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Full Length Research Paper

Application of sequence specific amplified polymorphism (SSAP) and simple sequence repeat (SSR) markers for variability and molecular assisted selection (MAS) studies of the Mexican guava

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Molecular markers have proven to be powerful tools in research related with diversity, variability, and improvement of economically important tropical crops. This study analyzed eight physiological and morphological fruit characters of economic interest in the cultivated Mexican guava (*Psidium guajava* L.), and assessed the suitability of two sequence specific amplified polymorphism (SSAP) and simple sequence repeat (SSR) markers developed for their use in early selection of individual plants with given fruit characteristics. Principal component analysis (PCA) explained 79% of the morphological variability observed among accessions. S-SAP was more informative than AFLP for studies of variability and diversity in guava, the former marker showing higher percentage of polymorphism (90%) and more intraspecific variability (0.58). It was analyzed by cluster analysis using the unweighted pair group with arithmetic means (UPGMA) method the relationships between accessions from nine guava varieties. S-SAP dendrograms clustered varieties in better agreement with pulp color and fruit shape, suggesting a possible association of the S-SAP marker with quantitative trait loci (QTL) related to fruit physiological and morphological fruit characters. According to results, the microsatellites mPgCIR131, mPgCIR136 and mPgCIR161 might also be linked to QTL related to internal and external pulp thickness, pulp color, and soluble solids, indicating that the SSR markers developed are appropriate for their use in early selection of guava individuals having specific fruit features, therefore being suitable for molecular marker assisted selection (MAS) of the crop.

Key words: Microsatellites, quantitative trait loci (QTL), sequence specific amplified polymorphism (SSAP), simple sequence repeat (SSR), *Psidium guajava* L.

INTRODUCTION

The guava (*Psidium guajava* L.) is a species of fruit tree belonging to the family Myrtaceae that is distributed in tropical and subtropical regions, mostly in Southeast Asia, Mexico, and Central and South America (Biffin et al., 2010). The trees and fruits of the species are worldwide known for their ecological and economic relevance (Grattapaglia et al., 2012; Woodrow et al., 2012), and in many countries guava fruits are highly valued, in some of them even being a staple food (Liu and Yang, 2011).

Some authors have proposed guava fruits are potentially nutraceutical due to their high contents of vitamins, minerals, and polyphenolic antioxidants (Hassimotto et al., 2005; Ho et al., 2012). Other parts of the guava plant have been used to treat diabetes, caries, wounds, diarrhea, inflammation, and hypertension (Gutiérrez et al., 2008), and have been reported as having anti-plasmodial, anti-inflammatory, hepatoprotective, anticancer, and antioxidant activities (Salib and Michael, 2004; Ojewole, 2006; Roy et al., 2006; Flores et al., 2015). The wide variety of applications and ecological importance of the species there is constant progress of numerous research efforts for improving its agronomical characteristics.

Conventional breeding methods to improve woody species as guava are limited (Rai et al., 2010; Liu and Yang, 2011). Selection of elite plants through the observation of phenotypic characters associated with traits of commercial importance continues to be favored among the methods for improvement of fruit tree crop production and the approach has in some cases proved to be effective, but breeding methods based on elite plant selection are also known to be extremely time consuming on average. In addition, the variation of the phenotypic characteristics in guava plantations has in many occasions proven to be mainly related to environmental factors, therefore being difficult to control (Srivastava and Narasimhan, 1967; Thaipong and Boonprakob, 2005).

In that context, molecular approaches have been not only useful for characterizing the genetic diversity among different guava cultivars, but also for identifying genes of commercial interest in the species. Among the most widely used molecular marker systems applied to studying the variability and genetic diversity of species in the genus *Psidium* are amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), and simple sequence repeats (SSRs) (Ferreira et al., 1994; Risterucci et al., 2005; Chen et al., 2007; Hernández-Delgado et al., 2007; Krishna and Singh, 2007). Data obtained from AFLP, SSR, and RAPD markers have revealed an ample genetic variability in accessions of guava from Mexico and other Latin American countries (Domínguez-Álvarez et al., 2005; Risterucci et al., 2005; Aranguren et al., 2010; Padilla-Ramírez and González-Gaona, 2010; Valdés-Infante et al., 2010). However, the perspective remains open for development of molecular markers for detecting more polymorphisms and variability among *Psidium guava* accessions. The sequence-specific amplified polymorphism (S-SAP) marker approach developed by Woodrow et al. (2012) in the myrtle can provide molecular markers adequate for genetic variation and breeding studies of guava accessions.

In addition, recent molecular marker assisted selection (MAS) studies have indicated that SSR markers might be associated with specific quantitative trait loci (QTL) and this approach was shown to be highly efficient for selection of elite plants with characteristics of commercial importance (de Oliveira et al., 2012; Nimisha et al., 2013). Additionally, application based on MAS using SSR primers for selection of cultivars with certain phenotypic characteristics has been described in commercially important crops including among other, pepper (Minamiyama et al., 2007), rice (Fu et al., 2010), and apple (Kenis et al., 2008).

About 50 QTL for fruit characters and nearly 150 primers related to SSR have been described in guava that could be used for selection of elite plants with fruit characteristics of commercially interest (Risterucci et al., 2005; Ritter et al., 2010; Valdés-Infante, 2012). Because S-SAP markers could be more informative for studies of variability in guava than previously used markers or markers related to SSR primers, they could be applied for selection of elite plants. In this study LTR retrotransposons was identified by means of molecular analysis of accessions of *P. guajava* from Mexico and designed S-SAP markers for the species. These markers were evaluated and the results compared with previously developed AFLP markers for guava. The application of primers associated with simple sequence repeats (SRR) was also evaluated for selection of plants with fruit characteristics of commercial importance in populations of Mexican guava. The present research demonstrated the importance of the application of S-SAP and SRR markers for studies of variability and future selection in guava populations of productive characters, such as fruit features including content of vitamin C, pulp color, number of seeds, soluble solid content, and internal and external pulp thickness.

MATERIALS AND METHODS

Plant materials

The present work used 91 accessions of guava collected from

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guava Germplasm Bank of the National Institute for Forestry, Agriculture and Livestock Research (INIFAP in its Spanish acronym), including five varieties (Calvillo siglo XXI, Caxcana, Huejucar, Hidrozac, and Merita) and three species (*Psidium guajava*, *Psidium friedrichsthalianum* and *Psidium cattleianum*). Nineteen accessions were used to assess variability through the SSAP marker and 70 accessions were used to validate the application of the use of SSR primers associated to external and internal pulp thickness (cm), pulp color, number of seeds, and soluble solid content (Table 1). Hernández-Delgado et al. (2007) described morphological characteristics of some of these accessions.

