be due to some replicates did not have the sufficient inoculums to occur infection. The highest density of cystosori of *P. betae***,** as possible in sterile soil with infected root treated with H_2O, where it is lowest in treatment of high salt concentration 8000 ppm.

The infection cystosorial frequency with *P. betae* were 65, 75, 54, 45, 35, 8% for non-sterile soil without infected root, root treated with H_2O, root treated with 2000 ppm NaCl, root treated with 4000 ppm NaCl, root treated with 6000 ppm NaCl and root treated with 8000 ppm NaCl respectively (Figure 5).

The frequency of virus infection depends on environmental conditions and inoculum densities of the viruliferous population of *P. betae* (Rush, 2003). The optimum temperature for infection with P. betae is 25°C (Asher and Blunt, 1987**).** It has been reported that where BNYVV reaches highest inoculums density in naturally infected soils there is usually greater colonization of the root system, and the virus that infects roots first usually reaches highest levels (Rush, 2003).

P. betae infection colonization intensity was changed with salt treatments. *P. betae* infection was high percentage in infected root treated H_2O with 64.6% while infected root treated with 2000 ppm NaCl was revealed high infection with 2000 ppm 35.5% compared to 9.25% with 8000 ppm.

From literature it was found that 31.4% of the roots were infested with aviruliferous *P. betae* cystosori while 14.3% of the roots did not contain any cystosori (Kutluk Yilmaz and Sokmen, 2010).

It was found that high salt concentration causing decrease in the cystosorial fungus density and virus concentration. It was also appeared to be a strong correlation between the number of attached zoospores and the virus content of the roots. Although this tends to indicate that attachment of viruliferous zoospores measurably and directly increase BNYVV-content, multiplication of the virus on its own cannot be excluded. According to Koenig and Stein (1990), once introduced the virus is able to spread throughout the plant without the aid of its vector. Nevertheless, these results shed new light on the role the vector plays in the course of the disease. To achieve a better understanding of the complex interactions between host, virus and vector further investigations are required.

The cystosorial colonization was increased in low salt concentration (2000 and 4000 ppm) while decrease in high salt concentration (6000 and 8000 ppm) especially in 8000 ppm. The same result was observed in virus concentration in roots where as the virus concentration was increased in low salt concentration (2000 and 4000 ppm) while decrease in high salt concentration (6000 and 8000 ppm). So the relation between capacity of fungal vector to infect the plant by virus and salinity concentration are antagonistic. Soil salinity extremes most often lead to decrease infection of BNYVV via effect on fungal zoospore.

Conflict of Interest

The authors have not declared any conflict of interest.

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Subsoil chemical amelioration and crop yields under continuous long-term no-till in a subtropical Oxisol

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Long-term no-till with shallow fertilizer input results in a chemical enriched topsoil but has minor effect of subsoil. The sub-optimal use of water and mobile nutrients stored in the subsoil layers is a frequent limiting factor to crop grain yield in tropical acid soils. This study aim assesses the corn and soybean grain yield response to gypsum combined with lime surface input as a tool for subsoil chemical quality improving. A Brazilian distrophic Oxisol located in Carazinho (RS State, BR) managed under continuous no-till with characteristics of good chemical soil quality in the topsoil and with a poor condition in the subsoil was select for this study. The experiment design was a randomized block with three replications. The following chemical treatments were broadcast on soil surface as followS: (a) control; (b) 2.5 Mg ha^{-1} of gypsum + 2.0 Mg ha^{-1} of dolomitic lime; and (c) 5.0 Mg ha^{-1} of gypsum + 2.0 Mg ha^{-1} of lime. Both chemical inputs were applied simultaneously, the rates were determined based on lime requirement according to South state fertilizer recommendation and gypsum according to Midwest (Savanna) recommendation. The soil samples were stratified in the 0.00 to 0.60 m soil layer with four sampling times at 0, 6, 22 and 34 months after experiment establishment. An increase in the calcium and magnesium soil contents, as well as soil base saturation, and a decrease in aluminum content, was verified in subsoil layers (0.15 to 0.25 m and 0.25 to 0.40 m) just after six months of chemicals application. These subsoil ameliorate effect was intensified with the conducting time of the experiment notedly at 22 months after chemical application. Moreover, it was found crop grain yield increments statistically significant ranging of 9 to 16%. The gypsum combined with lime was an effective alternative for improving vertically nutrient content of the Oxisol's decreasing the abrupt transition in chemical quality of topsoil and subsoil helping to the maintenance of competitive grain yield under non-disturbed long-term no-till.

Key words: Gypsum, subsoil acidity, soil fertility, calcium, sulfate.

INTRODUCTION

During the period of 1990/91 to 2012/13, the Brazilian agriculture had an increase of 37% in cropland area, but reaching an increase of 127% in grain production based on improvement in grain yield (CONAB, 2013). This way, the main cash crop yield has grown in an annually rate close to 3% in last three decades. However, achieve and sustain high grain crop yields is as important as obtain high efficiency in chemical inputs that is still a challenge in tropical environment.

The adoption of NT combined with cover crops and crop rotation is the main agriculture alternative for the sustainable use of soil in tropical environment (Amado et al., 2006). However, studies carried out in distrophic Oxisols have been suggesting that the NT chemical improvement associated to shallow fertilizer or surface lime application is restricted to topsoil, creating a profile non favorable for deep root growth, increasing the risk to crop water stress (Shainberg et al., 1989; Blanco-Canqui and Lal, 2008; Caires, 2012; Dalla Nora and Amado, 2013).

Naturally, the subsurface layers here defined as below 0.20 m, are acid and unfertile for most of the Brazilian tropical and subtropical Oxisols (Rampim et al., 2011). The high concentration of Al associate to low Ca concentration and basis saturation are the most frequent chemical impediments for deep root growth (Raij, 2010). The occurrence of short-term drought associate to shallow crop root growth has led to recurrent economic agricultural losses in Brazil (Caires et al., 2011a).

Lime, the main chemical input used for alleviate soil acidity, has low water solubility and its reaction is slow especially when soil surface-applied as in continuous NT. In this scenario, the improvement of the subsoil chemical attributes under NT is less probable, and depends on frequency and intensity of lime input, leaching of salts and organic compounds through the soil profile (Toma et al., 1999; Caires et al., 2003) and by the adoption of cover crops (Miyazawa et al., 2002). However, some studies where was investigated treatments with high rates and frequent lime application show the chemical soil quality improvement in the subsoil (Oliveira and Pavan, 1996; Caires et al., 2008). The NT disturbed aiming lime incorporation in 0-0.20 m increases the lime reaction but did not guarantee that subsoil layers deeper than tillage operation will be ameliorated (Farina et al., 2000a). Also, discontinuities of NT will have important environmental impact due the increase in soil erosion risk, high fuel consumption and soil organic matter oxidation (Amado et al., 2006).

The gypsum + lime surface-application is increasing gradually in Brazilian NT in conditions tropical and subtropical as an alternative for ameliorate subsoil chemical attributes and decrease short drought crop stress (Caires et al., 2003, 2011b; Rampim et al., 2011; Dalla Nora and Amado, 2013). After gypsum application there is a sharp increase in soil solution Ca content causing the displacement of Al, magnesium (Mg) and potassium (K) of the soil exchange complex (Farina et al., 2000a, b; Zambrosi et al., 2007; Favaretto et al., 2008). In wet tropical and subtropical climate under high precipitation there is a downward sulphate movement following water drainage causing basis leaching, mainly Ca and Mg, and boosts the formation of Al-sulfate, which is less toxic to plants (Carvalho and Raij, 1997; Favaretto et al., 2008). The consequence of this process is subsoil chemical amelioration preserving soil structure and soil organic matter (Dalla Nora and Amado, 2013).

The use of gypsum has been recommended preferably after or at least applied together with dolomitic lime, due to the synergistic effect of these chemical inputs (Raij, 2010). The gypsum enhances the action of the superficial application of lime enhancing the deep root growth (Caires et al., 2004).

The effect of gypsum on grain yield has been contradictory and crop type dependent (Farina et al., 2000a; Raij, 1994; Caires et al., 2004, 2011b). Farina et al. (2000a) evaluating long term gypsum effect (10 corn harvests which most had significant increase at $p<0.05$), reported an average grain yield increments of 135 kg ha^{-1} $year^{-1}$. Caires et al. (2004) report corn yield increments of 17% statistically significant at $p<0.01$. Rampim et al. (2011) report linear and statistically significant ($p<0.05$) wheat yield increments significance as a response to gypsum rates up to 5.0 Mg ha^{-1}. Raij (1994) found increases of 184 kg ha^{-1} ($p<0.05$) in soybean yield for the rate of 6.0 Mg ha^{-1} of gypsum. However, Caires et al. (2011b) did not find increases in soybean yield for gypsum rates varying from 0 to 9 Mg ha^{-1}, in agreement with data reported by Rampim et al. (2011). The authors reported that the soybean yield was less sensitive to gypsum than the corn and wheat. In addition, Reeve and Sumner (1972) and Dalla Nora et al. (2014) sustain that the gypsum positive effect on grain crop yields is more pronounced under water stress conditions.

There are few studies reporting the synergic effect of lime and gypsum on crop yields in long-term NT. In order to fill up this gap an Oxisol at Rio Grande do Sul State (RS), Brazil, with good chemical quality in topsoil but with poor in subsoil was selected to test the hypothesis that is possible ameliorate subsoil chemical quality allowing achieve high corn-soybean crop yields without soil disturbance.

MATERIALS AND METHODS

Field site description

The experiment was carried out during 2009 to 2012 in a cropland located at Carazinho, Rio Grande do Sul State, Brazil, with coordinates of 28° 17' S and 52° 47' W, in a distrophic Oxisol (Typic

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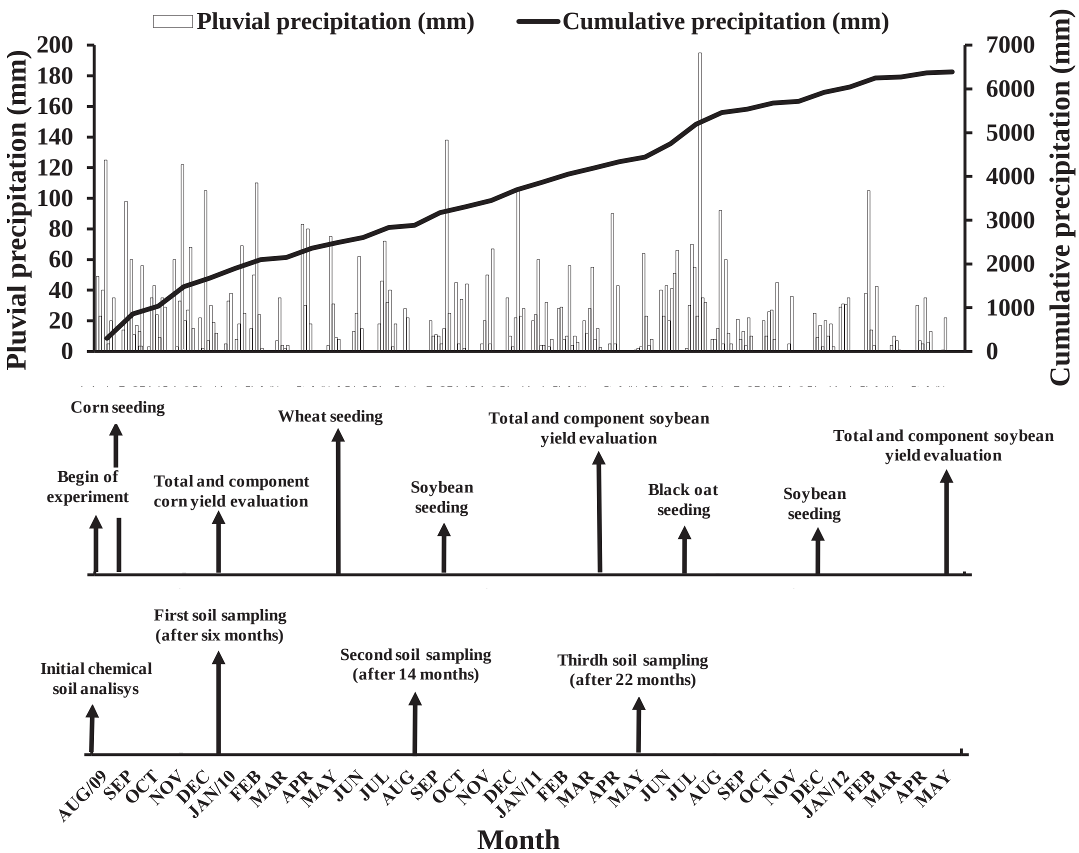

Figure 1. Daily and cumulative precipitation during experimental period and main experimental details.

Hapludox) (Soil Survey Staff, 2010). According to Köppen classification the climate of the region is wet subtropical (Cfa) with a mean annual temperature of 16°C, and mean annual rainfall of 2,020 mm. During the experimental period, rainfall accumulated are 1,864 mm after six months, 3,140 after 14 months, 4,440 after 22 months, and 6,389 after 34 months when the experiment was discontinued (Figure 1).

The experimental field site consists of 0.6 ha of a farm with 280 ha. The experimental field has been managed continuously under NT system for approximately 20 years with a cropping system composed of black oat (*Avena strigosa* Schreb)/maize (*Zea mays* L.)/wheat (*Triticum aestivum* L.)/soybean/black oat/soybean. This cropping system is typical in South Brazil. Liming rate applied in this field was 2.0 Mg ha^{-1} arbitrarily definied by farmers, with 75% effective calcium carbonate equivalent, input every five years always broadcast on soil surface. Before installing the experiment, the area was cultivated with black oats, as cover crop, which was managed with GLYPHOSATE [N-(phosphonomethyl)glycine] to, thereafter, homogeneously apply gypsum and lime (August, 2009). In sequence, a corn crop was seeded in September receiving 200 kg ha^{-1} of N (20 kg ha^{-1} at seeding + 180 kg ha^{-1} split in two equal doses as topdress N fertilization), 125 kg ha^{-1} of P_2O_5 and 130 kg ha^{-1} of K_2O. This corn crop was evaluated in relation to total grain and component yield. In the cropping system the wheat was seeded in July 2010, receiving: 250 kg ha^{-1} of the 5-25-25 fertilizer formulation and 30 kg ha^{-1} of N as a topdress N fertilization. This winter crop was not harvested due a severe frozen at flowering stage. In sequence a soybean crop was seeded in November 2010, which received 240 kg ha^{-1} of the 2-20-20 fertilizer formulation and was harvested in March 2011, when the evaluation of grain yield and component were done. During the winter the area had black oat as cover crop. In sequence, soybean crop was planted in December 2011, which received 240 kg ha^{-1} of the 2-20-20 fertilizer formulation and was harvested in May 2012, the soybean crop was evaluated in relation to total grain and components yield. The crop sequence and plant evaluations are shown in Figure 1.

Before installing the experiment the soil was sampled in four layers: 0.0-0.10; 0.10-0.20; 0.20-0.40 and 0.40-0.60 m soil depths with five replicates taken randomly in the experiment site.

Experimental design

A random block design with three replicates and plots size of 8 × 8 m was used in this study. Treatments consisted of: (1) T_0 - control without gypsum and lime applications; T_1 - 2.5 Mg ha^{-1} of gypsum + 2.0 Mg ha^{-1} of dolomitic lime; T_2 - 5.0 Mg ha^{-1} of gypsum + 2.0 Mg ha^{-1} of dolomitic lime. The gypsum applied had 29% of CaO, 16% of S and 1% of P. The dolomitic lime had 30% of CaO and 20% of MgO with 75% effective calcium carbonate equivalent and 75% reactivity.

Soil sampling and analysis

Six months after treatments application soils were sampled at the following depth layers: 0.00-0.05, 0.05-0.10, 0.10-0.15, 0.15-0.25, 0.25-0.40 and 0.40-0.60 m. A second and a third soil samplings were performed after 14 and 22 months of the beginning of the experiment, respectively, at the same soil depths.

Table 1. Soil chemical attributes prior to treatments application.

Depth	pH $_{H2O}$	Al	Ca	Mg	K	Ca/Mg	Ca+Mg/K	CEC	P	S	BS%	m%	Clay
m						cmol$_c$ dm^{-3}			mg dm^{-3}		%		g kg^{-1}
						Begin of experiment							
0.0-0.10	5.6	0.0	7.7	3.8	0.26	2.02	44.2	11.86	23.1	13.2	72.0	0.0	540.0
0.10-0.20	5.0	0.7	4.2	2.4	0.08	1.75	82.2	7.78	8.3	8.2	42.0	13.0	630.0
0.20-0.40	4.6	3.1	2.2	1.2	0.04	1.83	85.0	6.77	3.4	17.0	21.0	47.0	680.0
0.40-0.60	4.4	3.7	1.4	0.9	0.04	1.55	57.5	6.18	2.1	31.0	13.0	61.0	700.0

BS%, Basis saturation; m%, aluminium saturation.

The main soil chemical parameters analyzed were determined according to the standard methods described in Tedesco et al. (1995). Soil chemical analysis consisted of: pH water measured in a 1:1 proportion of soil/water; Al concentration obtained by KCl 1 mol L^{-1} extraction and titrated with NaOH 0.0125 mol L^{-1}, Ca and Mg concentrations also obtained from KCl 1 mol L^{-1} extraction and evaluated by atomic absorption spectrometry, P and K extracted with the double acid Mehlich-I extractor and measured by atomic spectrometry and flame photometry, respectively. The evaluation of S concentration was made in Ca phosphate extractor adopting the treatment with HNO_3-$HClO_4$ (Beaton et al., 1968), to determine sulfate after precipitation in a $BaCl_2$ gel solution (Tedesco et al., 1995).

At the physiologic maturation stage of corn and soybean crops, plant material was sampled close to the location of soil sampling pits, for grain total and components yield evaluation. The total area of Plant samples were 2 m^2 were 4 m (2 m from each planting row), and grain yield values were corrected for 13% water content.

For corn crop the following yield components were evaluated: Ears per meter of row; grain rows per ear; grain per row in ear; and weight of 1,000 grains. For soybean yield the components were: Pods per plant; grains per pod; and weight of 1,000 grains.

Statistical analysis

Results were submitted to analysis of variance by SISVAR (Sisvar, version 5.3) with the Tukey test at 5% probability. Regression analysis were made by JMP (JMP IN software, 3 ed., version 7.0.1). The temporal effect of the treatments for each layer of the soil sampled was evaluated by regression analysis between the concentrations of Ca, Mg, K and S and the sampling soil intervals of 0, 6, 14 and 22 months after the begin of the experiment.

RESULTS AND DISCUSSION

Soil chemical attributes at beginning of the experiment

For the topsoil (0.00-0.10 m) the values of pH and BS% were above the critical values (pH > 5.5; BS% ≥ 65%) while m% was bellow (m% < 10%) as established by two-state fertilizer and lime recommendation (CQFS-RS/SC, 2004) applied in Southern Brazil. This result implies in no need of lime input in experimental area based on topsoil chemical quality. However, already in the adjacent soil layer, 0.10-0.20 m, these chemical attributes were lower, being 5.0, 42 and 13% for pH, BS% and m%, respectively, indicating the need of lime input (Table 1). Therefore, there was an abrupt gradient of the acidity soil parameters through the soil profile. Comparing the soil layers of 0.0-0.10 and 0.10-0.20 m, there is decreases in BS%, P and K of 42, 64 and 71%, respectively, and comparing the topsoil with the typical diagnostic subsoil layer (0.20-0.40 m) the decreases were 72, 86 and 86%, respectively, characterizing a gradual loss of chemical soil quality with the increase in soil depth under continuous NT with lime application based on shallow topsoil (Amado et al., 2009).