Description of trait of commercial importance in guava accessions

Seventy guava accessions were morphologically characterized (Table 1). The specific characteristics that were subjected to descriptive statistical analysis were: External and internal pulp thickness (cm), pulp color, number of seeds, and soluble solid content (°Bx). Degrees Brix were measured using an ATAGO model N-1EBX refractometer (Mercado-Silva et al., 1988) and the values of other features of interest were determined according to the procedures described by Hernández-Delgado et al. (2007).

Using the statistical package SAS version 9.0, a principal component analysis (PCA) based on the correlation matrix was made on average values of the quantitative and qualitative features fruit weight (g), polar diameter, equatorial diameter, thickness of external pulp (cm), thickness internal pulp (cm), soluble solids (°Bx), number of seeds, and pulp recorded from 70 accessions of Mexican guava from the states of Aguascalientes (3), Colima (3), Estado de Mexico (3), Guanajuato (5), Jalisco (8), Michoacan (5), Nayarit (10), Puebla (3) San Luis Potosi (8), Tabasco (2), Tamaulipas (1), and Zacatecas (19).

Development of S-SAP markers in guava

Long terminal repeat (LTR) retrotransposons of guava were isolated with the following procedure. DNA was extracted from young leafs from 16 guava accessions using the ChargeSwich kit (Invitrogen USA). Based on the protocol of Pearce et al. (1999), the extracted DNA was pooled (Table 1). The final concentration of the mixed sample was 1 μg of DNA in a final volume of 20 μL. The pooled DNA was digested with 1 U of the enzyme *Mse*I at 37°C for 20 min. Afterwards, 50 pmol of MSE I adapters (5'-GACGATGAGTCCTGA G-3' and 5'-TACTCAGGACTCAT-3') were added to the digestion product, and ligated with 1 U of T4 ligase (New England Biolabs, USA) at 18°C during 16 h. Subsequently, 5 µL of the digested and linked DNA were used as a template for polymerase chain reaction (PCR) amplification with the RNase H1 (5'-MGNACNAARCAYATHGA-3') and the MSEI (5'-GATGAGTCCTGAGTAANNN-) primers. Amplification conditions were as follows: One denaturing step at 94°C for 3 min, 30 cycles with one denaturing step at 94°C for 1 min, annealing at 45°C for 2 min, extension at 72°C for 2 min, and final elongation at 72°C for 10 min. The PCR products were separated by electrophoresis in 0.8% agarose gels and fragments between 400 and 2000 bp were selected, purified with the Wizard SV Gel and PCR clean up System (Promega, USA), and used in a second PCR with the RNase H2 (5-GCNGAYATNYTNACNAA-3') primer and the previously described MSEI adapters (20 pmol.μL^{-1}). The amplified fragments were cloned in the vector PGEM (Promega, USA) and transformed in competent *E. coli* cells. Twenty transformants that tested positive to containing the amplified fragments were selected and sent to MACROGEN Seoul, Korea, for sequencing.

Specific primers were designed for S-SAP in guava using the sequences of retroelements. Using BLASTn (http://blast.ncbi.nlm.nih.gov/), 5 sequences from the obtained PCR products were identified that aligned with ty1/Copia retroelements deposited in the GenBank (www.ncbi.nlm.nih.gov; Figure 2). Polypurine tract and LTR sequences were identified from ty1/Copia retroelement sequences from guava, from which five oligonucleotides were designed. The software packages Mega v. 4.1 (Tamura et al., 2013) and Oligo analyzer (http://www.idtdna.com/analyzer/applications/oligoanalyzer/Default.aspx) were used for sequence analysis.

Screening for the better set of primers for development of AFLP and S-SAP molecular markers

For selecting the better set of primers developed for the AFLP and S-SAP methods, five accessions belonging to different varieties were used: Calvillo Siglo XXI (LS1-A4), Caxcana (LS1-A33), Huejucar (LS1-A20), Hidrozac (LS1-A24), and Merita (LS1-A45) (Table 1). The ADN from these accessions of guava was extracted with the ChargeSwich kit (Invitrogen USA).

AFLP analysis

The AFLP method was performed following Tamayo-Ordóñez et al. (2012). Approximately 100 ng of genomic DNA were subjected to double-digestion by *Eco*RI and *Mse*I endonucleases at 37°C for 1 h, and incubated at 65°C for 15 min. The DNA fragments were linked to EcoRI (5'-CTCGTAGACTGCGTACC-3' and 5'-AATTGGTACGCAGTC-3') and MseI (5'-GACGATGAGTCCTGAG-3' and 5'-TACTCAGGACTCAT-3') adapters at 15°C overnight. After pre-amplification with primers containing a single selective nucleotide (EcoRI primer 5'-GACTGCGTACCAATTCN-3' and MseI primer 5'-GATGAGTCCTGAGTAAN-3'), a selective amplification by PCR was performed with four combinations of the EcoR1 and MseI primers. AFLP reactions with selective primers were performed with a touchdown PCR program for most primer sets with an initial denaturing step for 30s at 94°C, 30s for annealing at 65°C, and 1 min extension at 72°C. In each cycle, a decrease of 0.7°C in the annealing temperature was applied. Final annealing temperature was of 56°C, which was used for 24 cycles, with a final extension step at 72°C for 7 min. PCR products were electrophoresed in a CEQ 8800 sequencer (Perkin–Elmer Inc., Foster City, CA). The obtained electropherograms were analyzed using the software GeneMarker v.1.75 (Perkin-Elmer, Inc., Boston, MA).

S-SAP analysis

The S-SAP method was performed as described by Porceddu et al. (2002) and Vos et al. (1995). The S-SAP adapters and pre-amplification adapter primers used were the same as in Vos et al. (1995). A total of 20 primer pairs, obtained by the combination of the 5 retroelement primers and the 4 EcoRI primers were used for S-SAP (Table 2). In order to evaluate the reproducibility of the method, experiments were run by triplicate. The resulting bands were detected and analyzed by electrophoresis in a CEQ 8800 sequencer (Perkin-Elmer Inc., Foster City, CA). The combinations of primers used in the present study (Table 2) were performed with the touchdown PCR program previously described for AFLP.

Data analysis

AFLP and S-SAP bands were scored as 1 (present) or 0 (absent) in a binary matrix for each primer pair combination. Only reproducible and robust bands in each of the replications were considered as

Table 1. Information about the 94 accessions of *Psidium* spp. collected in Mexico used in this research.

Accession	Species	Variety	State
Selections 1-1-SEL-10[a]	*P. guajava*	Sel-10	Aguascalientes and Zacatecas
Selections-1-3-SEL-11[a]	*P. friedrichsthalianum*	Calvillo S-XXI	Aguascalientes and Zacatecas
Selections-1-4-SEL-11[ad]	*P. friedrichsthalianum*	Calvillo S-XXI	Aguascalientes and Zacatecas
Selections-1-5-SEL-12[a]	*P. guajava*	Sel.-12	Aguascalientes and Zacatecas
Selections -2-15-SEL-46[a]	*P. guajava*	Sel.-46	Aguascalientes and Zacatecas
Selections-2-19-SEL-48[a]	*P. guajava*	Huejucar	Aguascalientes and Zacatecas
Selections-1-20-SEL-48[ad]	*P. guajava*	Huejucar	Aguascalientes and Zacatecas
Selections-2-21-SEL-51[a]	*P. Cattleianum*	Sel-51	Aguascalientes and Zacatecas
Selections-2-23-SEL-54[a]	*P. guajava*	Hidrozac	Aguascalientes and Zacatecas
Selections-2-24-SEL-54[ad]	*P. guajava*	Hidrozac	Aguascalientes and Zacatecas
Selections- 3-25-SEL-56[a]	*P. guajava*	Sel.-56	Aguascalientes and Zacatecas
Selections -3-27-SEL-57[a]	*P. guajava*	Sel.-57	Aguascalientes and Zacatecas
Selections-3-33-SEL-106[ad]	*P. guajava*	Caxcana	Aguascalientes and Zacatecas
Selections-3-34-SEL-106[a]	*P. guajava*	Caxcana	Aguascalientes and Zacatecas
Selections-4-45-SEL-126[a]	*P. guajava*	Merita	Zacatecas
Selections-4-46-SEL-126[acd]	*P. guajava*	Merita	Zacatecas
S-126[c]	*P. guajava*	Merita	Zacatecas
LS1-A45[c]	*P. guajava*	Merita	Zacatecas
LS1-A3[c]	*P. guajava*	Calvillo siglo XXI	Puebla
LS1-A4[bcd]	*P. guajava*	Calvillo siglo XXI	San Luis Potosí
LS1-A20[bc]	*p. guajava*	Huejucar	Guanajuato
LS1-A19[c]	*p. guajava*	Huejucar	Tamaulipas
LS1-A24[c]	*P. guajava*	Hidrozac	Zacatecas
LS1-A33[c]	*P. guajava*	Caxcana	Zacatecas
LS1-A34[c]	*P. guajava*	Caxcana	Zacatecas
LS1-A6[d]	*P. guajava*	nd	Jalisco
LS1-A12[d]	*P. guajava*	nd	San Luis Potosi
LS1-A16[d]	*P. guajava*	nd	Aguascalientes
LS2-A1[d]	*P. guajava*	nd	Puebla
LS2-A6[d]	*P. guajava*	nd	San Luis Potosi
LS2-A18[d]	*P. guajava*	nd	Michoacán
LS2-A23[d]	*P. guajava*	nd	Puebla
LS3-A1[d]	*P. guajava*	nd	Puebla
LS3-A3[d]	*P. guajava*	nd	Michoacán
LS3-A6[d]	*P. guajava*	nd	San Luis Potosi
LS3-A7[d]	*P. guajava*	nd	Guanajuato
LS3-A10[d]	*P. guajava*	nd	Nayarit
LS3-A14[d]	*P. guajava*	nd	Guanajuato
LS3-A17[d]	*P. guajava*	nd	San Luis Potosi
LS4-A10[d]	*P. guajava*	nd	Nayarit
LS4-A12[d]	*P. guajava*	nd	Michoacán
LS4-A14[d]	*P. guajava*	nd	Jalisco
LS5-A1[d]	*P. guajava*	nd	Zacatecas
LS5-A3[d]	*P. guajava*	nd.	Zacatecas
LS5-A4[d]	*P. guajava*	nd	Guanajuato
LS5-A5[d]	*P. guajava*	nd	Zacatecas
LS5-A8[d]	*P. guajava*	nd	Jalisco
LS5-A11[d]	*P. guajava*	nd	Jalisco
LS5-A21[d]	*P. guajava*	nd	San Luis Potosi.
LS5-A23[d]	*P. guajava*	nd	San Luis Potosi
LS6-A1[d]	*P. guajava*	nd	Zacatecas

Table 1. Contd.

LS6-A5[d]	*P. guajava*	nd	Guanajuato
LS6-A15[d]	*P. guajava*	nd	Michoacan
LS6-A18[d]	*P. guajava*	nd	Jalisco
LS6-A24[d]	*P. guajava*	nd	Jalisco
LS7-A1[d]	*P. guajava*	nd	Zacatecas
LS7-A11[d]	*P. guajava*	nd	Aguascalientes
LS7-A14[d]	*P. guajava*	nd	Jalisco
LS8-A2[d]	*P. guajava*	nd	Tabasco
LS8-A11[d]	*P. guajava*	nd	Nayarit
LS8-A15[d]	*P. guajava*	nd	Aguascalientes
LS8-A21[d]	*P. guajava*	nd	Guanajuato
LS8-A22[d]	*P. guajava*	nd	Nayarit
LS8-A23[d]	*P. guajava*	nd	San Luis Potosí
LS9-A1[d]	*P. guajava*	nd	Colima
LS9-A5[d]	*P. guajava*	nd	Colima
LS9-A11[d]	*P. guajava*	nd	Nayarit
LS9-A17[d]	*P. guajava*	nd	Nayarit
LS9-A19[d]	*P. guajava*	nd	Tamaulipas
LS11-A1[d]	*P. guajava*	nd	Michoacán
LS11-A5[d]	*P. guajava*	nd	Jalisco
LS11-A8[d]	*P. guajava*	nd	Nayarit
LS11-A9[d]	*P. guajava*	nd	Nayarit
LS11-A10[d]	*P. guajava*	nd	Nayarit
LS11-A11[d]	*P. guajava*	nd	Nayarit
LS11-A16[d]	*P. guajava*	nd	Colima
LS12-A1[d]	*P. guajava*	nd.	Zacatecas
LS12-A2[d]	*P. guajava*	nd	Zacatecas
LS12-A6[d]	*P. guajava*	nd	Zacatecas
LS12-A9[d]	*P. guajava*	nd	Zacatecas
LS12-A14[d]	*P. guajava*	nd	Zacatecas
LS12-A16[d]	*P. guajava*	nd	Zacatecas
LS12-A24[d]	*P. guajava*	nd	Zacatecas
LS13-A4[d]	*P. guajava*	nd	Zacatecas
LS14-A1[d]	*P. guajava*	nd	Zacatecas
LS14-A23[d]	*P. guajava*	nd	Estado de Mexico
LS14-A24[d]	*P. guajava*	nd	Estado de Mexico
LS15-A1[d]	*P. guajava*	nd	Tabasco
LS15-A9[d]	*P. guajava*	nd	Zacatecas
LS15-A14[d]	*P. guajava*	nd	Estado de Mexico
LS17-A21[d]	*P. guajava*	nd	Nayarit