For gypsum recommendation, Raij (2010) suggested as critical the value of m% > 40% for the layer 0.20-0.40 m (diagnostic subsoil layer), this way, as the soil of experimental site had m% = 47% (Table 1), it has a high probability to positive responses to gypsum input. Considering the clay content of 680 g kg^{-1}, the recommended gypsum rate would be equivalent to 4.1 Mg ha^{-1} that is in the range of gypsum rates investigated (2.5 and 5.0 Mg ha^{-1}).

In relation to the Ca and Mg contents and Ca/Mg ratio in the topsoil, the values are above the critical levels (Ca > 4.0 cmol$_c$ dm^{-3}, Mg > 1.0 cmol$_c$ dm^{-3} and Ca/Mg optimum range of 4-2:1) (Escosteguy, 2012). However, the Ca+Mg/K is out of the critical ratio (optimum range of 17-35:1) (Escosteguy, 2012), due to the low K concentration in relation to these two cations. The S concentration in topsoil is above the critical value of 5.0 and 10 mg dm^{-3} for corn and soybean, respectively, according to two-state South Brazil fertilizer recommendation (CQFS-RS/SC, 2004) (Table 1).

In summary, at the time of experiment implantation based on the chemical attributes of the topsoil layer the crop response to gypsum + lime treatments is unlikely. On the other hand, based on the chemical attributes of subsoil BS% these chemicals are needed.

Improvement of subsoil chemical attributes due to gypsum and lime input

The concentrations of Al, Ca, Mg, S and K, pH value, in addition of SB% and m% index, show significant relationships with gypsum + lime input as a function of

Table 2. Analysis of variance of chemical soil attributes under gypsum + lime rates, soil depth and soil sampling periods of six, 14 and 22 months after the begin of the experiment.

Causes of variation	pH H_2O	Al	m%	S	Ca	Mg	K	BS%
After six months		**$cmol_c$ dm^{-3}**	**%**	**mg dm^{-3}**		**cmolc dm^{-3}**		**%**
Gypsum + lime	*	*	*	*	*	*	ns	*
CV (%)	7.65	32.72	49.43	33.61	37.82	24.22	3.78	39.72
Soil depth	*	*	*	*	*	*	*	*
Gypsum + lime x depth	*	*	*	*	*	*	*	*
CV (%)	*3.00*	22.72	35.79	24.37	8.41	12.08	1.71	10.72
After 14 months								
Gypsum + lime	*	*	*	*	*	*	ns	*
CV (%)	9.21	33.21	41.22	13.61	41.27	13.21	31.1	22.94
Soil depth	*	*	*	*	*	*	*	*
Gypsum + lime x depth	*	*	*	*	*	*	*	*
CV (%)	1.84	8.12	23.43	10.84	14.2	4.12	3.42	6.69
After 22 months								
Gypsum + lime	*	*	*	*	*	*	ns	*
CV (%)	9.73	37.3	45.65	32.34	13.80	31.34	6.25	19.81
Soil depth	*	*	*	*	*	*	*	*
Gypsum + lime x depth	*	*	*	*	*	*	*	*
CV (%)	3.25	14.41	21.09	21.75	3.79	4.95	4.85	10.99

n.s, Non significative; *, Significative at 5%; CV, coefficient of variation.

soil depth, at all soil sampling periods (Table 2). For the topsoil the pH increases can be taken as an expected effect of liming (Figure 2) (Caires et al., 2005), however, due to its low water solubility and as a consequence slow down movement through the soil profile, the pH increase in the subsoil layers would not be expected (Ritchey et al., 1980; Pavan et al., 1984; Farina et al., 2000b). The movement of lime particles with drainage water may be also a mechanism of correction of soil subsurface soil acidity in continuous no-tillage notadely under high rates of lime input (Amaral et al., 2004). The pH increase in the subsoil layers verified in our study may be attributed to the gypsum effect, due to ligand exchange reaction, involving Fe and Al hydrated oxides with the sulfate, in this way dislocating hydroxides which partially promote the neutralization of soil acidity (Reeve and Sumner, 1972). Also, the plant uptake of high quantity of sulfate could result in hydroxide plant excretion increasing the pH (Soratto and Crusciol, 2008). Previously, Raij et al. (1994), Caires et al. (2003) and Rampim et al. (2011) reported increase in subsoil pH with lime + gypsum input similar to those here presented.

The high accumulated rainfall up to 14 and 22 months corresponding to 3,140 and 4,407 mm, respectively (Figure 1), must have contributed to the displacement of sulfate and to the increase in pH through the root growth zone (Figure 2). In this way, the subsoil layer of 0.25-0.40 m presented pH increases of 6 and 11% for the lowest and highest rates of gypsum + lime treatments, respectively, in relation to the control elapsed 22 months of experiment set up (Figure 2c). For this subsoil layer the decreases in Al concentration were 21 and 36% for the same rates and time elapsed (Figure 2c).The decrease of Al concentration in the subsoil noted in our study (Figure 2), is in agreement with previously reported by Pavan et al. (1984) and Farina et al. (2000b). The decrease in Al concentration probably is the result of ionic exchanges of Al by Ca, displacing the Al to the soil solution, being temporarily immobilized by sulfates (Pavan et al., 1984; Shamshuddin et al., 2009). Furthermore, even a small increased pH in the subsurface can reduce the concentration of Al.

Elapsed 22 months of the beginning of the experiment, the m% index for the 0.15-0.25 m subsoil layer presented decreases of 50 and 51% for the lowest and highest gypsum + lime rates, respectively, in relation to the control. For the 0.25-0.40 m these decreases were of 37 and 56%, respectively (Figure 2c). Farina et al. (2000b), in an experiment with gypsum input, also reported ameliorate in the chemical quality of a deep soil layer (0.45-0.75 m).

In treatments ameliorated with gypsum + lime the increase in S concentration through root zone growth was faster and intense (Figure 2). Previously, Farina et al. (2000b) reported S concentration increases up to 0.90 m soil depth, however, with a gypsum rate as high as 10 Mg ha^{-1}. The fast downward movement of S through soil profile in our study could have been favored by the lime application, because this input while increasing the pH promotes the increase of negative charges that reduce

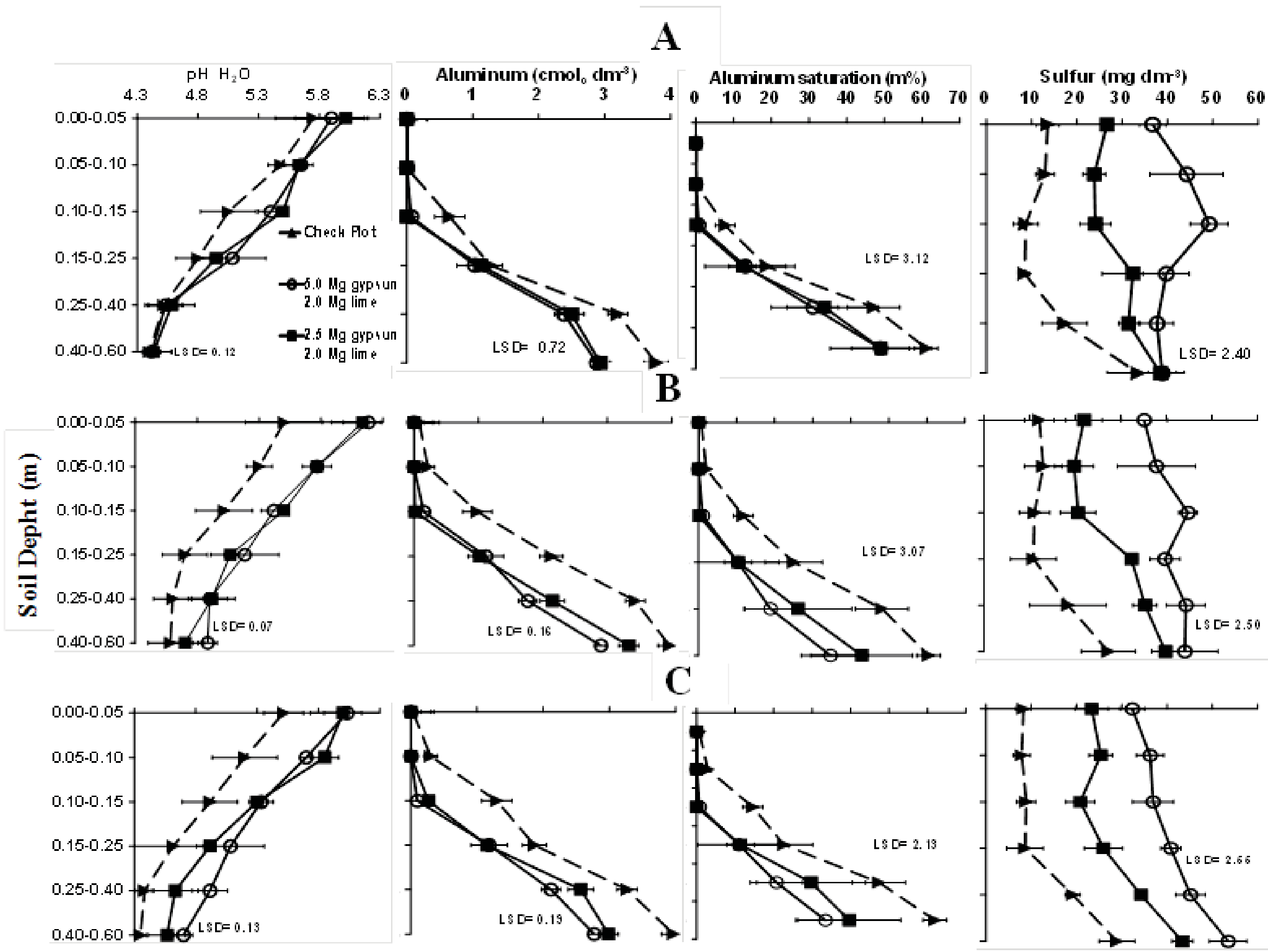

Figure 2. Values of pH, aluminium concentration, aluminium saturation (m%) and sulfur concentration affected by gypsum + lime after six (A), 14 (B) and 22 (C) months after the input. LSD by Tukey test ($p<0.05$).

the adsorption of the sulfate with the soil exchange complex (Farina et al., 2000b).

It was also observed that the application of gypsum + lime promoted an increase in the Ca and Mg concentrations along the soil profile (Figure 3). Elapsed only six months of the highest gypsum + lime rate treatment increments in the concentrations of Ca and Mg of 19 and 22%, respectively, were observed in the 0.40-0.60 m subsoil layer in relation to control (Figure 3a). Elapsed 14 months, these increments were amplified to 39 and 62%, respectively, and further to 48 and 64%, after 22 months (Figure 3b and c), in agreement with previously reported by Farina et al. (2000b). Therefore, in our study the relative increase in Mg was higher than Ca in subsoil.

As a consequence of the increases in the Ca and Mg content and at same time of the decrease in Al concentration, an expected increase of BS% was verified in subsoil (Figure 3). Similar results were previously presented by Caires et al. (2011a). So, after six elapsed months, increments of 32% and 34% in BS% were observed in the 0.25-0.40 m subsoil layer for the lowest and highest gypsum + lime rates, respectively in relation to the control (Figure 3a). After 22 elapsed months, these increments were 45 and 48%, respectively (Figure 3c).

The increments in Ca, Mg, BS%, S and pH, and the decreases in Al and the m% index (Figures 2 and 3) render a subsoil environment more adequate to plant root growth and, consequently, stimulate a better use of the soil water, as reported by Farina et al. (2000a, b), Caires et al. (2003); Favaretto et al. (2008); Shamshuddin et al. (2009). In this way, the lime + gypsum effect in ameliorate chemical soil quality through the root zone growth occurs in a gradual manner and is dependent of the rate of chemical inputs and of the accumulated rainfall volume. In addition, the long-term NT preserves

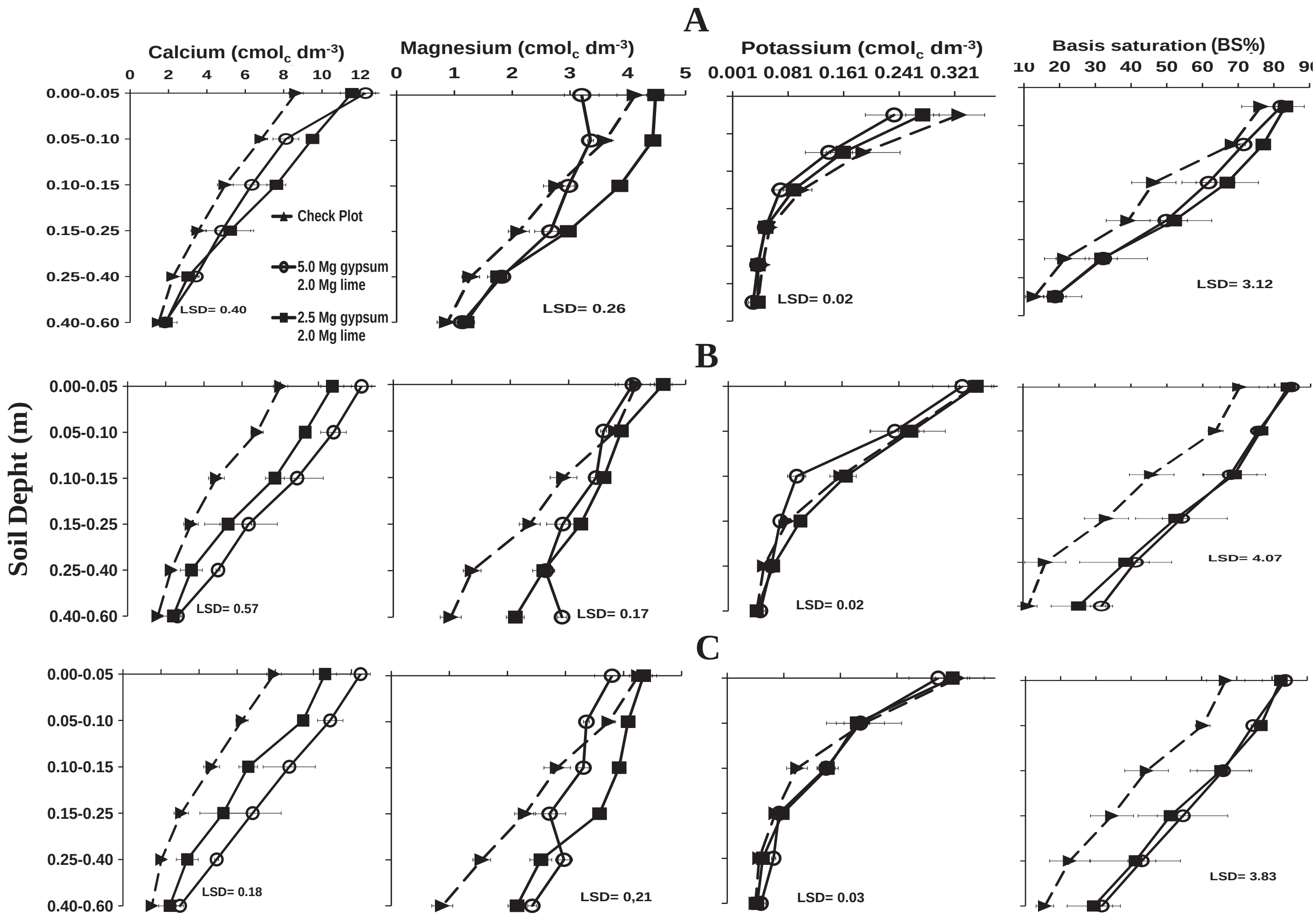

Figure 3. Values of calcium, magnesium, potassium concentration and basis saturation (BS%) affected by gypsum + lime after six (A), 14 (B) and 22 (C) months after the input. LSD by Tukey test ($p<0.05$).

the biopores that are vertically continuous through the root growth zone enhancing the downward solute movement following the preferential flow of water (Vervoort et al., 2001). The presence of biopores in long-term NT is a likely explanation for fast ameliorates in subsoil chemical quality under superficial gypsum + lime application.

The available K concentration in the soil layers investigated in general was not affected by rates of gypsum + lime input (Figure 3). It is important to note that the high clay contents (varying in depth from 540 to 700 g kg^{-1}), the increase of SOM in shallow soil layer and the CEC (11.86 $cmol_c$ dm^{-3}) (Table 1) might have disfavored the expected K leaching. Caires et al. (2011a) also reported that K leaching losses associated to the application of gypsum in Oxisol NT was reduced.

The concentration of Ca and SB%, in the soil layers of 0.15-0.25, 0.25-0.40 m and 0.40-0.60 m, and concentration of Mg in the layer of 0.15-0.25 m were related ($p < 0.05$) with the S concentration in the respective layers (Table 3). This relationship is probably due to neutral ion-pair formation with subsequent leaching through the soil profile (Caires et al., 2011a). Moreover, in our study no relationship was observed between the S and K concentrations in the subsoil layers (Table 3), as previously reported by Sumner et al. (1986) and Caires et al. (2011a) under Oxisols.

Corn and soybean grain yields affected by gypsum and lime input

The corn grain yield and some yield components (number of ears m^{-1} and number of grain $rows^{-1}$) shows significant relationships with gypsum + lime input (Table 4). Averaged of gypsum + lime treatments, the corn yield increase of 9% was found in relation to check plot (Figure 4a). Although, the check plot had achieved high corn yield (≅ 11 Mg ha^{-1}) as a result of good chemical soil quality of topsoil and long-term NT adoption. Farina et al.

Table 3. Regression equation between the results of all sampling times of calcium, magnesium, potassium and basis saturation (BS%) taken as dependent variable (y) with sulfur taken as the independent variable (x) affected by gypsum + lime in soil layers.