[a] Used for isolation of retroelements; [b] used for selection of better set of AFLP and SSAP primers; [c] used for evaluation of AFLP and SSAP markers; and [d] used for application of SRR-related primers and selection of individuals with traits of interest. nd = not determinable.

potential polymorphic markers. All calculations were performed using the NTSYS-pc 2.1 (Exeter Software Co., New York) software (Rohlf, 2000). In order to find out if the AFLP and S-SAP markers developed in this study could discriminate between accessions from different varieties of guava, nine accessions from five varieties were analyzed by the unweighted pair group with arithmetic means (UPGMA) method: Calvillo S-XXI (LS1-A3, LS1-A4), Huejucar (LS1-A19, LS1-A20), Hidrozac (LS1-A24), Caxcana (LS1-A33, LS1-A34), and Merita (LS1-A45, S126; Table 1). The genetic similarity between accessions was estimated by the simple matching genetic distance (*SM*) method calculated as: $SM = m/n$, where m is the number of matches and n is the total number of variables. Cluster analysis was performed from the similarity matrix by the UPGMA algorithm with the "FIND" option enabled to detect all possible trees.

The usefulness of AFLP and S-SAP markers for studying variability and polymorphisms was assessed using selected primers (Table 2) in 9 accessions of guava of which the variety was

Table 2. Eigenvalues of the most descriptive characteristics based on principal component analysis in Mexican guavas.

Variable	Eigenvectors	
	CP1	CP2
Fruit weight (g)	0.431029	-0.137558
Polar diameter (cm)	0.431600	0.036242
Equatorial diameter (cm)	0.449258	-0.016954
External pulp thickness (cm)	0.387100	-0.252010
Internal pulp thickness (cm)	0.441975	0.054375
Soluble solid content (°Brix)	-0.002116	-0.622743
Number of seeds	0.284236	0.411480
Pulp color	0.010915	0.596563

known, and which displayed phenotypic differences related to fruit features (Table 1).

Validation of the use of SSR primers as a tool for early selection in guava populations

The DNA from 70 guava accessions was extracted with the ChargeSwich (Invitrogen USA) kit (Table 1). Initially, a total of 18 SSR primers associated to 6 features of the fruit were chosen with the objective of selecting the best SSR primers for each fruit feature (Table 2). DNA from guava accessions having the highest and lowest values of each fruit characteristic, including internal and external thickness of the pulp, number of seed, soluble solid content, and pulp color was pooled and used as a template for amplification by PCR with the SSR primers specific to each described feature (Table 1). The values for each of the above-mentioned fruit features were subjected to analysis of frequencies in order to select the accessions of guava representing the minimum and maximum values of each feature to be used in the selection of SSR primers.

The PCR reaction conditions consisted of 2 ng of genomic DNA, PCR Buffer 1 X, 10 mM DNTPs, 10 pM each primer, 1.5 mM $MgCl_2$, and 1U Taq polymerase in a final volume of 20 µl. Amplification conditions were as follows: One denaturing step at 94°C for 3 min, 35 cycles with one denaturing step at 94°C for 45 s, annealing at 55°C for 1 min, extension at 72°C for 30 s, and a final elongation at 72°C for 20 min. Polymorphic primers able to discriminate between contrasting fruit features were selected for each combination of oligonucleotides associated with each fruit characteristic, and monomorphic primers that amplified in both contrasting features were discarded.

Three primers were finally selected: mPgCIR161 as a marker of white pulp and lower soluble solid content, mPgCIR136 as a marker for individuals with small external pulp thickness, and mPgCIR131 as a marker of individuals with thicker internal pulp. Primers were labelled with the fluorophores FAM, NED, and JOE and DNA from 70 guava accessions was amplified. The amplified fragments were separated by capillary electrophoresis in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and the visualization and classification by size of amplified fragments was performed by means of the software GeneMarker.

The results from GeneMarker were analyzed by means of a binary matrix with Microsoft Excel, indicating the absence and presence of fragments by the arbitrary values 0 and 1, respectively. The fragments that did not correspond to the expected size or having intensity peaks below 250 were eliminated from the matrix. Individuals were grouped according to allelic type (heterozygous or homozygous) and for each allelic type the percent efficiency of selection was calculated with the formula:

$$\text{Selection efficiency}=\left(\frac{\text{number of individuals with the expected feature}}{\text{individuals with the same allelic group}}\right)\times 100$$

RESULTS AND DISCUSSION

Characterization in individuals of guava of features of commercial interest

The descriptive statistics analyses of fruit characteristics in guava individuals indicated the following average values: 53.97±21.63 g of fruit weight, 10.76±1.52 °Bx of soluble solids, 184.28±69.95 seeds, 0.69±0.17 cm of external pulp thickness, and 3.69 ±0.48 cm of internal pulp thickness. Fruit weight and number of seeds proved to be the most variable morphological characteristics with coefficient of variation (CV) of 30%. The values of CV in the soluble solid content and in the external and internal pulp thicknesses varied from 10 to 24%.

The results of PCA considering eight morphological characteristics (Table 1) indicated that the first two components (PC1 and PC2) explained 79% of the observed morphological variability (Table 4). According to the values of the eigenvectors, the characteristics with greater weight in PC1, all having positive values, were internal pulp thickness, external pulp thicknesses, and polar and equatorial fruit diameter, while in CP2 these characteristics were soluble solid content with negative value and, with positive value, seed number and pulp color (Table 2).