Gypsum + lime	Chemical soil atributte	Soil depth	Equation	R^2
Mg ha^{-1}		m		
2.5 + 2.0	Ca	0.00-0.05	$\hat{y} = \bar{y} = 9.95$	-
		0.05-0.10	$\hat{y} = \bar{y} = 7.33$	-
		0.10-0.15	$\hat{y} = \bar{y} = 6.71$	-
		0.15-0.25	$\hat{y} = 1.7693 + 0.2478x - 0.0043x^2$	0.95*
		0.25-0.40	$\hat{y} = 2.2038 - 0.0283x + 0.0018x^2$	0.98**
		0.40-0.60	$\hat{y} = - 6.8353 + 0.3578x - 0.0033x^2$	0.88*
	Mg	0.00-0.05	$\hat{y} = \bar{y} = 3.80$	-
		0.05-0.10	$\hat{y} = \bar{y} = 3.48$	-
		0.10-0.15	$\hat{y} = 2.1354 + 0.0756x$	0.92*
		0.15-0.25	$\hat{y} = 1.4299 + 0.0961x - 0.0018x^2$	0.99**
		0.25-0.40	$\hat{y} = \bar{y} = 2.06$	-
		0.40-0.60	$\hat{y} = \bar{y} = 1.83$	-
	K	0.00-0.05	$\hat{y} = \bar{y} = 0.32$	-
		0.05-0.10	$\hat{y} = \bar{y} = 0.20$	-
		0.10-0.15	$\hat{y} = \bar{y} = 0.12$	-
		0.15-0.25	$\hat{y} = \bar{y} = 0.05$	-
		0.25-0.40	$\hat{y} = \bar{y} = 0.04$	-
		0.40-0.60	$\hat{y} = \bar{y} = 0.03$	-
	BS%	0.00-0.05	$\hat{y} = \bar{y} = 83.9$	-
		0.05-0.10	$\hat{y} = \bar{y} = 75.6$	-
		0.10-0.15	$\hat{y} = \bar{y} = 61.9$	-
		0.15-0.25	$y = 28.761 + 1.4121x - 0.0212x^2$	0.98*
		0.25-0.40	$y = 42.921 - 2.3725x + 0.0653x^2$	0.93*
		0.40-0.60	$y = - 19.13 + 0.3391x + 0.0182x^2$	0.94*
5.0 + 2.0	Ca	0.00-0.05	$\hat{y} = \bar{y} = 10.72$	-
		0.05-0.10	$\hat{y} = \bar{y} = 8.45$	-
		0.10-0.15	$\hat{y} = \bar{y} = 7.23$	-
		0.15-0.25	$\hat{y} = 50.268 - 3.1463x + 0.0511x^2$	0.95*
		0.25-0.40	$\hat{y} = 4.5186 - 0.2192x + 0.0051x^2$	0.99**
		0.40-0.60	$\hat{y} = - 4.9876 + 0.2602x - 0.0021x^2$	0.95*
	Mg	0.00-0.05	$\hat{y} = \bar{y} = 4.34$	-
		0.05-0.10	$\hat{y} = \bar{y} = 4.00$	-
		0.10-0.15	$\hat{y} = \bar{y} = 3.54$	-
		0.15-0.25	$\hat{y} = 1.9681 + 0.0166x + 8e\text{-}05x^2$	0.92*
		0.25-0.40	$\hat{y} = 2.397 - 0.1486x + 0.0035x^2$	0.97*
		0.40-0.60	$\hat{y} = \bar{y} = 2.06$	-
	K	0.00-0.05	$\hat{y} = \bar{y} = 0.30$	-
		0.05-0.10	$\hat{y} = \bar{y} = 0.19$	-
		0.10-0.15	$\hat{y} = \bar{y} = 0.10$	-
		0.15-0.25	$\hat{y} = \bar{y} = 0.05$	-
		0.25-0.40	$\hat{y} = \bar{y} = 0.05$	-
		0.40-0.60	$\hat{y} = \bar{y} = 0.04$	-
	BS%	0.00-0.05	$\hat{y} = \bar{y} = 84.18$	-
		0.05-0.10	$\hat{y} = \bar{y} = 72.02$	-
		0.10-0.15	$\hat{y} = \bar{y} = 60.46$	-
		0.15-0.25	$\hat{y} = 39.829 - 0.1974x + 0.0139x^2$	0.99**
		0.25-0.40	$\hat{y} = 33.402 - 1.2482x + 0.0323x^2$	0.99**
		0.40-0.60	$\hat{y} = - 142.55 + 6.8332x - 0.0667x^2$	0.91*

R^2, Coefficient of determination; $^*p<0.05$; $^{**}p<0.01$.

Table 4. Analysis of variance of yield and yield components of corn (six months), soybean (22 months) and soybean (34 months) affected by gypsum + lime rates.

Corn (after six months)					
Cause of variation	**Yield (kg ha^{-1})**	**Ears m^{-1}**	**Rows ear^{-1}**	**Grain row^{-1}**	**Weight of 1000 grains (kg)**
Gypsum + lime	*	*	ns	*	ns
CV (%)	2.77	3.47	5.79	4.73	14.23
Soybean (after 22 months)					
	Yield (kg ha^{-1})	**Pods $plant^{-1}$**		**Grains pod^{-1}**	**Weight of 1000 grains (kg)**
Gypsum + lime	*	*		ns	ns
CV (%)	2.98	10.96		2.56	12.96
Soybean (after 34 months)					
Gypsum + lime	*	ns		ns	*
CV (%)	3.06	13.41		3.00	3.22

ns, Non significative; cv, variation coefficient.

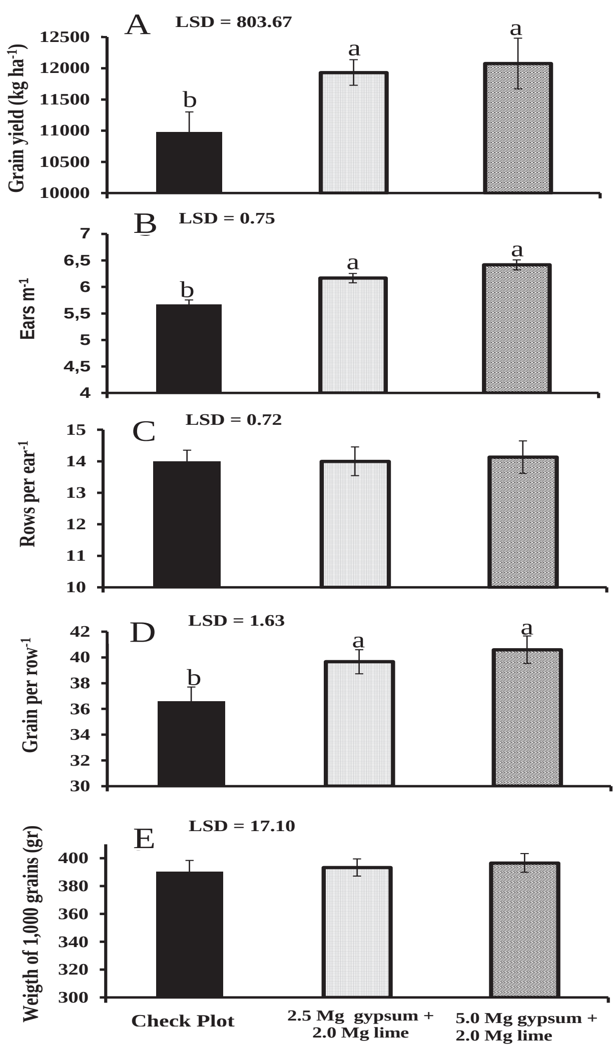

Figure 4. Corn yield (kg ha^{-1}) (A), number of ears m^{-1} (B), rows ear^{-1} (C), grain row^{-1} (D), weight of 1,000 grains (E) affected by gypsum + lime treatments after six months. LSD by Tukey test ($p<0.05$).

(2000a) reported an average increase of 25% in corn yield by gypsum input in an Oxisol, resulting in 3.8 Mg ha^{-1} of grain increase in accumulate production of 11 crop-seasons. These authors reported that the largest corn yield increases were observed in years with water stress. Caires et al. (2004, 2011b) also reported increases in corn yields in distrophic Oxisols ameliorated with gypsum + lime.

The soybean grain yield and following yield components: Pods per plant and weight of 1,000 grains show significant relationships with gypsum + lime input (Table 4). In our study it was found increments of 13 and 16% in soybean grain yield for rates of 2.5 to 5.0 Mg ha^{-1} gypsum + lime, respectively, compared to control (Figure 5a), in first soybean crop (elapsed 22 months after of the experiment beginning). In the second soybean crop, elapsed 34 months, these increments were amplified to 16 and 18% (Figure 5b). Similar results were reported by Raij et al. (1994) and Sousa et al. (1996) under water stress conditions, in which the combined effect of gypsum and lime allowed better efficiency use of soil water.

Among soybean yield components, the number of pods per plant shows an increase of 12% in highest gypsum + lime rate compared to check plot (Figure 5c) in first soybean year under satisfactory rainfall. Moreover, the number of grains per pod and the weight of 1,000 grains were not altered by gypsum + lime treatments (Figure 5e and g). In the second soybean crop, under water stress conditions, the component weight of 1,000 grains shows an increase of 4% ($p<0.05$) in highest gypsum + lime rate compared to check plot (Figure 5h). This result maybe was associate to water scarcity at soybean grain fill (Salinas et al., 1996; Desclaux et al., 2000) (Figure 1). Although, the number of grains per pod and the pods per plant were not affected by gypsum + lime treatments (Figure 5d and f).

In this study the highests crop grain yield achieved (12.1 Mg ha^{-1} to corn and 4.2 Mg ha^{-1} to soybean) under

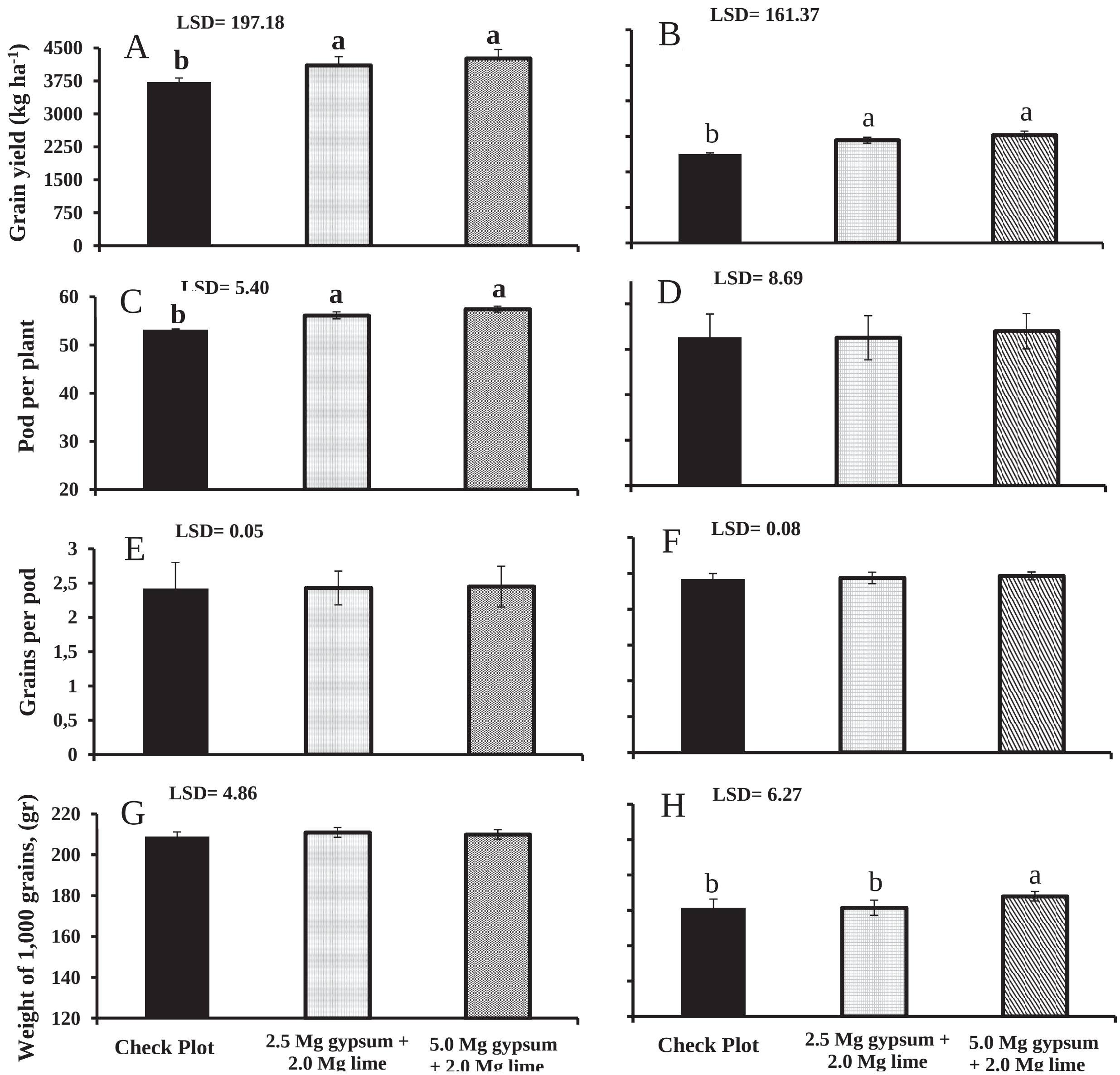

Figure 5. Soybean yield (kg ha^{-1}) (A), number of pods per plant (C), grains per pod^{-1} (E), weight of 1,000 grains (G) affected by gypsum + lime treatments after 22 months and soybean yield (kg/ha) (B), number of pods plant^{-1} (D), grain pod^{-1} (F), weight of 1,000 grains (H) affected by gypsum + lime treatments after 34 months. LSD by Tukey test ($p<0.05$).

NT with ameliorate chemical subsoil quality could be classified as high for non-irrigated cropland. With this, it is inferred that the soil management systems here evaluated were satisfactory to plant growth and, in this case, the adoption of practices such as conventional tillage to physically mix soil layers in order to reduce the chemical attributes gradient between top and subsoil layers and stimulate deeper root growth could be dispensable saving time, fuel, CO_2 emissions, decreasing soil loss, mantaining soil organic matter and preserving soil structure. In this scenario, the use of gypsum + lime in Oxisols could be a promising strategy to mantain continuous NT with competitive crop grain yields.

Conclusions

The long-term Oxisol NT with superficial lime input showed good chemical soil quality in the topsoil but had poor in the subsoil due acidity characteristics. The lime + gypsum input resulted in ameliorate subsoil, expressed by increases in concentration of Ca and basis saturation associate to reduction in concentration of Al and Al saturation. This amelioration was linked to sulfate movement following the preferential flow of water through the soil profile. In response to the amelioration of Oxisol subsoil, corn and soybean yields were increased in the range of 9 to 18% supporting the maintenance of undisturbed no-till with high crop grain yields.

Abbreviations: NT, No-till; **Al,** aluminum; **Ca,** calcium; **Mg,** magnesium; **NH_4,** ammonia; **K,** potassium; **RS,** Rio Grande do Sul State; **N,** nitrogen; **F,** fluor; **m%,** aluminum saturation; **S,** surfur; **BS%,** basis saturation; **Br,** Brazil.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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Anthropometric model and obesity index of agricultural tractor operators

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This work evaluates the anthropometric model of agricultural tractor operators, presenting a diagnosis of the control instruments' position and the nutritional state of the operators. One hundred and twenty-seven agricultural tractors operators were interviewed, of which 23 were selected for the anthropometric evaluation. A rural company, a farm, a farmers' meeting and a tractor dealership were selected for the data collection. Twenty-three anthropometric measurements of each operator were collected and arranged in a table with the dimensions of all operators, presenting statistical results in a 90% confidence interval. Two types of tractors of medium mass and similar power, representing the largest portion of the market, were used. The positioning of the tractors' controls and their interactions with the operators' area of reach, as well as their accessibility in the command position, were evaluated. The nutritional state of the tractor operators was studied, as well as the relationship of their body mass index with their age. The results show that there was no difference between the general operator types in South Brazil in regard to the measures of their height when standing erect. Seven of the operators who presented BMIs with characteristics of obesity within the normal range were less than 38 years old, and those with characteristics of pre-obesity and obesity were more than 38 years old.

Key words: Ergonomics, control instruments, command position, body mass index.

INTRODUCTION

Technological development and the changes evoked by the globalized economy have demanded that rural owners strive for better quality to make agriculture and cattle-raising more competitive, increasing the productivity and reducing the costs. The region of Toledo - PR has a territorial area of 1,198,607 Km^2 (urban and

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rural area) and is considered to be one of the most productive grain areas of the state. With the progressive mechanization and an increased number of work processes, it is essential that agricultural machinery meets the demands of safety, operator comfort and productivity (Hansson, 1991). In these terms, current machinery must be designed ergonomically when human physicists are looking for a balance between the technical and economic demands. Ergonomy is the science that offers a positive basis for the modern forms of production administration, reducing the incidence of pathologies, especially those that are the consequences of fatigue and of repetitive motion, cumulative trauma and postural injuries (Yadav and Tewari, 1998; Iida, 2005).

Nkakine et al. (2008) reported that the amount of physical effort required for control of the machine components may limit performance efficiency and operator comfort. Tewari et al. (2002) stated that if tractor controls are not properly adapted to the operator’s anatomy, the performance demanded of him may quickly reach and even exceed the limit of tolerance.

Anthropometry is the Science dealing with dimensions of the human body; it is basically the listing of data and information on human body size. Hansson (1991) reminded that man-machine relationship must be studied using Anthropometric and ergonomic principles, to improve efficiency of machine, reduce risks and enhance operator comfort.

An anthropometric evaluation of agricultural tractor operators requires a referential system for the ergonomic design of their work stations. Although some modern tractors are still financially inaccessible for small- and medium-sized farmers, comfort items have already been added for the operator (electronic control devices, cabins, systems of shock absorption and protection in the event of accidents), incorporated and tied to fluctuations in costs, they still do not address the optimal physiological integration that is the basis of ergonomics.

Few anthropometric studies have been conducted in developing countries. In research that was developed in India (Yadav et al., 1999), the authors concluded that the operators analysed were smaller than the American and European operators in most of the dimensions appraised. In Brazil, studies have been performed to determine the anthropometric measures of agricultural tractor operators, revealing little attention given towards the efficiency, comfort and safety of the operators engaged in the operation of agricultural machines. However, the maximization of agricultural production systems is associated with the modernization of agricultural mechanization. Thus, considering that the anthropometric measures of operators of agricultural machines varies from region to region, and that these measures are ergonomically important in projects of tractors (Schlosser et al., 2000, Victor et al., 2002; Nkakine et al., 2008; Mehta et al., 2008; Kumar et al., 2009) with anthropometry.

A study by Schlosser et al. (2000) reports that the correct disposition and dimensions of the components of the operator's work station, such as the steering wheel, seat and means of entrance and exit, is important for the process of adapting the machine to the operator. In principle, they do not greatly increase production costs and help to improve the operator’s comfort.

The dimensions of the steps for accessing agricultural tractors should be from 0.20 to 0.30 m, with equal heights, landings of 0.10 m, distance of the tractor of 0.15 m and an inclination from 65 to 70°, to avoid superposition of the steps (Hansson, 1991, Yisa, 2002).

Mathematical models can be used in anthropometry to estimate certain measures of the human body in function with other known measures, as statistical correlations between the variables have been significant. In Brazil, these models can be a very important tool in each region due to the existence of a great variety of physical types as a result of the many ethnic groups, in addition to the quite different nutritional and health conditions between the regions (Iida, 2005).

The body mass index (BMI) is the anthropometric measure most commonly recommended and widely used for classifying overweight and obesity in adults which is defined as weight in kg/height in meters squared (World Health Organization - WHO, 1995; .Rani et al., 1999; Mei et al., 2002).

According to the report of the World Health Organization/Food and Agriculture Organization - WHO/FAO (2003), in many regions of the world, especially but not exclusively in the rural areas of developing countries, an appreciable proportion of the population is still engaged in physically demanding activities relating to agricultural practices and domestic tasks performed without mechanization or with rudimentary tools. Also according to this report, body mass index (BMI) can be used to estimate, albeit crudely, the nutritional state and the prevalence of overweight and obesity within a population and the risks associated with it. It does not, however, account for the wide variations in obesity between different individuals and populations.

Though some research gives general information indicating that a body mass index (weight (kg)/height (m^2)) in the obese range has been associated with an increased risk of injury resulting from motor vehicle crashes, falls and sports activities (Boulanger et al., 1992; Xiang et al., 2005; Pollack et al., 2007), these papers have not been interested in its specific effect on the risks of injury in tractor operators resulting from activities with tractors and agricultural machines.

The study of Virginia agricultural health and safety survey, Mariger et al. (2008) found that the age-adjusted mean BMI of Virginia farmers was 28.5, indicating that they are overweight.

Determining obesity-related differences can provide useful information for understanding the impact of obesity on the performance of occupational activities and the subsequent risk of injury, particularly for those tasks requiring static postures or repetitive motions that can be limited by the development of fatigue (Cavuoto and Nussbaum, 2013).

The present work has as its objective the evaluation of the anthropometric model of the agricultural tractor operators in the Toledo - Parana-Brazil region and the nutritional state of these operators.

MATERIALS AND METHODS

The work was conducted in Toledo Parana-Brazil from September 2003 to June 2004 with 23 workers who were selected from 127 workers and anthropometrically analysed. The tractors analysed in this region, with different models and similar power, represent two thirds of the total tractors sold in the region (ANFAVEA, 2004). The preliminary analysis investigated the characteristic problems of ergonomic concepts based on the large collection of anthropometric information offering support for the development of this research.