Figure 1 shows seven groups of guava accessions each group with the following average values for fruit characteristics: Group I with 11 accessions from Nayarit (7), Zacatecas (3), and State of Mexico (1), mostly with pink pulp, 64 g fruits, 4 cm thick inner pulp, 10 °Bx, and 284 seeds; Group II including two larger fruit accessions from Tabasco with white and creamy white pulp, 453 g fruits, 4 cm thick inner pulp, 10.7 °Bx, and 239 seeds;

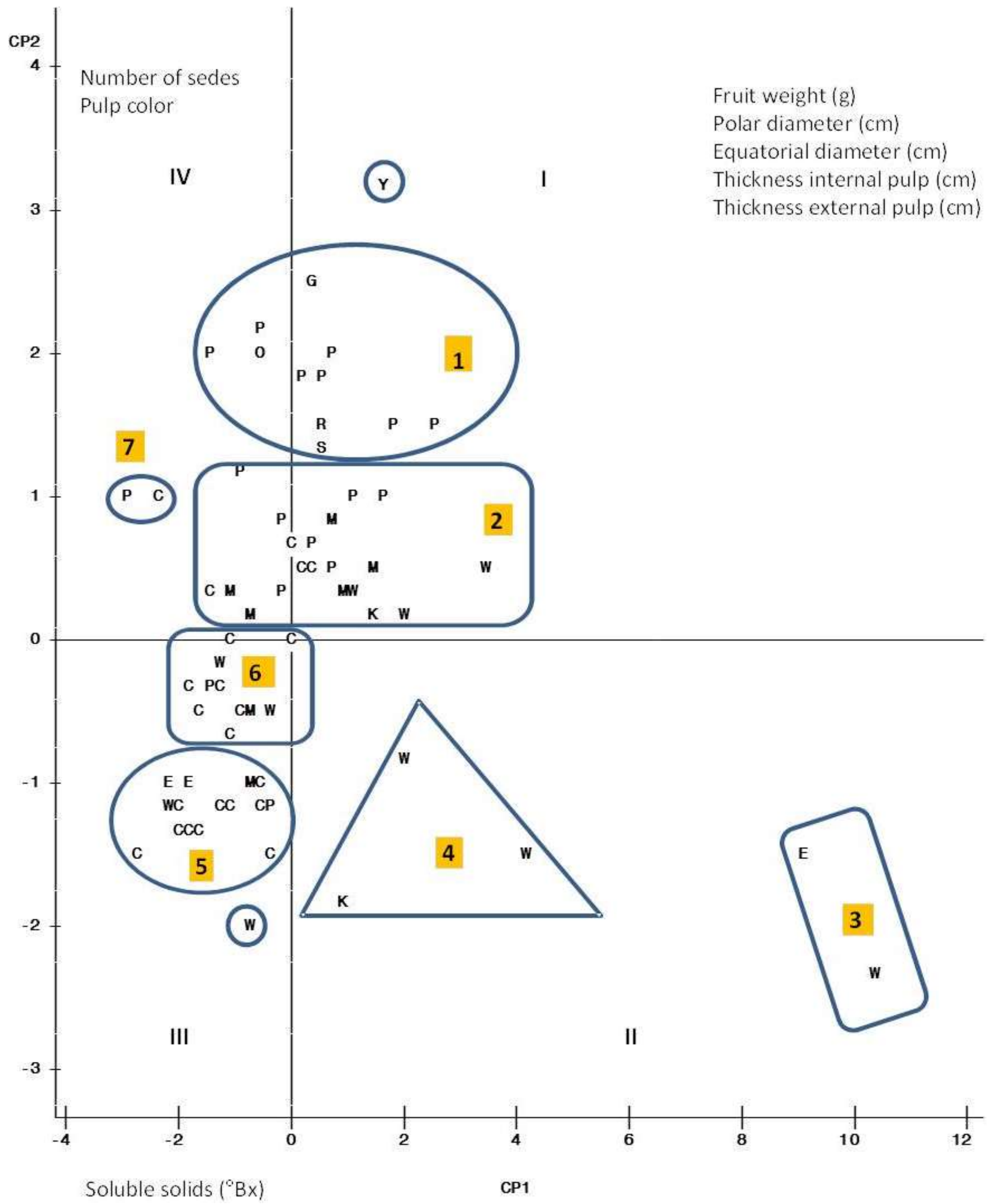

Figure 1. Dispersion of guava individuals based on the three principal components of the PCA of morphological data. The letters represent pulp color of individuals: White (W), white-cream (E), Cream (C), cream-pink (O), pink-cream (H), pink-white (K), pink (P), cream-yellow-orange (R), orange (O), orange-pink (G), speckled-orange pink (L), yellowish-orange (Y).

Group III with 16 accessions from Zacatecas (8), Jalisco (3), State of Mexico (2), Michoacán (1), Tamaulipas (1), and Guanajuato (2) with mostly with pink to creamy pink pulp, 72 g fruits, 7 cm thick inner pulp, 13.9 °Bx, and 402 seeds; Group IV with 3 accessions from Jalisco, Guanajuato, and Zacatecas with white and whitish pink pulp, 127 g fruits, 4.8 cm thick inner pulp, 13.5 °Bx, and 172 seeds; Group V with 15 accessions from Jalisco (4), Zacatecas (4), San Luis Potosí (2), Nayarit (2), Guanajuato (1), Michoacán (1), and Puebla (1) mostly with cream to whitish cream pulp, 37 g fruits, 14 °Bx, and 134 seeds; Group VI with 11 accessions from San Luis

Potosi (3), Zacatecas (2), Guanajuato (2), Nayarit (1), Colima (1), Michoacan (1), and Puebla (1) mostly with cream pulp, 41 g fruits, 2.7 cm thick inner pulp, 8.6 °Bx, and 153 seeds; and Group VII including two accessions having the smallest and less sweet fruits from Puebla and Colima with pink to cream pulp, 20 g fruits, 2.7 cm thick inner pulp, 8.5 °Bx, and 123 seeds.

Identification of retroelements and selection of the better primers for development of AFLP and S-SAP molecular markers in guava

The bioinformatics analysis indicated presence of LTR-retroelements corresponding to the Ty1/Copia retrotransposon. From the obtained sequences it was possible to identify the ADIFTK RNase H2 motif, which is highly conserved in *P. sativum*, *Diospyros kaki*, and *Vicia faba*, among other species (Figure 2) (Pearce et al., 1999; Du et al., 2009). Also, ADIFTK RNaseH domains were shown to be present in myrtle (*Myrtus communis* L.), which belongs in the same plant family of *P. sativum* (Woodrow et al., 2012).

Polypurine tract (PPT) and long terminal repeat (LTR) sequences were identified in the isolated nucleotidic sequences. The Ty1/Copia retroelement sequences of studied guava accessions displayed a 90 to 100% similarity compared to the corresponding sequences of *Diospyros kaki*, with an average of 100% in nucleotide sequences. The Ty1/Copia retrotransposon sequences of *P. guajava* were submitted to the GenBank with the accessions numbers KF991100.1, KF991104.1, KF991103.1, KF991102.1, and KF991101.1.

From the latter sequences, the following 5 retroelement primers were designed: GUA1-5'-ATTGGGTCCATCAGTTTC-3', GUA2-5'-ACACGAAATACGGCTACG-3', GUA3-5'-CTGCGACTTCACCAAGCCAT-3', GUA4-5'-CTTGAGGGGGAGTGTTGAG-3', and GUA5-5'-AGGGAGGTCTAAACTGAGGAAA-3'.

AFLP showed a polymorphism of 78% (ACA/CTA) and 86% (AAG/CGC), and the tested combinations for S-SAP had a polymorphism of between 73 and 100% (Table 2). A total of 1,763 bands were analyzed for S-SAP, of which 1,560 (88.48%) were polymorphic. The primer pairs showing 100% polymorphism in S-SAP were GUA2/EcoR1(AAG), GUA3/EcoR1(AAC), GUA5/EcoR1(AAC), and GUA5/EcoR1(AAG) (Table 2). Unlike GUA2 and GUA3, primer GUA5 resulted in 97 to 100% polymorphism when being combined with any of the four EcoRI primers in the analyzed accessions.

Potential use of S-SAP markers for study of guava germplasm

Table 3 contains the comparison of information provided by AFLP and S-SAP in the nine analyzed accessions for which the variety they belong to had been identified. Despite AFLP allowed for amplification of more bands than S-SAP (82 and 72, respectively), the number of polymorphic bands was similar for both compared methods (68 and 65, respectively). The percentage of polymorphism was higher when using the S-SAP method (90%) than when using the AFLP method (82%). The similarity indices between the nine analyzed accessions were 0.65 for AFLP and 0.58 for S-SAP.

The AFLP marker grouped eight of the nine analyzed accessions in a single cluster, while accession S-126 was grouped outside that cluster (Figure 3A).

Contrastingly, the dendrogram obtained from the S-SAP marker showed two clusters (designated clusters I and II). Cluster I includes accessions S-126, LS1-A4, and LS1-A20, and cluster II groups accessions LS1-A24, LS1-A45, LS1-A19, LS1-A34, LS1-A3, and LS1-A33 (Figure 3B). No correlation was observed between the latter two clusters and the provenances of the accessions, but rather to morphological characteristics of the fruit such as pulp color and fruit shape, which are distinctive of each variety. Group I clustered together accessions with cream pulp and ovoid fruits. Group II with two subgroups clustered together varieties with white speckled pulp and truncate or semi-rounded fruits.

The results of the analysis of two sets of primers for developing S-SAP and AFLP markers (Table 3) showed that S-SAP produces 12% less bands than AFLP. However, despite the lower number of bands produced by S-SAP compared to AFLP, the former marker is more informative than the latter for studies of intraspecific variability and polymorphism in *P. guajava*. Previous studies indicated that the EcoRI/MseI (ACA/CTA and AAG/CGC) primer pair selected for AFLP showed a polymorphism of 84.2 and 92.4% in 68 accessions of *P. guajava.* However, the use of the latter set of primers in the present work in five varieties resulted in lower levels of polymorphisms than previously reported of 78 and 86%, respectively (Table 3), these values were nevertheless consider to be high enough for studying polymorphism and variability in guava. The level of polymorphism obtained for S-SAP (100%) is higher than that previously reported for AFLP (Hernández-Delgado et al., 2007). This means that S-SAP results in higher levels of polymorphism compared to AFLP. The observed indices of similarity between the nine analyzed accessions for AFLP and S-SAP indicate high intraspecific genetic variability. S-SAP showed lower similarity values than AFLP, which indicates that S-SAP allows for detecting more intraspecific variability in guava than AFLP. The results obtained indicate that S-SAP is more informative for studies of variability and polymorphism of *P. guajava*, as has been reported for other crops of economic relevance such as barley (Soleimani et al., 2005; Shan et al., 2012), grapevine (Stajner et al., 2015), holly (Levina et al., 2010), and