The information collection was divided into two sub-systems: The basic information supplied by specialized bibliographies, databases and suppliers of materials and equipment and the analytical compilation produced by experiments and direct observations in the management of agricultural machinery.

To follow criteria similar professional characteristics in order to obtain good representation of the universe of tractor drivers, this research used four sources, geographically distinct, whose operators have knowledge of both types of agricultural tractors analyzed.

The first field sampling was carried out on a farm, which has five MF tractors manufactured between 2003 and 2004, within the established standard for this search. From a population of 18 operators of agricultural machinery farm, a sample of four operators was selected within the established criteria. The second field sampling was performed at another farm, which has five NH tractors manufactured between 2003 and 2004. In this farm, with a population of nine operators of agricultural machinery, a sample of four operators was also selected obeying the same criteria. The third sample was taken at a meeting of farmers (field day) in the region. At this meeting, in a population of 72 operators analyzed a sample of 12 operators was selected. The fourth data collection was carried out at a dealership for new and used tractors and among 28 operators of agricultural machinery only three met the selection criteria. Thus, to represent the group studied in a total of 127 operators analyzed, 23 agricultural tractor operators were selected obeying criteria anthropometry with a margin of error of 5% significance.

In the field experiment, the anthropometric evaluation was aimed exclusively at the operators of middle power tractors, similar to the analysed models and with analogous measurements.

Twenty-two measurements of the anthropometric features of 23 tractor operators (Table 1) were selected from two farm tractors. The observations were taken carefully to measure all the dimensions in a correct posture and precise manner. Standing height (stature), body mass, eye height, shoulder height, elbow height, etc. were measured in standing posture for that the subjects were asked to stand on a flat surface; their arms were adjusted according to their height, with their feet closed and their body vertically erected, while their heels, buttocks and shoulders touched the same vertical plane. Similarly, other measurements were recorded in sitting posture, for that the subjects were asked.

An anthropological instrument (anthropometer) was used for taking measurements with an accuracy of 70.25 mm. The data recorded for the subject was mean of four readings. The linear dimensions, taken with the subject standing, were measured directly with an electronic metric system. The dimensions of the seated operators were measured in the seat of the tractor following the procedure used previously with the ISO 7250-1. The anthropometric determination of the operators was conducted following criteria similar to those adopted by Iida (2005). The measurements followed a normal distribution, as represented by the average and the standard deviation.

The ISO 7250-1 norm defined anthropometric patterns in 1996. However, this rule is not specific to machine operators and was developed with data on persons of European origin. Operators from foreign countries can differ (Nkakine et al., 2008; Mehta et al., 2008; Kumar et al., 2009), and anthropometric variations can occur within an individual country according to its extent. Thus, an agricultural tractor whose workplace measurements are in accord with international norms will not be comfortable for Brazilian operators.

From the application of the calculation, a 90% confidence interval (between 5 and 95%) was determined for the sample of the 23 operators, which differentiates 5% who are below 1.68 m in height and 5% who are above 1.72 m in height, as well as other dimensions. The principal dimensions (in the projection of the longitudinal vertical plan) measured in the agricultural tractors were the tractor's floor height and foot step height, the least distance of the operator's vision to the ground in front of the tractor and the seat reference point (Figure 1).

The nutritional state was defined based on the WHO's standard for Body Mass Index (BMI) WHO/FAO (2003), considering that a BMI between 18.5 and 24.9 kg/m^2 is in the "normal range"; a BMI between 24.9 and 29.9 kg/m^2 indicates an " Overweight" pre-obese condition; and a BMI above 30.0 kg/m^2 indicates class I "obesity".

RESULTS AND DISCUSSION

Anthropometric measurements

The operators' measurements of obtained on the two tractors were compared with the corresponding parameters of the 5th and 95th percentiles to ascertain their appropriateness. In Table 1, the anthropometric data obtained from the body's members that are associated with the act of getting on and off the tractor are presented. The corresponding dimensions are shown in Figure 2.

The results show that taller operators can be at a reasonable advantage for the visibility at various stages, during tasks performed over a long period such as ploughing and sowing (up to 12 h per day) (Table 1). The regression analysis shows a positive linear statistically significant relationship ($p < 0.01$) between the operator's height and the dimensions of their members (Figure 3). The statistical analysis revealed a correlation of 0.9057 between the variables of body height and knee height and of 0.8547 between the variables of body height and full hand length. At a height of 60 cm of access to the tractor, the operators around the margin of the 5th percentile can exert more effort than those around the 95th percentile. Although there is no recommended value available for the ideal step access height, the general opinion of all of the operators is that higher steps cause more discomfort.

Though the overall evaluations were homogeneous,

Table 1. Measurements of the anthropometric characteristics of 23 tractor operators in Toledo - PR in the static body.

Operators		Standard deviation	Percentile 5th	Mean	Percentile 95th	Difference between 5th and 95th percentiles
a.1	Age (years)	12.1	22.5	42.5	62.5	
a.2	Body mass (kg)	10.3	62.8	79.7	96.7	
	Measurements taken with subject stands (mm)					
b.1	Height (erect)	51.0	1,649.0	1,733.0	1,817.0	168.0
b.2	Eye height	51.6	1,533.0	1,618.0	1,703.0	170.0
b.3	Shoulder height	44.6	1,354.0	1,427.0	1,500.0	146.0
b.4	Elbow height	26.3	1,012.0	1,056.0	1,099.0	87.0
b.5	Functional lower arm height	35.1	682.0	740.0	797.0	115.0
b.6	Functional upper arm height	69.2	1,935.0	2,049.0	2,162.0	227.0
b.7	Full-hand length	18.4	702.0	733.0	763.0	61.0
	Measurements taken with subject sits (mm)					
c.1	Popliteal height	6.7	447.0	458.0	469.0	22.0
c.2	Height (from the floor)	26.7	1,259.0	1,303.0	1,347.0	88.0
c.3	Eye height (from the floor)	30.7	1,162.0	1,212.0	1,263.0	101.0
c.4	Shoulder height (from the floor)	27.4	969.0	1,014.0	1,059.0	90.0
c.5	Elbow height (from the floor)	24.1	610.0	650.0	690.0	80.0
c.6	Knee height	16.2	516.0	543.0	570.0	54.0
c.7	Thigh length	31.0	458.0	509.0	560.0	102.0
c.8	Forearm	12.8	338.0	359.0	380.0.	42.0
c.9	Buttock-knee length	21.2	555.0	590.0	624.0	69.0
c.10	Elbow-to-elbow width	14.7	495.0	519.0	543.0	48.0
c.11	Seat breadth	18.2	343.0	373.0	403.0	60.0
	Hand (mm)					
d.1	Functional length	5.0	99.0	107.0	115.0	16.0
d.2	pan width	5.9	202.0	212.0	222.0	20.0
d.3	Functional grip	1.8	27.0	30.0	33.0	6.0

The basic anthropometrics measurements were defined by Wilkinson (1991).

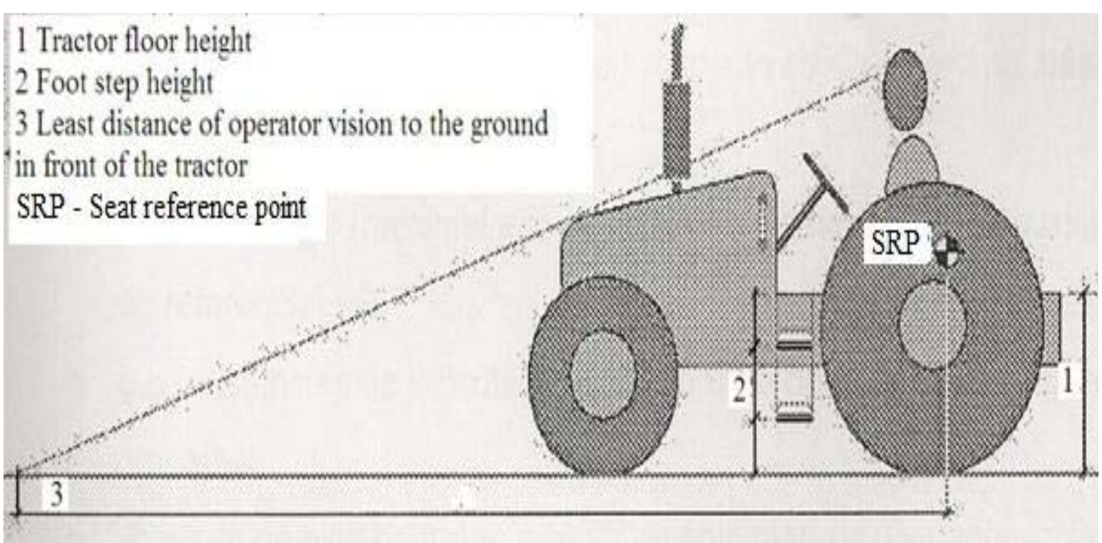

Figure 1. Measurements of selected tractor dimensions.

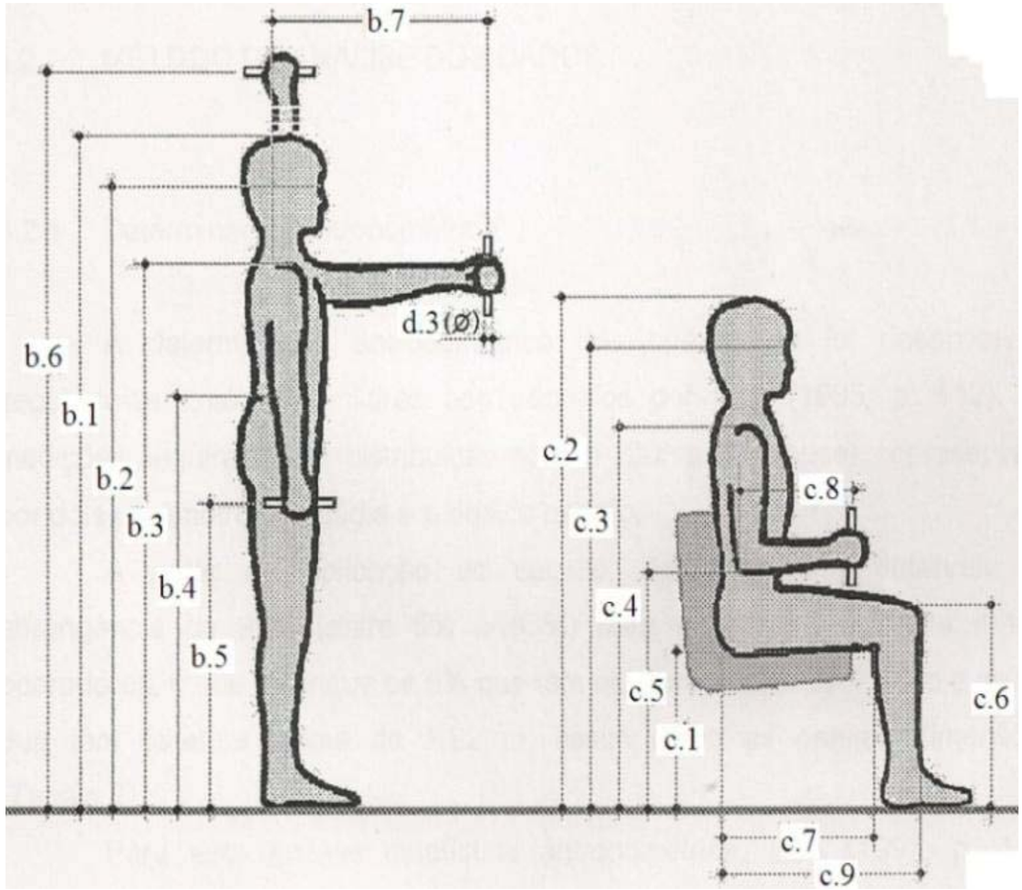

Figure 2. Anthropometric data of tractor operators (Wilkinson, 1991).

considerable variations in stature were observed, from 1,649 to 1,817 mm in height. Body weight also presented significant variations, from 61.8 to 96.7 kg.

In accordance with Iida (2005), these results allow the development of a mathematical model for the variables of the full hand length and the forearm in the function of the knee height. They are presented in Figure 3 and show the dependent proportionality between these measures. It is also observed that the dimensions of the members are proportional to the knee height of the operators. We performed a Pearson's correlation analysis to identify the dependence of the anthropometric variables of full hand l ength and forearm on knee height. It provided evidence of a strong positive correlation between these variables (0.78 and 0.77, respectively). The seat-man-pedal assembly can be represented by a kinematic chain of linkages at a horizontal distance in front of the seat reference point (SRP), a vertical distance above and below the SRP and direction of thrust and the use of the steering wheel for bracing (Pheasant and Harris, 1982). The variables investigated in the models can be very useful in making decisions about the design of the tractor's workspace to improve both efficiency and operator comfort.

The specific dimensions of the tractors evaluated are presented in Table 2 and elucidated in Figure 4. The results show that the steering wheel of the NH tractor at the full back adjustment has a distance from the seat reference point (SRP) of 695 and of 760 mm in the MF tractor. The subjects with the 95th percentile full hand reach (763 mm) have a comfortable reach, whereas subjects with the 5th percentile full hand reach (702 mm) do not have a similar comfortable hand reach with the MF tractor. The same dynamic can be observed when we compare the seat height of the tractors (Table 2, h) with the popliteal height of the operators (Table 1, c1). The linear regression models, in Figure 3, show that the height of all of the subjects analysed is sufficient for working comfortably while operating the NH tractor. For this way, the steering wheel can be adjusted to the operator's height. The safety features in the design of the operator's seat are of prime importance in reducing the static work of the muscles. The ability to adjust the seat and controls is necessary to accommodate operators of different statures and physiques comfortably (Yadav and Tewari, 1998).

In accordance with Schlosser et al. (2000), the knee height is used to define the seat height in regard to the

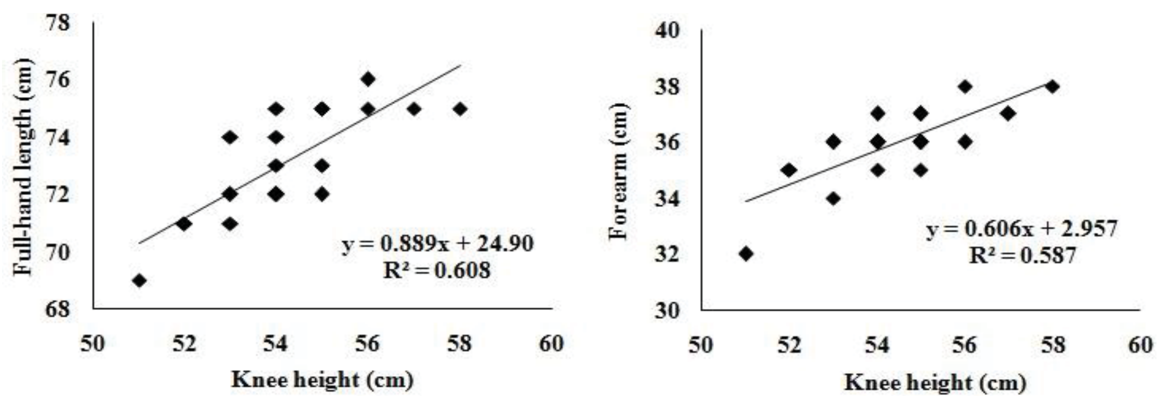

Figure 3. Dimensions of full hand length and forearm as in function of the knee height.

Table 2. Dimensions and characteristics evaluated for two tractors.

Tractors	NH	MF
Model	7630	292
External dimensions	4,115	4,250
Mass (kg)	3,039	3,575
Power (kW) rpm	(75.8) 2100	(77.2) 2200
D (mm)	545-695	610-760
d (mm)	150	150
h (mm)	460	470
b (mm)	360	390
a (mm)	40°	40°
e (mm)	26	26
ϕ (mm)	390	390
x (mm)	720	730
mPR (mm)	540-630	620-720
MPR (mm)	470-620	610-700
Tractor floor height	1090	920
Steps (mm)		
1° step	600	590
2° step	260	330
3° step	230	There is not
Floor	1,090	920

operating platform. This height must be such that the operator's feet are always supported and that the operator has easy access to the foot controls. In this scenario, it is possible to infer that the vertical position of the seat, defined by international standards (ISO 7250-1, Schlosser et al., 2000), is in accord with the standards of the operators of the Toledo region (Figure 3). In all of the analysed cases, the operators had to alter their seated positions to use the gearshift, hydraulic controls and many other controls, still that eventually. It was observed that the distance of the operator to the front panel and to the gearshift provokes flexion of the spinal column in some of the operators (Table 2).

Table 2 presents the dimensions of the intervals between the access steps in the evaluated tractors. The initial interval is measured between the ground and the first step, successively, up to the tractor's floor. The access steps checked are vertical. The analysed tractors' access steps do not meet ergonomic standards (Hansson, 1991) regarding their measurements and their angles of inclination. For Hansson (1991), the process of getting on and off machinery that must be mounted or dismounted frequently should be convenient and risk-free. The machine must be designed so that mounting and dismounting are safe and convenient, without the need for uncomfortable body movements. The steps should be

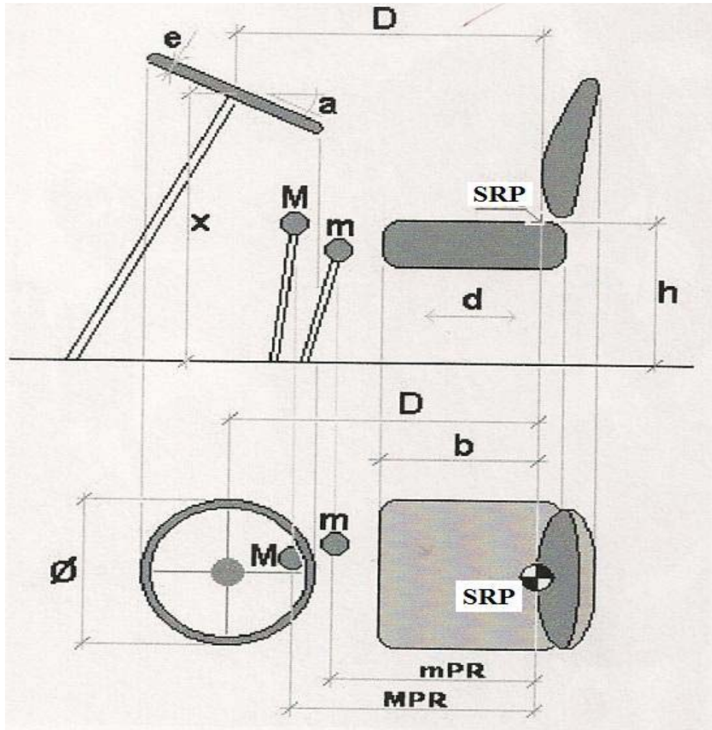

Figure 4. Dimensions and locations of controls and workspace: (a) horizontally inclined steering wheel (b) seat pan depth, (d) seat reach adjustment, (D) distance of steering wheel from seat reference point (PR), (e) steering wheel thickness, (h) seat height, (x) steering wheel centre height, (ϕ) steering wheel diameter, (mPR) distance between the speed reduction lever and the seat reference point, (MPR) distance between the speed lever and the seat reference point (Table 2).

designed or positioned so that they are unlikely to be damaged during work.

The steps have different spacing, with variations of 230 to 600 mm. There are reports of frequent accidents due to falls. It was observed that the height of the access steps exceed the ergonomic limits, which are from 200 to 300 mm (Hansson, 1991), with equal heights and a minimum width of 100 mm. The minimum horizontal reach should be 150 mm with an inclination of 65° to 70°, avoiding the superposition of the steps.