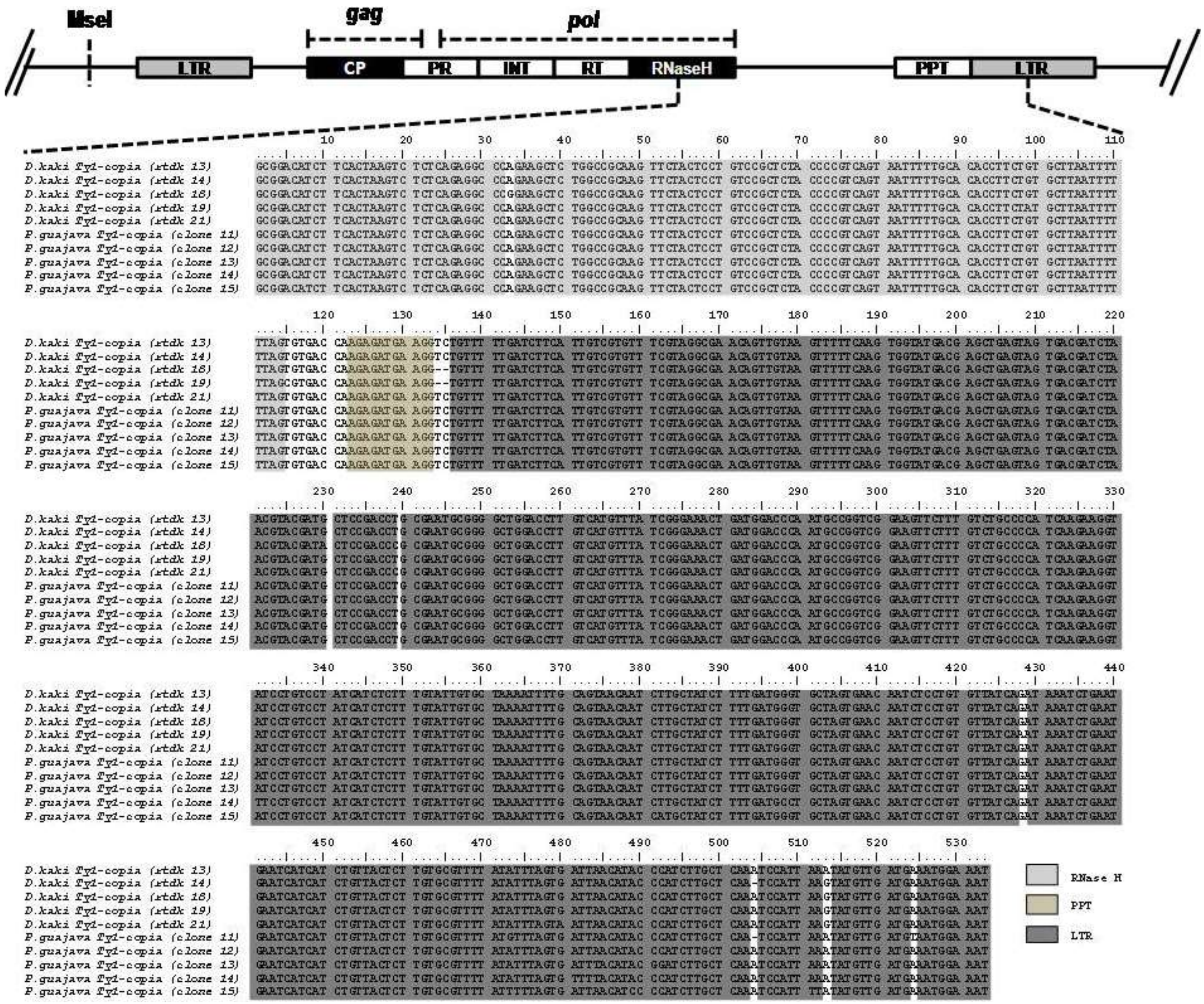

Figure 2. Genetic diagram of a LTR retrotransposon according to Pearce et al. (2000) indicating conserved motifs that were used for isolation and identification of retrotransposon clones of *P. guajava*. **(**A) The Ty1-copia LTR retrotransposons have LTRs at each end; *gag* gene encodes capsid-like protein (CP); *pol* gene encodes protease (PR), integrase (INT), reverse transcriptase (RT), and RNase H. Solid arrows indicate position of primers for S-SAP in the left LTR and the *Mse* I adapter; dashed arrow indicates the position in sequence of the LTR and PPT primers. Nucleotide and amino acid residues represent consensus sequences obtained for five clones isolated in this work. (B) *Ty1-copia* retrotransposons alignment to sequences obtained from the GenBank database. Accessions EU068710.1, EU068711.1, EU068718.1, and EU068716.1 correspond to *Diospyros kaki* sequences in GenBank. Accessions KF991100.1, KF991104.1, KF991103.1, KF991102.1, and KF991101.1 correspond to sequences in GenBank of retroelements obtained in this study. RNase H gene sequences are highlighted in yellow, poly purine tract (PPT) gene sequences are highlighted in grey and long terminal repeat (LTR) gene sequences are highlighted in blue.

citrus (Biswas et al., 2011), among others.

The high level of polymorphism obtained with marker S-SAP in comparison with AFLP was possibly due to the ubiquitous nature of retroelements (Flavell et al., 1992; Hirochika and Hirochika, 1993; Han, 2010), which suggests that the S-SAP marker could be well suited for studies of variability and diversity of Mexican guava germplasm. Otherwise, the resulting UPGMA dendrograms of nine accessions of guava showed discrepancies between the AFLP and the S-SAP approaches. AFLP formed a single cluster, while S-SAP formed two groups with the five analyzed varieties. S-SAP clustered varieties according to pulp color and fruit shape (Figure 3). Clustering of the cream, ovoid fruit

Table 3. Comparison of informativeness obtained with AFLP and S-SAP in nine accessions of *P. guajava.*

Indicator	AFLP	SSAP
Number of analyzed accessions	9	9
Polymorphism		
Total number of bands	72	82
Number of non-polymorphic bands	7	14
Number of polymorphic bands	65	68
Percentage of polymorphism	90	82
Variability		
Similarity index	0.58	0.55
Maximum	0.77	0.78
Minimum	0.38	0.49

varieties Calvillo Siglo XX-I and Merita in groups I and II could be indicative of intraspecific variability between these two varieties. Varieties Caxcana, Huejucar, and Hidrozac with white, speckled and pink pulp, respectively, clustered within group II. According to the distribution of varieties in the dendrogram, except for accession LS1-A3, most accessions with cream pulp tend to be located in the upper part of the dendrogram, accessions with speckled and pink pulp occupy the central part of the dendrogram, and accessions with white pulp were plotted at the bottom of the dendrogram.

PCA considering eight morphological characteristics indicated that the two first components explain 79% of the morphological variability observed among the accessions. Accessions grouped in five main groups in which pulp color showed not being determinant for discrimination of PCA groups. In contrast, clustering of S-SAP results suggests that the chosen primers hold great potential for their use in selection in guava of crops with specific pulp color, and could be used in early selection of elite material according to the agronomic characteristics of interest.

The grouping of accessions resulting from S-SAP according to pulp color and fruit shape may reflect an association of retroelements with a quantitative trait loci (QTL) conferring pulp color and fruit shape in guava. The association of retroelements with QTL related to fruit color has been described in economically important fruit crops such as peach (Falchi et al., 2013), grapevine (Kobayashi et al., 2004; Pelsy, 2010), and blood orange (Butelli et al., 2012). It has been suggested that high levels of activity of mobile elements (retroelements) can contribute to increment allelic diversity related to fruit color (Tao et al., 2005; Rico-Cabañas and Martinez-Izquierdo, 2007, De Felice et al., 2009; Butelli et al., 2012).