Five anthropometric measures were compared with the results obtained in other literature (Table 3).

The results show that there was no difference between the general types of the operators of the South region of Brazil (Rio Grande do Sul and Botucatu – SP) obtained, respectively, in the work of Schlosser et al. (2000) and those of Rossi and Santos (2009) for the measures of height when standing. Our results show also that the Toledo operators are taller than the English operators described by Dul and Weerdmeester (1995). When we compare only the operators of the southern region of Brazil, we observe that for the measures of sitting eye height and arm reach, these dimensions were less for the Toledo region operators; however, on examination, the Toledo operators had longer thigh lengths than those that were studied by Schlosser et al. (2000), Rossi and Santos (2009) and Dul and Weerdmeester (1995). This characteristic of our regions tractor operators allows them to be more comfortable because longer thighs gives them better access to the clutch pedals, the brakes and the accelerator, quicker with less effort. This enables the operator to maintain his normal position. The results of the sitting knee height were similar.

Body mass index

The body mass index (BMI) in relation to the age of the tractor operators is shown in Figure 5. The relationship between the body mass and the square of the height allowed the Body Mass Index to be valued in relation to the obesity classification of the tractor operators classified according to the WHO/FAO (2003) criteria.

It was confirmed that the operators' body mass varies from normal to obese. Approximately 35% of the operators in this sample were in the normal range, with a BMI between 18.5 and 24.9 kg/m^2, 48% were overweight (pre-obese, with an index between 25 and 29.9 kg/m^2)

Table 3. Comparison of the anthropometric standards of the agricultural tractor operators of the Toledo region – Paraná.

Body measurements (mm)	Toledo - PR	Botucatu - SP	England - UK	Rio Grande do Sul
Standing height	1,733	1,721	1,675	1,739
Eye height from the seat	752	782	765	820
Sitting knee height	543	535	n.d	552
Arm reach	733	793	n.d	896
Thigh length	509	482	488	458

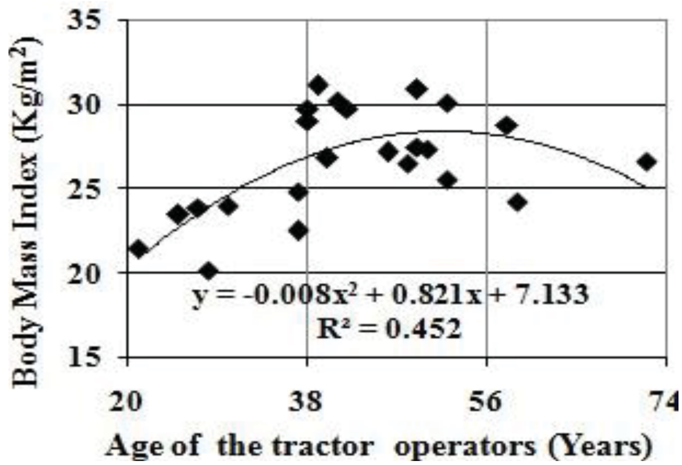

Figure 5. Obesity index of the agricultural tractor operators.

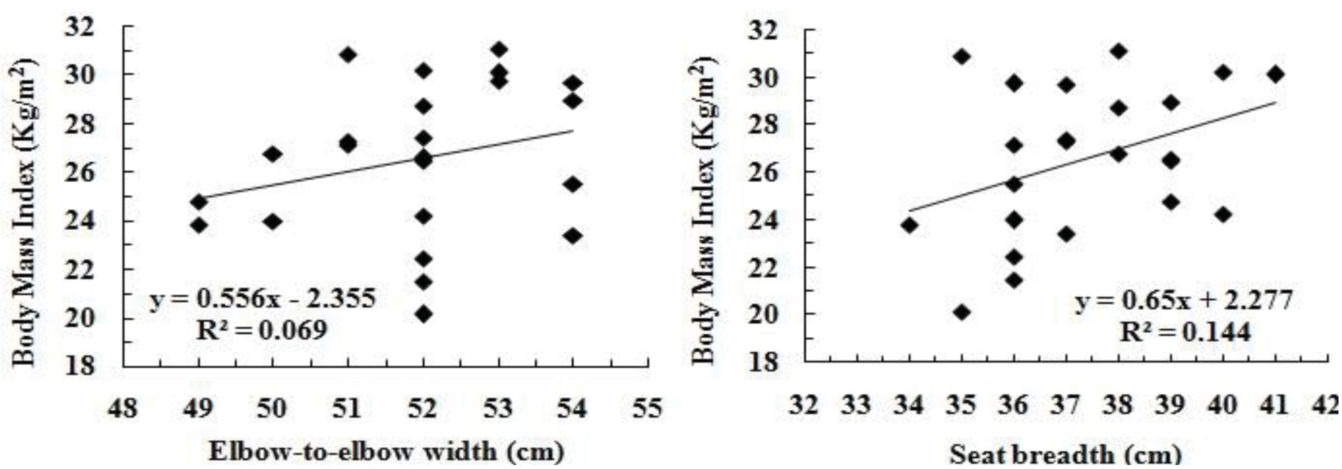

Figure 6. Relationships between the body mass index and elbow-to-elbow width and seat breadth fit with regression linear curves.

and 17% qualified as obese (30.1 to 30.9 kg/m^2) (WHO/FAO (2003)).

It was observed also that of the operators with BMI characteristics in the normal range, approximately 30% were less than 38 years old, and those with characteristics of pre-obesity and obesity were more than 38 years old.

Linear regression curves of the Body Mass Index (BMI) in relation to elbow-to-elbow width and seat breadth variables were developed (Figure 6). The data showed moderate positive correlations between the BMI and these variables, 0.3 to elbow-to-elbow width and 0.4 to seat breadth, respectively.

Current evidence shows obesity-related differences in the elbows' extension and in the quadriceps (Maffiuletti et al., 2007).

BMI can be used to estimate, albeit crudely, the prevalence of overweight and obesity within a population and the risks associated with it. It does not, however, account for the wide variations in obesity among different individuals and populations.

In recent years, different ranges of BMI cut-off points for overweight and obesity have been proposed, in particular for the Asia-Pacific region (WHO, 2000). Nevertheless, the consensus was that the median BMI for the adult population should be in the range of 21 to 23 kg/m^2 to achieve optimum health, and the goal for individuals should be to maintain a BMI in the range of 18.5 to 24.9 kg/m^2 (WHO/FAO, 2003).

Conclusion

The median stature of the operators inside the analysed parameters is similar to that quoted in the literature of Brazil's southern region and within the confidence intervals of anthropometric evaluations. The vertical position of the seat, defined by international standards, is in accordance with the standard Toledo region operators. The heights between the access steps are varied and unsuitable, as is the inclination of the staircase. The results show that there was no difference between the general types of the operators of Brazil's South region regarding the measure of standing height. Of the operators who presented BMIs with characteristics in the normal weight range, seven were less than 38 years old, and those with characteristics of pre-obesity and obesity were more than 38 years old.

Conflict of Interest

The authors have not declared any conflict of interest.

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Logarithmic scaling and effects of severity levels of ringspot disease on sensory quality of coffee brew

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A logarithmic scale was developed to evaluate the severity of the ringspot caused by *Coffee ringspot virus* (CoRSV) in coffee berries to investigate the effect of disease severity on the quality of brew. The scale comprised seven severity levels (0, 1 to 4%, 4.1 to 8%, 8.1 to 15%, 15.1 to 25%, 25.1 to 50%, 50.1 to 75%, 75.1 to 90%) and was evaluated for accuracy, precision, and reproducibility of severity estimation. The accuracy and precision were determined by simple linear regression between the actual and the estimated severity, considering twelve raters. Brew quality was assessed in berries with three severity levels (4.1 to 8%, 50.1 to 75%, 75.1 to 90%) and healthy berries. The selected fruits were processed and analyzed for electrical conductivity, total sugars, reducing sugars, non-reducing sugars, activity of polyphenol oxidase, and total phenol. The logarithmic scale obtained was easy to use and able to provide quick estimates of disease, good accuracy and good precision. Biochemical analysis of berries showed that polyphenol oxidase activity decreased with increasing severity, whereas total sugars and total phenol levels increased with increasing severity. This result indicated that the higher the CoRSV severity level, the greater the change in compounds directly related to the final quality of brew, which consequently contribute to coffee depreciation.

Key words: CoRSV, *Coffee ringspot virus*, phenolic compounds, polyphenol oxidase.

INTRODUCTION

Coffee industry in Brazil accounts more than 30% of the world's supply of coffee, which represents 2.70 million tons of coffee in 2011, and confirms its great potential for generating foreign exchange (FAO, 2012).However, Brazil has reported fall in exports due to inability to meet international market requirements. Effect of genotype and

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lack of appropriate methodology for processing berries and climatic conditions favoring the occurrence of pests and diseases caused by fungi and bacteria are considered the main causes of depreciation in quality of coffee brew (Silva et al., 2000, 2005, 2013; Batista et al., 2003; Bertrand et al., 2003, 2012; Poltronieri et al., 2011).

In the past ten years, beside fungi and bacteria, the *Coffee ring spot virus* (CoRSV) has been related to depreciation in quality of coffee brew (Reis and Chagas, 2001; Boari et al., 2006). Although it has been described and reported in Brazil since the 1940s, only in the mid-1990s did high incidences begin to show in commercial crops in the State of Minas Gerais and later in several other states where the coffee is grown. The virus causes considerable damage, such as defoliation, which can reduce plant yield and cause discoloration of ripe fruit up to 100% of its surface, increasing berry susceptibility to fungus attack and consequently premature fruit falling.

Studies conducted to determine the influence of ring spot on coffee biochemical compounds reported that the disease can change sugar, total phenol, and PPO contents (Reis and Chagas, 2001; Boari et al., 2006). Reis and Chagas (2001) reported increase in total sugar content in berries affected by coffee ring spot and hypothesized that disease could have increased the susceptibility to fungal infection. It is known that the secondary metabolites resulting from fungus infection, such as ochratoxin, could also have serious implication on human health (Bryden, 2007); therefore, the virus damage is beyond the simple depreciation of coffee brew.

In their paper, however, Reis and Chagas (2001) did not investigate whether changes in brew quality could only be related to ring spot or whether it could also be influenced by fungal infection. Thus, there is a need to develop logarithmic scales which allow berry separation by disease levels severity with greater accuracy, precision, and reproducibility in severity estimates (Berger, 1980; Nutter Junior and Schultz, 1995).

In this study, a logarithmic scale was initially constructed to classify berries according to ring spot severity, whereby the berries were analyzed with three severity levels along with healthy fruit to determine disease influence on the final coffee brew quality.

MATERIAL AND METHODS

Development of the logarithmic scale

Coffee cherries cv. Acaiá Cerrado were harvested from crops at the campus of Federal University of Lavras (UFLA), Lavras, Minas Gerais, with different severity levels of coffee ring spot and free of fungal infection. The berries were photographed with an 8-megapixel digital camera at DFP/UFLA laboratory, and images were processed with *IT-2.0-version UTHCSA Image tool* program. After obtaining the actual severity level of ring spot on each berry, the seven-point severity scale with intervals based on logarithmic increments was designed. Definition of track severity followed the criteria recommended by Horsfall and Cowling (1978), where the upper scale limit should correspond to the maximum intensity observed in field, while determination of actual intensity of disease in field and its scale representation should have the high precision. In addition, scale subdivisions should comply with the limitations of human visual acuity defined by the stimulus-response law of Weber-Fechner, where visual acuity is proportional to the logarithm of stimulus intensity. With the designed scale, the levels of precision and accuracy of estimates were calculated (Kranz, 1988; Campbell and Madden, 1990).

For validating the logarithmic scale, a panel of 12 raters inexperienced in quantifying coffee ring spot measured the severity of 84 images of berries with different severity levels. First, the severity level was measured without using a scale. Next, two evaluations were done using the logarithmic scale: the first was performed seven days after the assessment without scale and the second, seven days after the first evaluation with scale. Accuracy and precision of each rater were determined through simple linear regression, by using the actual severity level obtained electronically as independent variable, and the severity estimated by the rater as dependent variable. The accuracy of estimates determined by each rater and by the panel of raters was determined by *t* test applied to the intercept of the linear regression (a) to verify the hypothesis H_0: $a = 0$, and the slope of the line (b) to test the hypothesis H_1: $b = 1$, at 5% probability. Intercept values significantly different from zero described the presence of constant deviation, while values of the slope significantly different from one indicated the presence of systematic deviations. Accuracy of estimates was obtained through the regression coefficient of determination (R^2), by absolute errors (estimated severity minus actual severity), and by the repeatability of estimates determined by regression of the second assessment in relation to the first sample of the same unit. Reproducibility of the estimates was determined by R^2 values obtained from linear regressions between the estimated severity levels among the raters combined in pairs (Campbell and Madden, 1990; Nutter Junior et al., 1993; Nutter Junior and Schultz, 1995). Regression analyses were performed with SAS ® software (2009).

Classification and analysis of berries

Coffee cherries from cultivar Acaiá Cerrado collected from UFLA crops were properly conditioned in containers and transported to the Department of Plant Pathology for evaluation and classification into three severity levels (4.1 to 8; 50.1 to 75; and 75.1 to 90%), plus healthy cherries, using the logarithmic scale developed and validated previously. Subsequently, berries were sun-dried with special care to prevent it from undesirable changes. After drying and processing (light roasting and grinding), 300 g aliquots of ground coffee were taken from each severity level, with three repetitions, for analysis of the total phenolic content (TPC) by the Goldstein and Swan method (1963) described by the AOAC (1990), reducing sugars (RS), non-reducing sugars (NRS), and total sugars (TS) by the Lane-Enyon method cited by the AOAC (1990); electrical conductivity (EC) according to a method adapted from Loeffler et al. (1988) and polyphenol oxidase activity (PPO) was determined by the method of Ponting and Joslyng (1948). The analyses were performed in Lavras, at the Laboratory of Coffee Brew Quality from EPAMIG (Minas Gerais State Agricultural Research Corporation), and the comparison test was carried out in the Laboratory of Coffee Quality, UFLA. Results were statistically

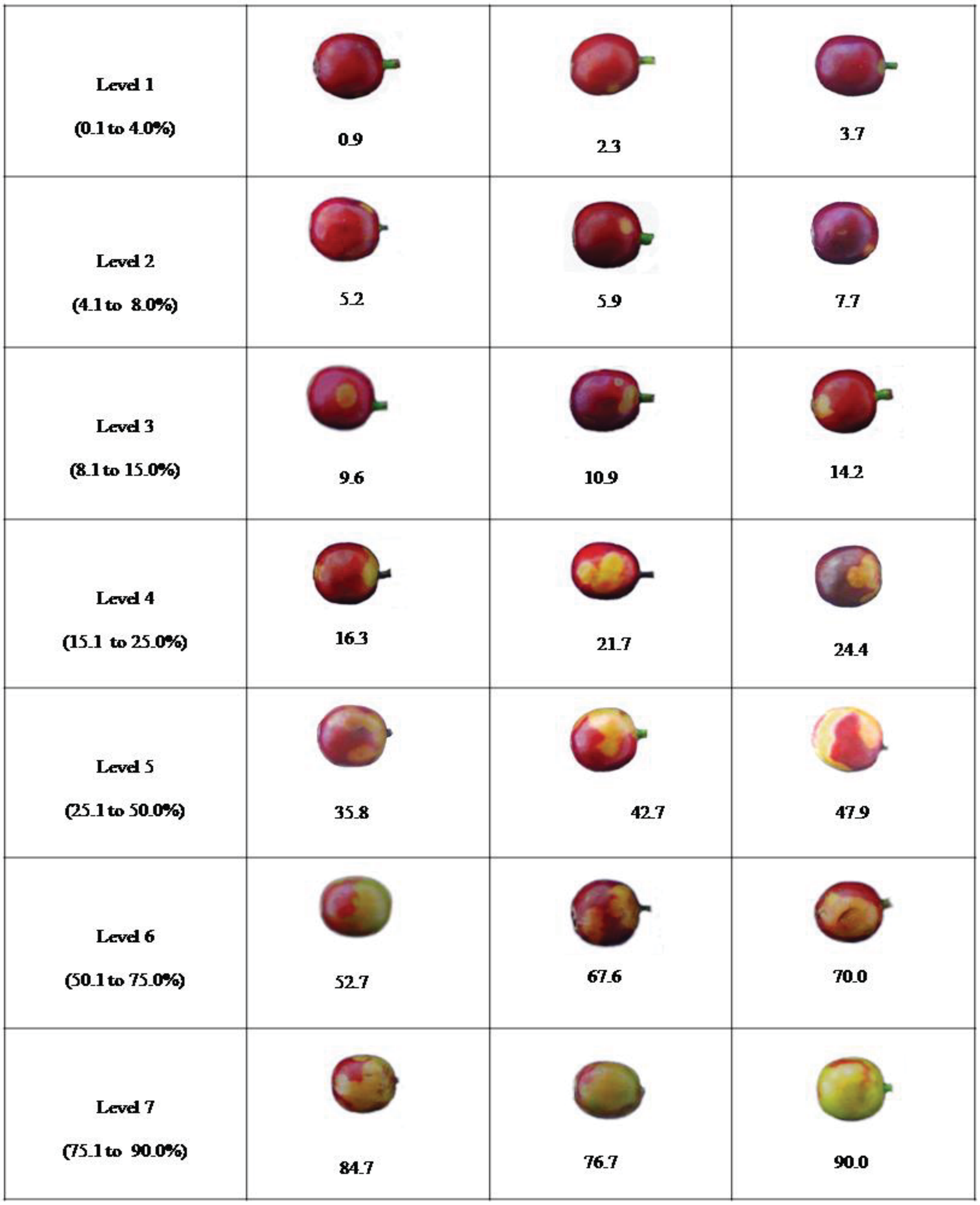

Figure 1. Logarithmic scale for coffee ringspot disease in berries with severity levels 0.1 to 4%; 4.1 to 8.0%; 8.1 to 15%; 15.1 to 25%; 25.1 to 50%; 50.1 to 75%; 75.1 to 90%. UFLA, Lavras, MG (2005).

analyzed with the Sisvar ® program.

RESULTS

The maximum and minimum severity values used for the scale development were 0.1 and 90%, respectively. Values above 90% were not found. The logarithmic scale to evaluate severity of coffee ring spot was designed with seven intervals: 0, 1 to 4%, 4.1 to 8%, 8.1 to 15%, 15.1 to 25%, 25.1 to 50%, 50.1 to 75% and 75.1 to 90% of the injured area of the berries (Figure 1).

The majority of raters (75%) showed inaccurate estimates in the first evaluation without scale (Table 1). Constant deviations were observed for 41% of the raters H, I, J, K and L, who overestimated the injured. When

Table 1. Intercept (a), slope (b), and coefficient of determination (R^2) of linear regression equations between actual severity and estimated severity of coffee ringspot disease in the evaluations performed by raters with and without a logarithmic scale. UFLA, Lavras, MG (2005).

Rater	Without scale			With scale					
				1st Evaluation			2nd Evaluation		
	a	*b*	*R²*	*a*	*b*	*R²*	*a*	*b*	*R²*
A	-1.2	1.02	0.85	-2.8	1.04	0.89	-1.4	1.03	0.88
B	-0.9	1.03	0.91	-1.5	1.06	0.91	-1	0.99	0.92
C	3.87	1.16**	0.85	0.11	1.12*	0.87	-0.3	1.08	0.88
D	2.67	1.1*	0.91	0.74	1.02	0.89	2.08	1.05	0.92
E	3.11	1.05	0.89	4.88*	1.03	0.86	5.72**	0.97	0.9
F	1.44	1.09*	0.9	-1	0.97	0.92	0.06	1.04	0.92
G	1.66	1.13**	0.89	-4.1	1.05	0.83	-1.3	1.08	0.88
H	3.78**	1.04	0.94	-0.2	1.03	0.93	-0.4	1.02	0.95
I	2.7*	1.09*	0.93	0.18	1.06	0.94	0.87	1.04	0.93
J	4.73**	1.03	0.92	1.67	1.1	0.87	-1.2	1.02	0.92
K	10.2**	0.92	0.85	3.24*	1.04	0.92	1.98	1.02	0.88
L	3.7*	0.95	0.87	-0.8	1.04	0.92	0.17	1.05	0.91
Mean			0.89			0.9			0.91

* Indicates that the null hypothesis (a=0 or b=1) was rejected by the *t* test (P <0.05); ** Indicates that the null hypothesis (a=0 or b=1) was rejected by the *t* test (P <0.01).

using the scale, only two raters (E and K) overestimated the severity in the first assessment, while one rater (E) consistently overestimated the severity in the second evaluation, according to intercept values significantly different from zero (P ≤ 0.05). Regarding the slope values, systematic deviation was observed in 41% of the raters (C, D, F, G, and I) which showed values significantly different from 1 (P ≤ 0.05), without using the diagrammatic scale. With the scale, only one rater in the first evaluation (C) showed a slope value significantly different from 1 (P ≤ 0.05).

The absolute errors reduced with the logarithmic scale (Figure 2) when compared with the residual distribution of estimates obtained without scale. It did not show a definite undesirable pattern in spite of the wide variation range in the first, second, and third evaluations (28.61 to -45.07; 32.05 to -59.59; 25.13 to -44.78, respectively). The percentage of absolute error values found in the range of -10 to 10 rose from 75% in the evaluation without scale to 85 and 87%, respectively in the first and second evaluations with scale.

The raters showed good repeatability in the estimates, since the mean amount of variation in the first evaluation explained by the second one was 91% (Table 2). Only 17% of raters showed slope values of regression between the two evaluations significantly different from 1 (P ≤ 0.05) and intercept values different from zero (P ≤ 0.05) (Table 2). Reproducibility of estimates among raters was also used as an indicator of accuracy analysis of the scale. Without the logarithmic scale, the coefficient of determination (R^2) of the estimate regressions between pairs of raters ranged from 74 to 93% with a mean of 87% (Table 3). With the logarithmic scale, R^2 values ranged from 80 to 94% with a mean of 88%.

The biochemical analysis showed that the increasing of ring spot severity in coffee fruits also increased the levels of total phenol (TPC), reducing sugars (RS), and electrical conductivity (EC) contents, while reduced the activity of polyphenol oxidase (PPO) (Table 4). Non-reducing sugar content (NRS) and total sugar (TS) contents did not differ statistically in the Scott-Knott test at 5% probability (Table 4).

DISCUSSION

In this paper, a diagrammatic scale was devised to assess the severity of the ring spot in coffee berries. This scale was efficient when it was later applied to separate the berries with three severity degrees of virus disease, which were submitted to the biochemical tests considered indicators of the coffee brew quality. The information obtained was quite interesting, because it allowed correlating the severity of the ringspot disease with the quality of coffee brew, considering that the evaluated coffee berries were free of any apparent secondary infection by fungi.

Defining criteria to standardize evaluation of coffee ring

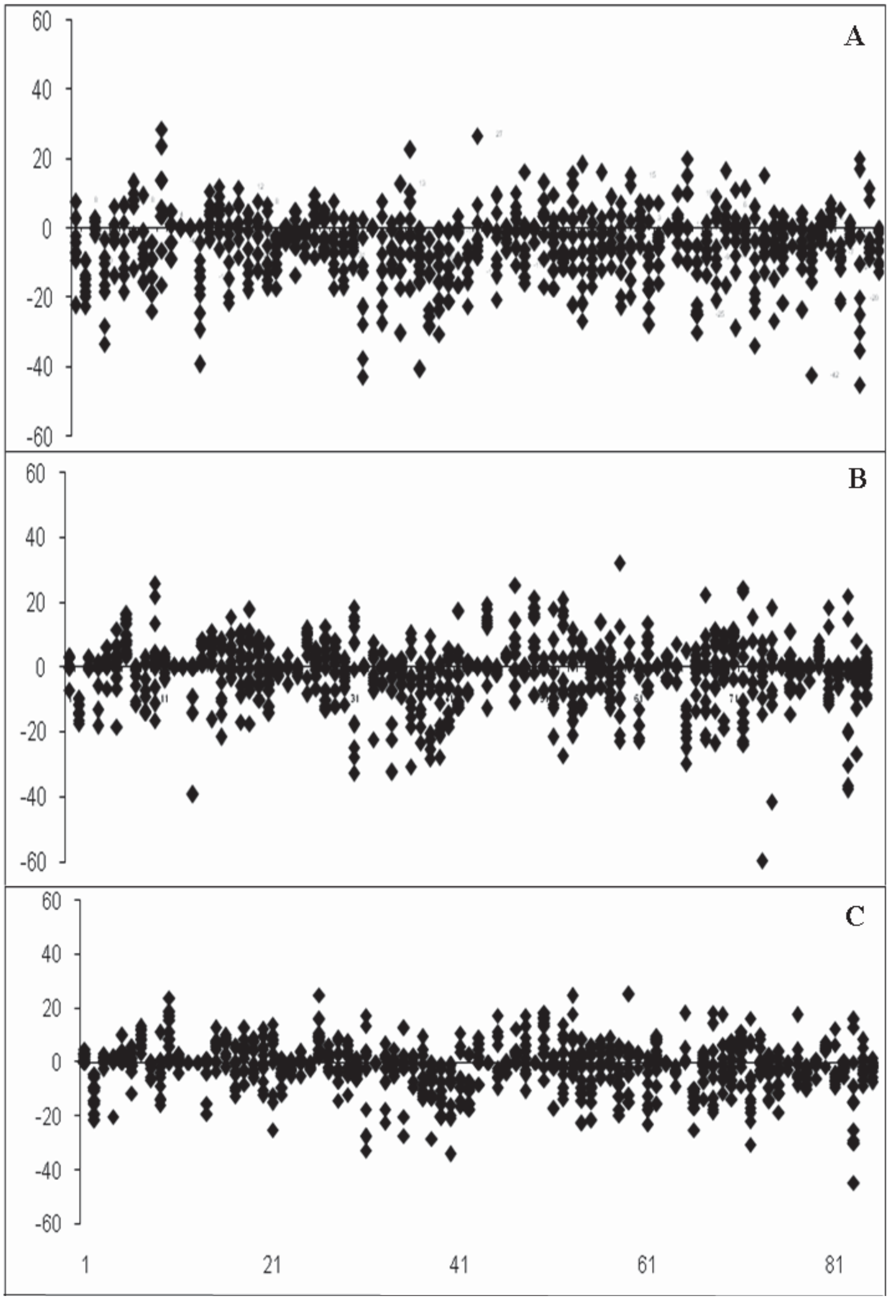

Figure 2. Absolute errors (differences between actual and estimated severity) of all raters in evaluation without logarithmic scale (a) and with logarithmic scale (b and c). Points represent the 85 estimates for each rater. UFLA, Lavras, MG (2005).

spot is necessary due to the wide variation in the severity of disease. During the evaluation, using the diagrammatic scale, there was improvement in precision in the second evaluation with scale in relation to the first, showing that training and constant use of the logarithmic scale positively affected accuracy and precision of the estimates (Corrêa et al., 2009). The percentage of absolute errors was considered a good value according to the criteria adopted in studies on logarithmic scales (Amorim et al., 1993; Corrêa et al., 2009). Some levels of absolute errors in the estimates were compensated by the speed and standardization provided by logarithmic

Table 2. Intercept (a), slope (b) and coefficient of determination (R^2) of linear regression equations correlating second and first estimates of coffee ringspot disease by the same rater, using logarithmic scale. UFLA, Lavras, MG (2005).

Rater	*a*	*b*	R^2
A	0.58	0.95	0.89
B	0.84	1.02	0.92
C	2.99	0.96	0.85
D	0.1	0.94	0.9
E	-0.4	1.04	0.91
F	0.19	0.9**	0.92
G	-2.4	0.96	0.92
H	0.16	1.01	0.98
I	0.42	0.99	0.95
J	4.33*	1.03	0.87
K	4.14*	0.93	0.88
L	1.04	0.92*	0.9
Mean			0.91

*Indicates that the null hypothesis (a=0 or b=1) was rejected by the *t* test (P <0.05); **Indicates that the null hypothesis (a=0 or b=1) was rejected by the *t* test (P <0.01).

Table 3. Coefficient of determination (R^2) of linear regression equations correlating the estimates of coffee ringspot disease between raters, with and without logarithmic scale (first and second evaluations). UFLA, Lavras, MG (2005).

Rater	Without scale										
	B	C	D	E	F	G	H	I	J	K	L
A	**0.90**	**0.80**	**0.84**	**0.82**	**0.83**	**0.79**	**0.87**	**0.84**	**0.82**	**0.74**	**0.78**
B		0.83	0.87	0.87	0.88	0.84	0.92	0.87	0.86	0.81	0.83
C			0.91	0.89	0.85	0.88	0.86	0.89	0.86	0.82	0.82
D				0.92	0.86	0.89	0.90	0.93	0.90	0.85	0.88
E					0.90	0.91	0.90	0.91	0.93	0.84	0.86
F						0.87	0.93	0.90	0.89	0.86	0.83
G							0.87	0.92	0.87	0.84	0.86
H								0.92	0.92	0.86	0.87
I									0.91	0.87	0.86
J										0.84	0.86
K											0.80

Rater	With scale – 1st Evaluation										
	B	C	D	E	F	G	H	I	J	K	L
A	0.86	0.77	0.81	0.78	0.88	0.78	0.90	0.86	0.81	0.79	0.88
B		0.83	0.87	0.81	0.88	0.80	0.88	0.91	0.85	0.89	0.87
C			0.82	0.79	0.81	0.77	0.82	0.85	0.87	0.86	0.80
D				0.81	0.84	0.81	0.83	0.86	0.85	0.85	0.88
E					0.82	0.78	0.82	0.88	0.80	0.86	0.86
F						0.84	0.91	0.92	0.83	0.86	0.89
G							0.80	0.84	0.77	0.75	0.85
H								0.91	0.82	0.83	0.90
I									0.86	0.93	0.92

Table 3. Contd.

J										0.85	0.82
K											0.87

Rater	With scale – 2nd Evaluation										
	B	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
A	0.91	0.81	0.82	0.83	0.86	0.86	0.87	0.87	0.88	0.82	0.83
B		0.86	0.91	0.86	0.91	0.85	0.89	0.88	0.92	0.84	0.89
C			0.89	0.91	0.91	0.86	0.86	0.91	0.88	0.86	0.88
D				0.93	0.93	0.85	0.89	0.91	0.88	0.89	0.89
E					0.90	0.88	0.86	0.93	0.86	0.91	0.87
F						0.88	0.92	0.94	0.93	0.89	0.94
G							0.86	0.88	0.89	0.81	0.86
H								0.90	0.89	0.84	0.89
I									0.93	0.90	0.92
J										0.85	0.90
K											0.85

Table 4. Mean of values obtained in the biochemical analysis of coffee berries with different disease severity levels. UFLA**,** Lavras, MG (2005).

Treatments	TPC [(1)]	RS [(2)]	NRS [(3)]	TS [(4)]	EC [(5)]	PPO[(6)]
Healthy Fruit	6.0 b	0,53 d	6.26 a	7.26 a	135.9 c	64.93 a
4.1 - 8%	6.33 b	0.60 c	6.4 a	7.4 a	137.66b	64.2 b
50.1 – 75%	7.53 a	0.66 b	6.46 a	7.46 a	145.9 b	62.93 b
75.1 – 90 %	7.63 a	0.83 a	6.6 a	7.53 a	150.23 a	62.2 c
CV	1.61	18.95	2.39	2.45	2.37	0.72

Means followed by the same letter do not differ by Tukey test at 5% significance. [1]Total phenolic content; [2] Reducing sugars; [3] Non-reducing sugars; [4] Total sugars; [5]Electrical conductivity; [6] Polyphenol oxidase activity.

scales. Moreover, like most methods for quantifying disease severity, the use of this tool is subject to a certain degree of subjectivity, which can be minimized with rater training (Nutter Jr. and Schultz, 1995). The R^2 values, which presented a mean of 88%, were similar to the results found in the validation of logarithmic scale to other pathosystems. According to Nutter Junior et al. (1993), different raters using the same scale to evaluate the same material should estimate the same severity values whose significance is checked with linear regressions between pairs of values estimated by the raters.

The diagrammatic scale enables determination of severity levels for the pathosystem, provides a standardized method for quantifying disease severity, and makes it possible to correlate severity variation in berries with different fruit-related variables, such as brew quality. The logarithmic scale proposed to evaluate coffee ring spot was easy to use, able to provide a quick estimate of the disease, and provided good accuracy and precision of estimates.

Results of the biochemical analysis of berries showed a significant alteration in several components, such as the increasing of total phenol, reducing sugars, and electrical conductivity, and the activity decreasing of polyphenol oxidase (PPO), which are considered the key parameters in the quality of brew. Considering that those alterations were related to the increasing of ring spot severity on coffee berries, it leads to conclusion that the quality of coffee brew depends on the quantity of symptoms induced by CoRSV, being inversely proportional to the severity of ring spot in coffee berries. Similar results had already been found by Reis and Chagas (2001) and Boari et al. (2006) for most of the items evaluated herein. Boari et al. (2006), however, found a slight decrease in electrical conductivity levels in grains affected by ring spot, whereas the current results showed increase in these values as severity levels increased. There is evidence that brew quality increases as electrical conductivity levels decrease (Prete and Abraão, 2000). However, other authors found no correlation between EC values and sensory analysis of coffee (Favarin et al., 2004). On the other hand, Malta et al. (2005) evaluated the influence of grain size and type of grain defect on electrical conductivity and potassium leaching of exudates of coffee beans and concluded that defective beans may affect test results.

It is known that coffee taste is highly dependent on organic compounds such as acids, aldehydes, ketones, sugars, proteins, amino acids, fatty acids, and phenol contents, as enzyme activity may lead to depreciation in final quality of coffee brew in cupping test (Amorim and Silva, 1968; Oliveira et al., 1977; Amorim and Melo, 1991; Carvalho et al., 1994; Mazzafera and Robinson, 2000; Silva et al., 2013). Several authors have reported a correlation between chemical composition and activity of PPO in berries, as well as peroxidase and phenolic contents in taste, aroma, and therefore in quality of coffee brew (Amorim and Silva, 1968; Oliveira et al., 1977; Amorim and Mello, 1991; Farah and Donangelo, 2006).

Phenolic compounds are considered responsible for the most significant changes in taste and aroma of coffee brew. Pinto et al. (2001) found a direct correlation between polyphenol content and brew quality. According to these authors, the higher the polyphenol content, the worse the brew quality; they found that *rio* brew and *soft* brew had the highest and lowest polyphenol contents, respectively. The authors also reported that *strictly soft* and *riada* brew had higher levels of total and non-reducing sugars. PPO activity can also significantly affect brew quality (Amorim and Silva, 1968; Oliveira et al., 1977; Amorim and Amorim, 1977).

Carvalho et al. (1994) developed a table correlating polyphenol content with coffee classification. PPO levels above 67.66 U g^{-1} of processed grains (U is the unit of enzyme activity equivalent to 0.001 optical density per minute) would be found in *extra fine* coffee, *strictly soft* brew; 62.99 to 67.66 U g^{-1} of processed grains in *fine* coffee, *soft* brew, and *only soft* brew; 55.99 to 62.99 U g^{-1} in *acceptable* coffee, *hard* brew; and under 55.99 U g^{-1} in *not acceptable* coffee, *riada* and *rio* brew. These data corroborate previous reports by several authors (Amorim and Amorim, 1977; Oliveira et al., 1977; Amorim and Mello, 1991). Based on this table, the results obtained in this work showed that grains with 0 and 8% of ring spot severity were classified as *soft* brew, while those with 75 and 90% severity were classified as a *riada* brew and *rio* brew. It demonstrated that severity of ring spot was directly related to coffee brew quality, which shows that ring spot importance goes beyond damage and losses caused by defoliation and fruit drop. Even when there is no loss in quantity, the quality loss caused by depreciation of coffee brew is certainly an additional factor in the damage caused by CoRSV infection in coffee plants.

Conclusion

This article provided a new tool for ringspot evaluation in coffee berry and also showed that the quality of coffee brew decrease when the disease severity increase. Besides that, it was possible to observe that this depreciation was not influenced by any secondary fungal infection that can be seen under visual inspection.

Conflict of Interest

The authors have not declared any conflict of interest.

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Assessment of soil salinity using electrical resistivity imaging and induced polarization methods

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2D imaging involving geoelectrical resistivity and time domain induced polarization has been used to assess the spatial variability of the physical properties of subsurface soil in Covenant University Farm, southwestern Nigeria. Apparent resistivity and chargeability of the induced polarization effect were concurrently measured along six traverses using Wenner array. The observed data were processed to produced 2D inverse models of the subsoil resistivity and chargeability. Soil samples were also collected and analysed for conductivity and salinity levels. The results show that the salinity level in the soil is within the range for normal soil and therefore healthy for plant growth. The inverse model sections were integrated with the laboratory test to qualitatively assess the salinity, degree of compaction, and thickness of the soil in the farm. Other petrophysical properties such as clay volume, moisture content and organic matter which are related to soil conductivity were also inferred. The study demonstrates that geoelectrical resistivity imaging can be a useful tool for effectively assessing the variations of soil condition in large tracts of land for precision agriculture.

Key words: Soil salinity, 2D imaging, geoelectrical resistivity, induced polarization, precision farming.

INTRODUCTION

The knowledge of soil properties is useful for agricultural practices and environmental impact analysis. Agricultural practice has always indicated that there are differences in the soil among nearby parcels of land; these differences are usually manifested in crops productivity. Many techniques have been used in determining subsurface soil properties, and their spatial and temporal variability. The distribution of these properties is often exploited in a more efficient way, allowing increased crops yield without necessarily using chemical fertilizers and pesticides (Robert, 2002; Rodriguez et al., 2010). Consequently, the environmental impacts on soils, surface water and groundwater due to agricultural activities can be considerably reduced. However, the mapping and characterization of soil properties as rapidly and accurately as possible can be very challenging. Geophysical techniques are effective, fast, and relatively inexpensive tools that can be used for rapid and accurate characterization of soil parameters. Geoelectrical resistivity survey is one of such geophysical methods that can be used to map and characterize the spatial and temporal variability of soil parameters (Williams and Baker, 1982; Mckenzie et al., 1989; Corwin and Lesch, 2003; Amezketa, 2007; Sudha et al., 2009). Geoelectrical

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resistivity survey makes use of the variations in the electrical properties of rocks and minerals in the subsurface.

Soil conductivity (or its inverse, resistivity) is influenced by a variety of factors including the salinity, clay volume, moisture content, porosity, mineralogy, organic matter and temperature of the soil. Thus, soil conductivity is a complex physiochemical property that results from the inter-relationship and interaction of these soil properties. Geoelectrical resistivity measurements can be used to assess the spatial distribution and temporal variability of any or a combination of these properties. Soil conductivity has been used to map and characterize spatial distribution of soil salinity (or total solute concentration) and assess other soil properties such as clay content, porosity and Cation Exchange Capacity (CEC) (Shevnin et al., 2006, 2007) which correlate well with conductivity.

Soil salinity is basically the amount of major dissolved inorganic solute present in the soil aqueous phase, which consists of soluble and readily dissolvable salts. These include charged species such as Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cl^{-}, HCO_3^{-}, NO_3^{-}, SO_4^{-2} and CO_3^{2}, non-ionic solutes, and ions that can combine to form pairs of ions (Corwin and Lesch, 2003). Soil salinity tends to increase over time due to various factors which are either natural or artificial. The natural factors include processes such as mineral weathering and saline water intrusion, while the artificial factors include practices such as irrigation, application of fertilizers and other anthropogenic activities. Soil salinity has been shown to have detrimental effect on plant growth; this effect is usually manifested in loss of stand, reduced and thwarted plant growth, and reduced yield and crop failure (Rhoades and Corwin, 1990; Rhoades and Loveday, 1990). Plant growth is important to humans and the ecosystems as plants serve a lot of purposes including food supply, protection of soil from erosion, prevention of desertification, provision of oxygen for respiration, and the reduction of carbon dioxide in the atmosphere through photosynthesis.

Soil salinity limits the amount of water and nutrients uptakes by plants from the soil by significantly reducing the osmotic potential, thus making it difficult for plants to extract water from the soil. Consequently, this results in low yield or complete destruction of the plants (Corwin and Lesch, 2003). Soils salinity may cause specific ion-toxicity thereby upsetting the nutritional balance of plants. Also, the salt composition of the soil water influences the composition of the cations on the exchange complex of the soil particles and consequently influences soil permeability. Apart from limiting crops yield and adversely affecting soil hydraulic parameters, soil salinity can negatively impact groundwater system as well as causing damages to infrastructures in the area through corrosion.

Different methods have been used for the assessment of soil salinity. Traditionally, soil salinity is assessed by visual crop observation. This traditional technique of visual crop observation is fast and economic; but its major disadvantage is that the plants would have been damaged or low yield would have been recorded before salinity is detected. Geophysical techniques can be used to detect or monitor salinity and other soil properties before having detrimental effects on plants (Rhoades et al., 1990, 1999). Soil salinity is commonly quantified in terms of the total concentration of dissolved soluble salts as measured by the electrical conductivity of the solution in dSm^{-1} (Corwin and Lesch, 2003). For a pure solution, the electrical conductivity σ_w is a function of the chemical composition and is characterized by the relation

$$\sigma_w = k\sum_{i=1}^{n}\lambda_i M_i |v_i|, \quad (1)$$

where k is the cell constant which accounts for the geometric factor of the electrodes, λ is the molar limiting ion conductivity ($S\,m^2\,mol^{-1}$), M is the molar concentration ($mol\,m^{-3}$), v is the absolute value of the ion charge, and i is the ion species in the solution. In practice, soil conductivity is determined for an aqueous extract of a soil sample.

In this study, electrical resistivity and time domain induced polarization geophysical techniques together with laboratory analysis were used to map and characterize the resistivity and chargeability of the subsurface soil in Covenant University Farm. Most part of the farm is usually water logged during the raining season due to underlying relatively impermeable near-surface lateritic clay layer. This is expected to lead to increased soil salinization in the area. The soil samples collected were analysed for conductivity and salinity. Similarly, the inverse model sections of the observed apparent resistivity and chargeability were used to assess the salinity level, degree of compaction, as well as the thickness of the soil. Other petrophysical properties including clay volume, moisture content and organic matter which are related to soil conductivity are inferred from the resistivity models and laboratory results. This is because the ionic charges in the salts can significantly affect the flow of electric current in the soil.

SITE DISCRIPTION AND GEOLOGICAL SETTING

The study site (Covenant University Farm, Lat. 6.67° N and Long. 3.16° E) is located in the eastern part of the Dahomey Basin, southwestern Nigeria. The basin is a combination of inland, coastal and offshore basins, and stretches along the continental margin of the Gulf of Guinea (Figures 1 and 2). The area is generally gently sloping low-lying and is characterized by two main

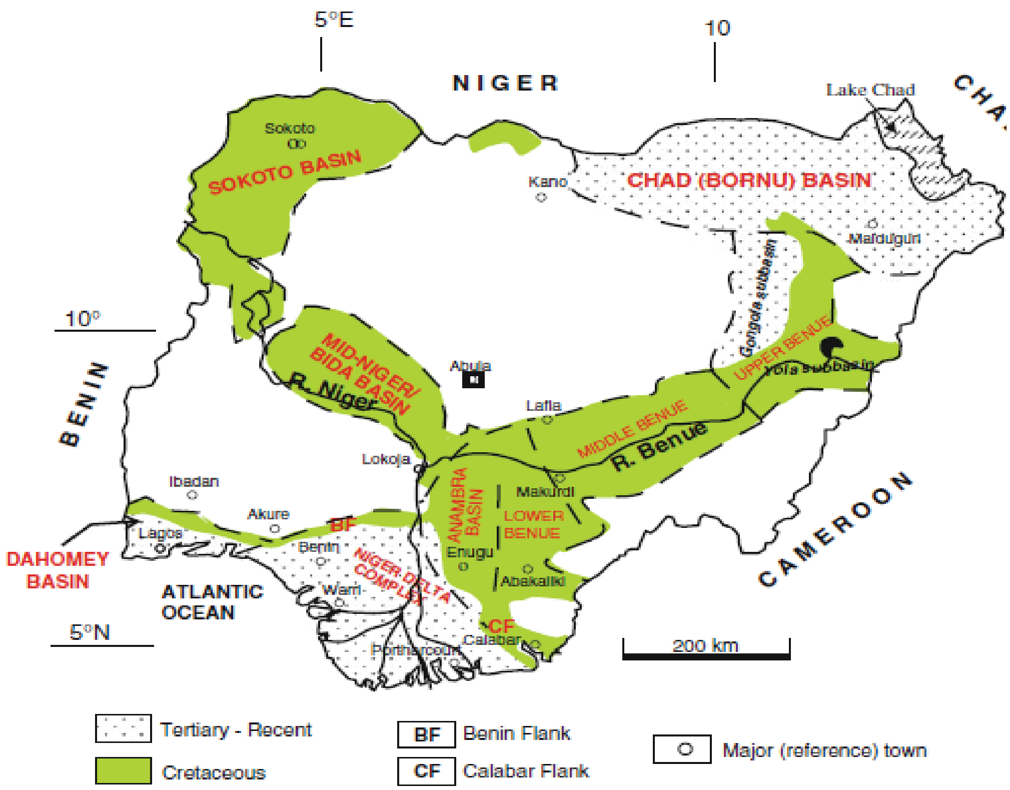

Figure 1. Geological map of Nigeria showing the major geological components: Basement, Younger Granites, and Sedimentary Basins (after Obaje, 2009).

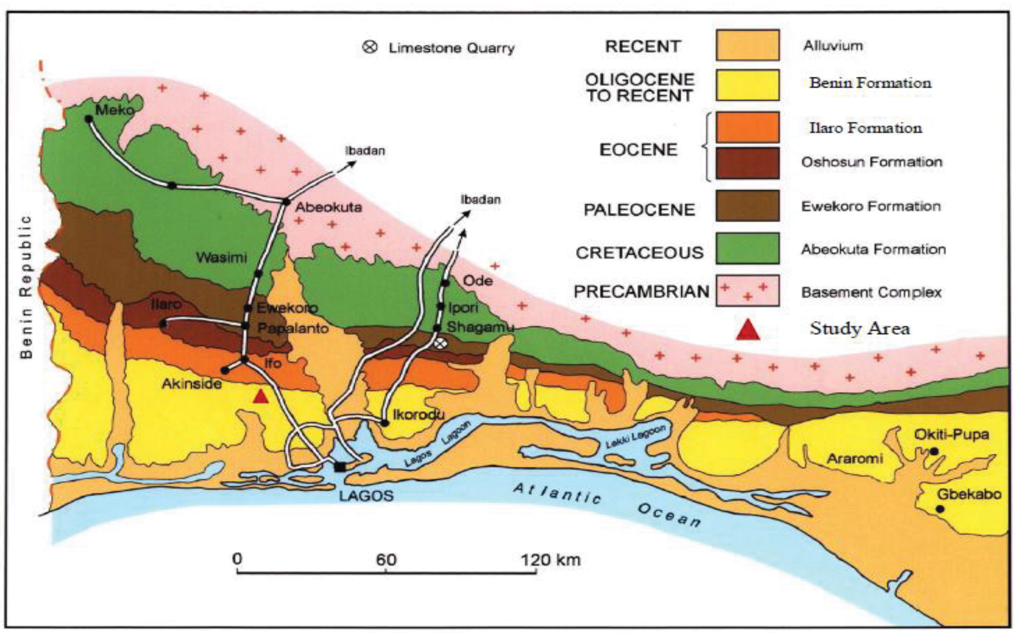

Figure 2. Geological map of the Nigerian part of the Dahomey embayment (modified after Gebhardt et al., 2010).

climatic seasons – the dry season that spans from November to March and raining (or wet) season between April and October. Occasional rainfalls are often witnessed within the dry season due to its proximity to the

Atlantic Ocean. Rainfall forms the major source of groundwater recharge in the area; mean annual rainfall is greater than 2000 mm. The mean monthly temperature ranges from 23°C in July to 32°C in February. Because of its proximity to the coast, the area is under the influx of sea salt and other types of aerosols sprayed from the Atlantic Ocean; this can potentially increase the salinity of the subsoil.

The rocks in the basin are Late Cretaceous to Early Tertiary in age (Jones and Hockey, 1964; Ogbe, 1970; Omatsola and Adegoke, 1981; Okosun, 1990; Billman, 1992; Olabode, 2006). The stratigraphy of the basin has been grouped into six lithostratigraphic formations namely, from oldest to youngest, Abeokuta, Ewekoro, Akinbo, Oshosun, Ilaro and Benin Formations. However, some workers have described the Cretaceous Abeokuta Formation as Abeokuta Group consisting of Ise, Afowo and Araromi Formations (Omatsola and Adegoke, 1981). The Cretaceous Abeokuta Formation mainly composed of poorly sorted sequence of continental grits and pebbly sands over the entire basin with occasional siltstones, mudstones, shale-clay with thin limestone beds due to marine transgression. Overlying the Abeokuta Formation is the Ewekoro Formation which is predominantly composed of shallow marine limestone due to the contamination of the marine transgression. The Ewekoro limestones are Palaeocene in age. The Ewekoro Formation is overlain by the shale-dominated Akinbo Formation of Late Palaeocene to Early Eocene (Ogbe, 1970; Okosun, 1990). The Akinbo Formation is overlain by the Oshosun Formation which composed of Eocene shale and then Ilaro Formation which is predominantly a sequence of coarse sandy estuarine, deltaic and continental beds; the Ilaro Formation is characterized with rapid lateral facies changes. Overlying the Ilaro Formation is the Benin Formation which is predominantly coastal plain sands and Tertiary alluvium deposits.

The local geology is consistent with the regional geology and is predominantly Coastal Plain Sands and Recent sediments. The Coastal Plain sands consists of poorly sorted clayey sand, reddish mud/mudstone, clay lenses, sandy clay with lignite of Miocene to Recent underlain by a sequence of coarse sandy estuarine, deltaic and continental beds characterised by rapid changes in facies. The top soil is mainly sandy loam which is rich in organic matter and underlain by unconsolidated sand with varying thickness ranging from about 1.0 to 2.5 m across the farm land. This unconsolidated sand is underlain by more consolidated and relatively impermeable lateritic clay unit. This causes runoff water to settle in most parts of the area after rainfall for periods ranging from few days to weeks. At the time of this study, the farm land has been tilled and cultivated with maize and plantain already growing on it. Organic fertilizer was applied on the farm. The aquifer system is confined and relatively deep with depth ranging from about 45 m to >65 m as characterized from previous studies (Aizebeokhai and Oyebanjo, 2013; Aizebeokhai and Oyeyemi, 2014).

METHODOLOGY

Laboratory measurements

A total of twelve soil samples were collected from the study site and analyzed in laboratory. The samples were collected from two locations, at 20 m and 70 m marks, on each profile except for Traverses 5 and 6 where the samples were collected at 20 m and 50 m. The soil samples were limited to the top soil within 15 cm range. The samples were visually observed so as to determine their physical characteristics which can be used as aids to grain size identification. The samples were dried to remove the moisture content left in the soil samples. During the laboratory testing, distilled water was used in every step that involved liquid. The beakers, measuring cylinders and spatulas were washed with distilled water and oven dried so as to remove traces of ions and water molecules present in the apparatus. A small amount of each sample collected, approximately 2 g, was placed in the beakers and 100 ml of distilled water was added to it. The mixture was then stirred properly to accelerate the dissolving of the traces of salt present in the samples. The solutions were covered and left for about 70 h so that any salt present in the soil samples could dissolve properly.

The conductivity meter was calibrated using two solutions 1413 and 848 µsKm, respectively so as to sterilize the sensing part of the conductivity meter. The JENWAY 4510 conductivity meter, which applies an alternating current (I) at a specified frequency to two active electrodes and measures the potential (V) was used for determining the conductance. The conductivity meter then uses the conductance and cell constant to determine the conductivity displayed. The current source was adjusted so that the measured potential (V) equals the reference potential (approximately ± 200 mV). The HANNA salinity meter was used to measure the salinity level in the samples. The temperature of the samples was also determined. The conductivity and salinity meters were re-calibrated after each reading before using it for the next sample. The conductivity of the samples was measured in micro-Siemens per meter (µS/m) and converted to deci-Siemens per meter (dS/m) and the salinity levels are expressed in percentage.

Geophysical survey

Six 2D geoelectrical resistivity and time domain induced polarization profiles were conducted with the aid of ABEM Terrameter (SAS 1000/4000 series). Traverses 1 to 4 were 100 m in length, while Traverses 5 and 6 were 70 m and 80 m in length respectively due to limitation of space. The 2D traverses were conducted in the west-east direction and are separated from each other with a distance of 15 m. Wenner electrode configuration with minimum electrode spacing of 1.0 m was used for the data measurements, and a data level of 5 (maximum electrode spacing of 5.0 m) was achieved in each of the profiles. The minimum electrode spacing and data level reached ensures that the effective depth of investigation is confined to the root zone (about 2.0 m depth). Care was taken to minimize electrode positioning error in the measurements throughout the survey. To ensure quality and minimized error in the data collection, the measurements were stacked for each observation and the data stacking range between 3 and 6. The root-mean-squares error in the measurement was generally less than 0.3%. Data measurements with root-mean-squares error up to 0.5% or higher were repeated, after ensuring that the electrodes were in good contact with the ground. The apparent resistivity and apparent

Table 1. Conductivity and salinity level from laboratory observations.

Samples	Temperature (°C)	Weight (g)	Conductivity (dS/m)	Salinity (%)
T_1 (20 m)	28.0	2.0037	6.2×10^{-4}	0.5
T_1 (70 m)	28.0	2.0057	1.085×10^{-4}	0.2
T_2 (20 m)	28.0	2.0023	1.156×10^{-4}	0.2
T_2 (70 m)	27.9	2.0041	2.41×10^{-4}	0.2
T_2 (20 m)	27.9	2.0003	7.80×10^{-5}	0.2
T_3 (70m)	28.1	2.0054	1.069×10^{-4}	0.2
T_4 (20m)	28.1	2.0058	7.12×10^{-5}	0.5
T_4 (70m)	28.0	2.0068	1.243×10^{-4}	0.2
T_5 (20m)	28.0	2.0047	1.106×10^{-4}	0.4
T_5 (50m)	27.8	2.0057	8.11×10^{-5}	0.3
T_6 (20m)	27.8	2.0680	8.40×10^{-5}	0.4
T_6 (50m)	27.8	2.0467	1.649×10^{-4}	0.4

Table 2. Standard salinity level for soil (Richards, 1954).

Electrical conductivity (dS/m)	Interpretation	Inference
0 – 2	Normal soil	Little or no effect on growth of plant
2 – 4	Saline	Affects only very sensitive plants
4 – 8	Slightly saline	Affects many plants
8 – 16	Moderately saline	Affect tolerant plants
> 16	Severely saline	Affects even very tolerant plants

chargeability were measured concurrently. The chargeability of IP effect was measured by integrating the area under the IP decay curve according to the relation

$$M = \frac{1}{V_0}\int_{t_1}^{t_2} V(t)dt \, , \qquad (2)$$

where V_0 is the voltage measured before the current is turned off, t_1 and t_2 is the start and stop time interval respectively, and $V(t)$ is the decaying voltage.

The observed apparent resistivity and chargeability data sets for each of the 2D profiles were processed with RES2DINV computer code (Loke and Barker, 1996). The RES2DINV computer program uses a nonlinear optimization technique which automatically determines a 2D resistivity model of the subsurface for the input apparent resistivity data (Griffiths and Barker, 1993; Loke and Barker, 1996). The program divides the subsurface into a number of rectangular blocks according to the spread of the observed data. Least-squares inversion with standard least-squares constraint which attempt to minimize the square of the difference between the observed and the calculated apparent resistivity values was used to invert all the 2D traverses. Smoothness constraint was applied to the model perturbation vector only and appropriate damping factors were selected. Apparent resistivity datum points with greater than 50% RMS errors were eliminated from the 2D data set before the final inversion.

RESULTS AND DISCUSSION

The JENWAY conductivity meter and HANNA salinity meter were used to measure the conductivity and salinity level for each sample solution, respectively. The salinity levels obtained are given in percentages. Similarly, the conductivity values were measured in micro-Siemens per meter (µS/m) and converted to deci-Siemens per meter (dS/m). The observed salinity level and conductivity in soil samples are presented in Table 1. The standard values for salinity levels in soils are presented in Table 2 (Richards, 1954). The laboratory observations showed that the conductivities and salinity levels of the soil were generally low and within the limit for normal soil for plant growth.

The inverse resistivity and chargeability models obtained from the data inversion are presented in Figures 3 to 8. The inverse model resistivity and chargeability sections presented were achieved after the fifth iteration, except for Traverse 2 which converges after the third iteration. The effective depth of investigation for the model sections is about 2.8 m. The root-mean-squares errors observed in the inverse resistivity models range between 4.2 and 7.2%. Correlation between measured chargeability data and calculated ones shows low noise in IP data. The root-mean-squares errors observed in the chargeability inverse models are much lower than those of the resistivity inverse models, and range from 0.11 to 0.29%.

The tilled layer is largely characterized with very low resistivity values and can thus be easily discriminated from the more compacted region. The inverse resistivity

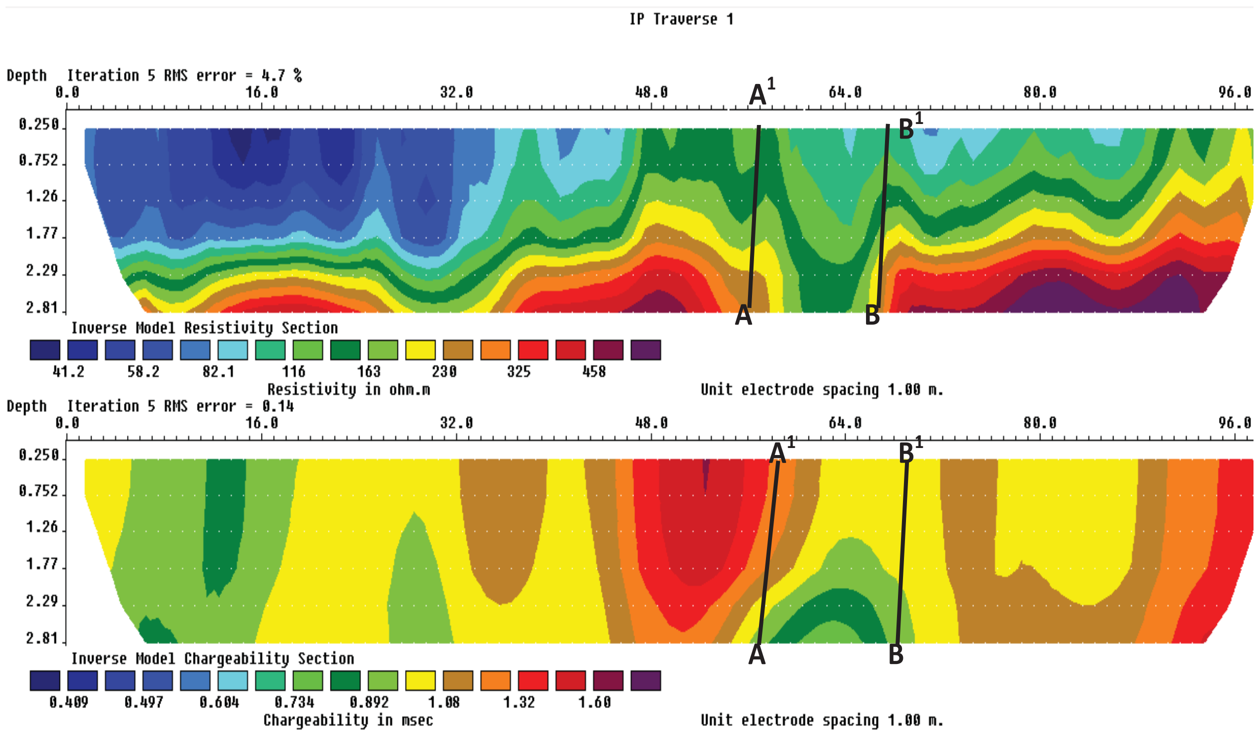

Figure 3. Inverse resistivity and chargeability model sections for Traverse 1.

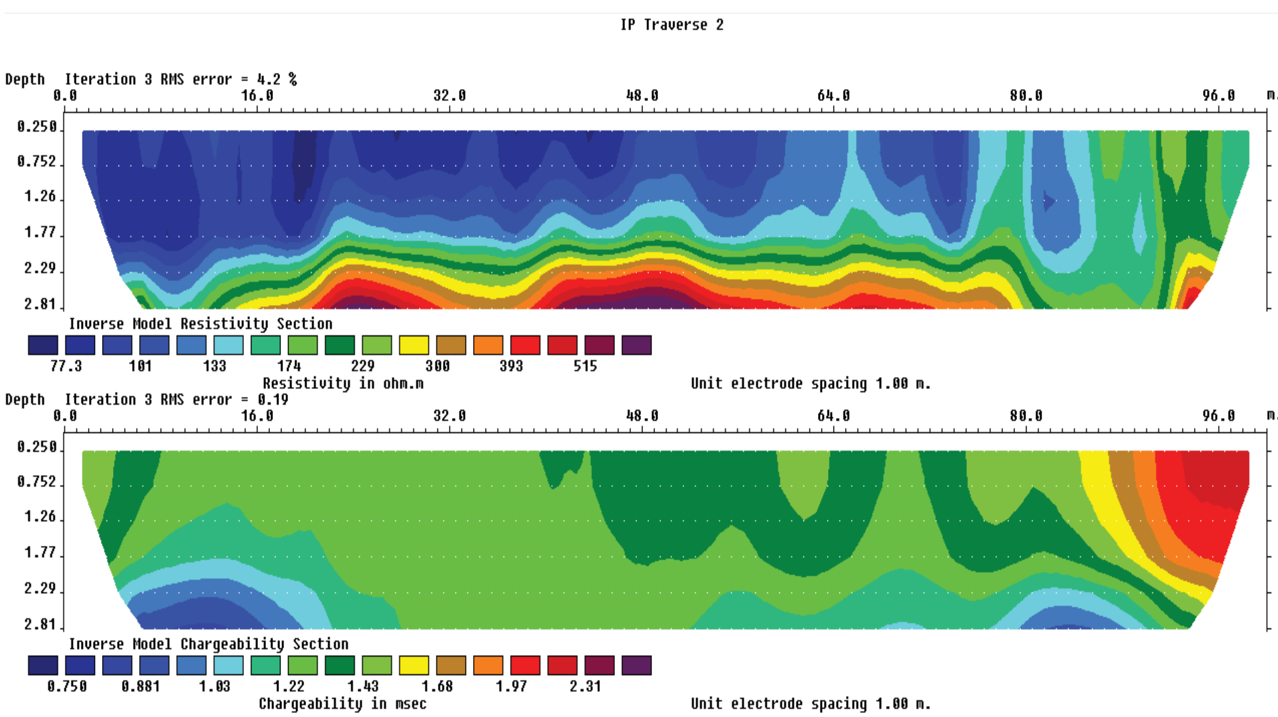

Figure 4. Inverse resistivity and chargeability model sections for Traverse 2.

models are generally characterized with low resistivity values in all the traverses, ranging from about 40 to 700 Ωm. Low resistivity (<$100\,\Omega m$) values are particularly pronounced in the west end of the farm. On the whole,

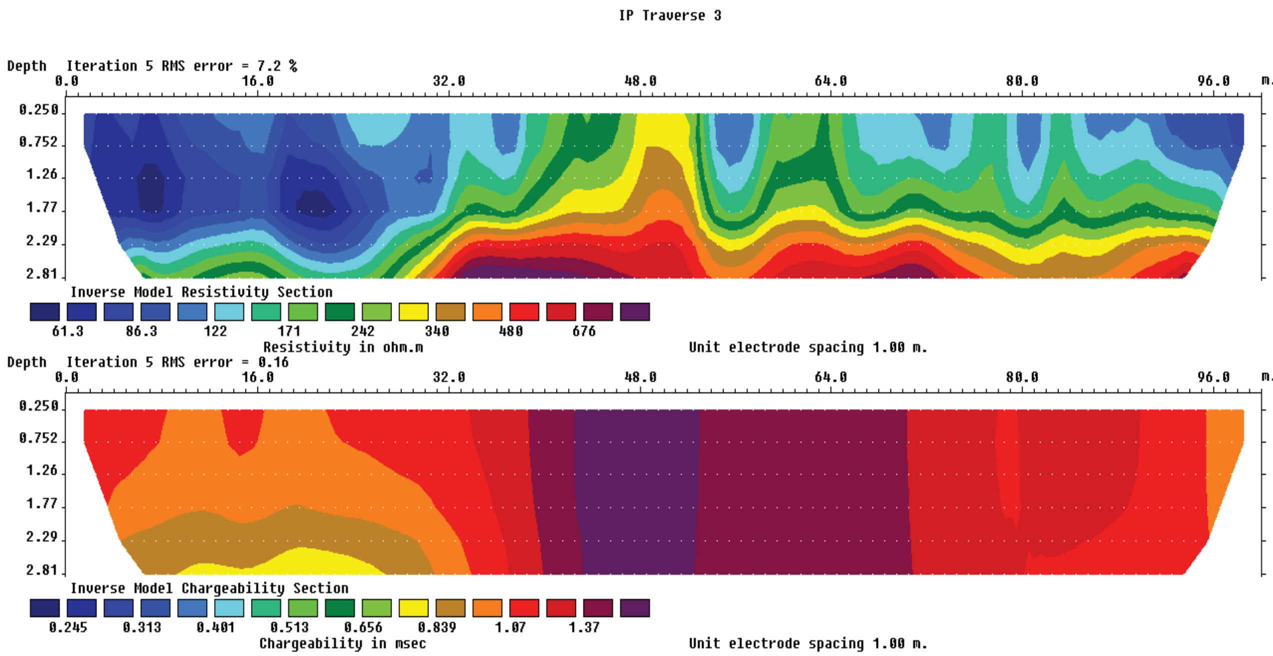

Figure 5. Inverse resistivity and chargeability model sections for Traverse 3.

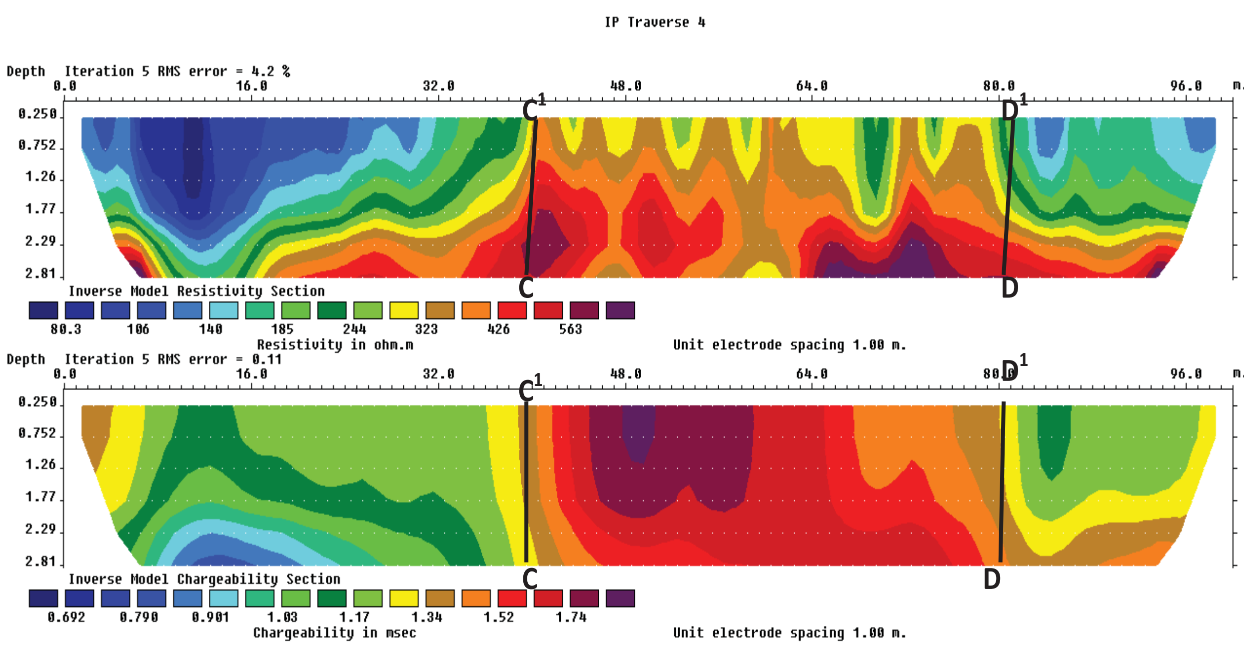

Figure 6. Inverse resistivity and chargeability model sections for Traverse 4.

the inverse model resistivity is averagely less than $100\Omega m$ to an average model depth of about 2.2 m, indicating high moisture content (and/or clay mineral) and less consolidated soil within this depth. However, relatively higher model resistivity values are observed in Traverse 4. Field observation shows that the subsoil in this area is more compacted than most parts of the survey farm. This indicates that the subsoil in the study site is generally conductive.

Soil moisture, porosity, degree of consolidation and organic matter are thought to be the dominant factors that determine the observed inverse model resistivity. Areas

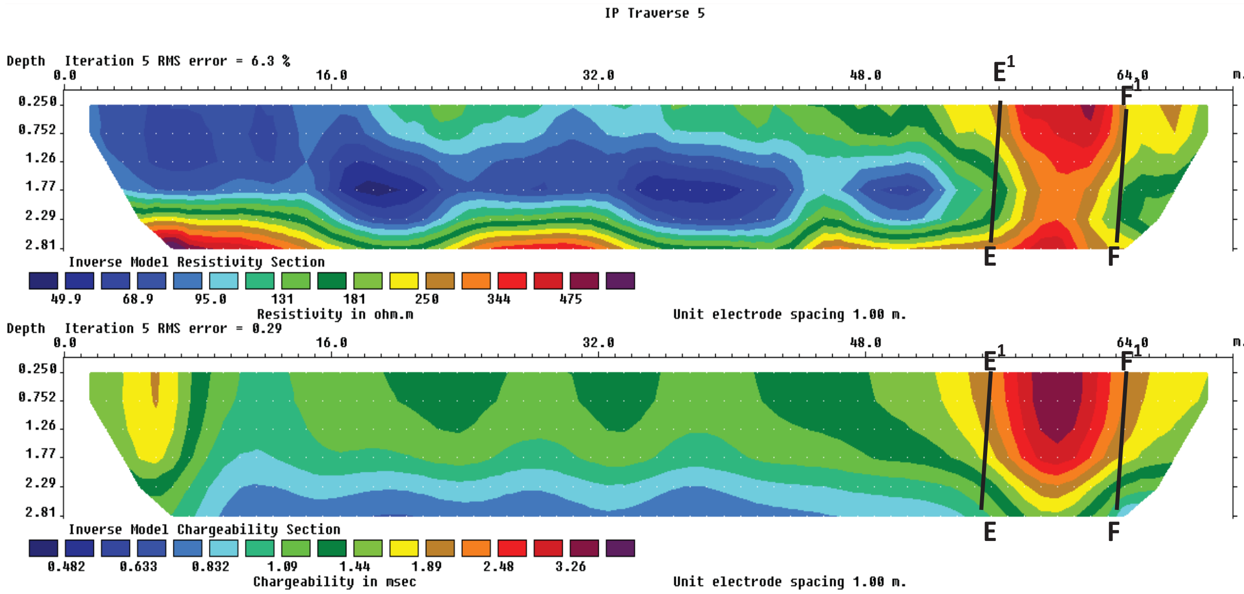

Figure 7. Inverse resistivity and chargeability model sections for Traverse 5.

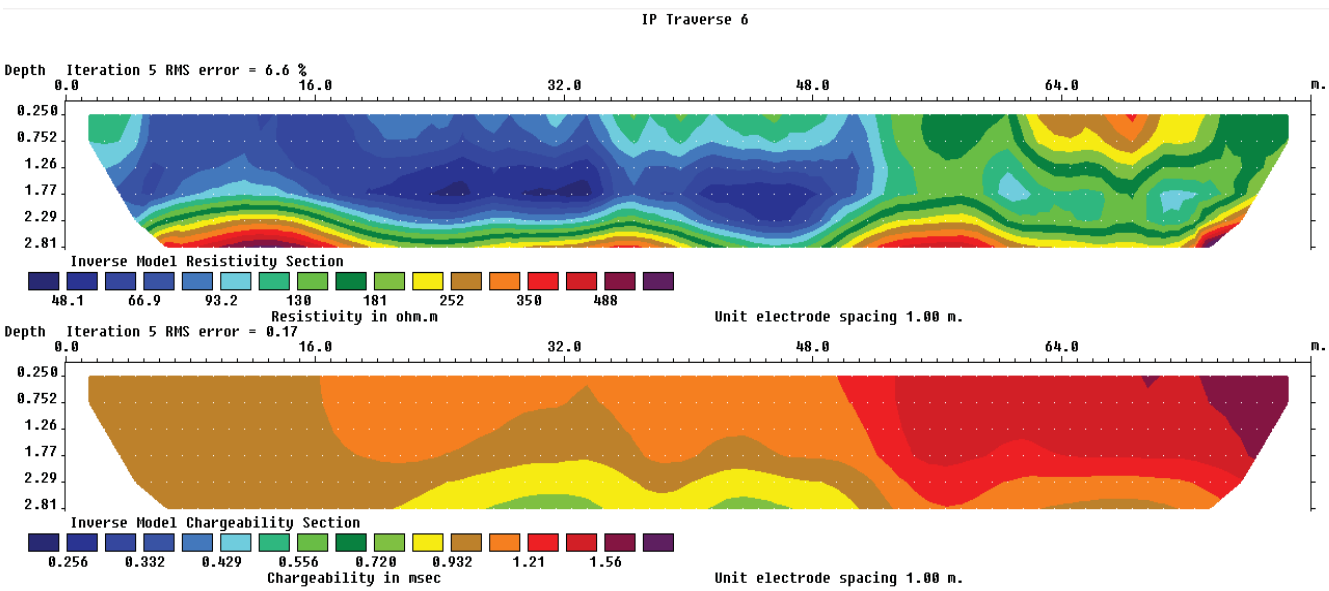

Figure 8. Inverse resistivity and chargeability model sections for Traverse 6.

with loosed soil are generally more porous and contain higher soil moisture content. Low resistivity anomalies $(<100\,\Omega m)$ were generally observed in these areas. Although, soil salinity could significantly decrease model resistivity values, the observed low resistivity values in the inverse model sections are not attributed to soil salinity. Results from the laboratory test, indicating low salinity level in the sub soil, further confirmed that the impact of salinity in the observed model resistivity is minimal.

The model chargeability observed in the inverse model sections is generally low, ranging from 0.4 to 3.5 ms. Strong correlations are observed between the resistivity and chargeability anomalies in the inverse model sections for Traverses 2, 5 and 6 with high resistivity values corresponding to relatively low chargeability values. Areas with relatively high resistivity anomalies $(>100\,\Omega m)$ are thought to be regions with more consolidated soil materials, consisting mainly of lateritic soil. However, some anomalies with high resistivity values also produced high chargeability values; for example, anomaly marked CC^1 and DD^1 in Traverse 4 and that marked EE[1] and FF[1] in Traverse 5. The anomaly marked AA[1] and BB[1] in the inverse model section for Traverse 1 is thought to be a fractured zone which serves as conduit path for fluid flow.

The chargeability of a given medium is a measure of the discharge of the polarization in the medium. Thus, it is related to the permittivity and resistivity of the subsurface materials as well as the porosity and moisture/water content in the subsurface media. Other factors that can significantly influence the chargeability of surface materials are grain size and shape of the constituent particles, mineral volume fraction and mineral conductivity. Strong IP effects are commonly observed in sediments containing clays disseminated on the surface of larger grains. Hence, clayey sand and clayey sandstone typically displays large IP effects. In contrast, compacted clays are usually associated with low IP effects, as the ohmic conduction dominates current flow. Small measurable IP effects are associated with clean sand and gravel (Vanhala, 1997).

The model sections generally shows low chargeability ranging from 0.4 to 3.5 ms. This indicates that the soils are mainly composed of sandy materials and less disseminated clayey materials. A careful analysis of the resistivity and chargeability in the inverse model sections show that observed low resistivity anomalies did not show distinct and relatively high chargeability anomalies. Thus, the observed low resistivity anomalies are not principally due to increased clay volume in the subsoil. This is because clay is expected to produce high chargeability anomaly due to cationic exchange capacity. Hence, the observed low resistivity anomaly is mainly due to increased porosity and high moisture content in the subsoil.

Conclusions

The knowledge of the spatial distribution of soil petrophysical properties is useful for precision agriculture as well as environmental impact analysis. In this study, apparent resistivity and chargeability of the subsoil were concurrently measured along six traverses using Wenner electrode configuration. 2D images of the inverse models of geoelectrical resistivity and chargeability of the induced polarization effects of the area investigated are produced. The 2D model sections of the geoelectrical resistivity and chargeability were used to qualitatively assess the spatial variability of the salinity, degree of compaction, and horizontal thickness of the subsoil. Other soil properties including clay volume, moisture content and organic matter, which are related to the conductivity, were equally inferred from the inverse model sections. Soil samples analysed for conductivity and salinity showed that the salinity level in the study area is within range for normal soil and therefore healthy for plant growth. Consequently, the low resistivities observed in the inverse model sections are not attributed to increased salinization in the soil but due principally to the effect of tillage, moisture content and presence of organic matter in the soil. The study demonstrates that geoelectrical resistivity imaging can be effectively used to map and assess the saptial variability of soil properties in large tracts of land for precision agricultures and environmental impact analysis. The degree of relaibility of the subsoil resistivity model can be significantly improved if the technique is combined with other geophysical methods such as self potential, induced potential and electromagnetic methods, which are equally sensitive to these petrophysical parameters.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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