The color and shape of fruits are among the most popular commercial criteria for consumer acceptance of guava. However, low uniformity and quality in fruit have economic consequences for production and export of guava fruits. Despite the increasing interest in improving cultivars with desirable characteristics for consumers, little is known about the genetic factors that are related to the color and shape of fruit in guava, complicating the development of studies focused in marker assisted selection (MAS) of this crop (Collard and Mackill, 2008; Xu and Crouch, 2008). The possible association of retroelements with QTL related to pulp color and fruit shape in guava shown by the use of S-SAP marker could in the future be used for molecular mapping, marker assisted selection (MAS), and better understanding of gene function and gene regulation in guava, which would be of aid to the breeding of this crop species.

Simple sequence repeat (SSR) related to features of commercial importance

The evaluation of eighteen primer pairs associated to SSR related to six features of commercial importance (Table 3) indicated that only four primer pairs were polymorphic (discriminative between guava accessions with high and low values according to the feature analyzed), six were monomorphic (not discriminative between guava accessions with high and low values according to the feature analyzed discriminative), and six did not show amplification products.

Three primers pairs (mPgCIR131, mPgCIR243, and mPgCIR208) were analyzed for evaluating the thickness of internal pulp: The mPgCIR131 pair was polymorphic for greater internal thickness; the mPgCIR243 pair showed no discrimination between accessions with higher or lower internal pulp thickness (was monomorphic); and the primer pair mPgCIR208 failed to amplify. Of the four primers pairs tested for association to external pulp thickness, three did not amplify (mPgCIR020, mPgCIR284, and mPgCIR243), only mPgCIR136 proving to be polymorphic for lower external pulp thickness (Figure 1A).

Regarding the two primers pairs (mPgCIR161 and

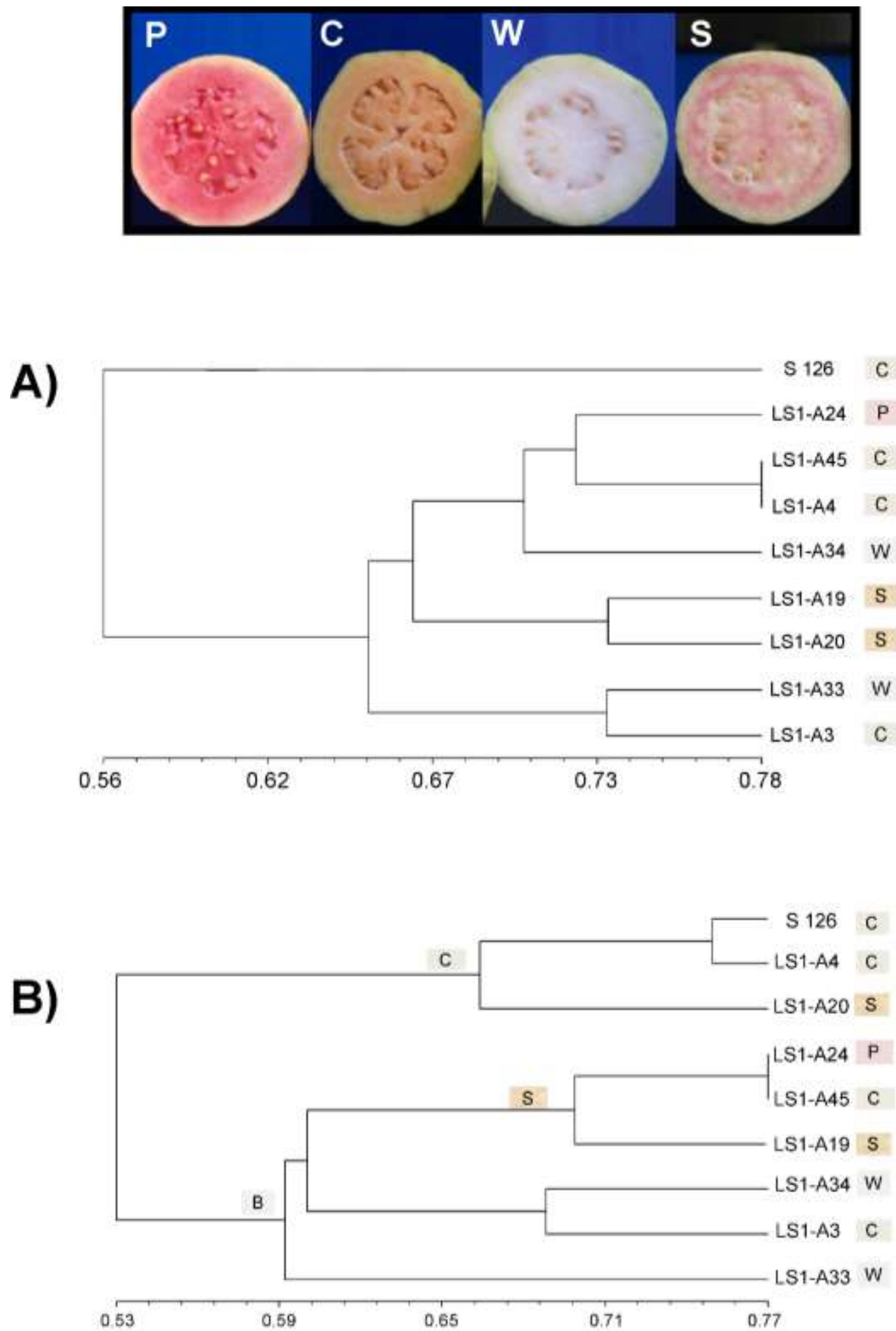

Figure 3. Genetic dissimilarity dendrograms of 9 guava accessions generated by the UPGMA algorithm. (A) Dendrogram based on the AFLP marker; (B) Dendrogram based on the S-SAP marker. The letters C (cream), S (speckled), P (pink), and W (white) make reference to the pulp color of the analyzed accessions, as illustrated in photograph above dendrograms. This study included five varieties of guava: Calvillo S-XXI (LS1-A3, LS1-A4), Huejucar (LS1-A19, LS1-A20), Hidrozac (LS1-A24), Caxcana (LS1-A33, LS1-A34), and Merita (LS1-A45, S126).

mPgCIR254) associated with soluble solid content, mPgCIR161 only amplified in individuals having lower values of soluble solid content, while mPgCIR254 was found to be unable to discriminate between low and high soluble solid content values (Figure 1B). All of the three primer pairs (mPgCIR034, mPgCIR161, and mPgCIR208) evaluated for seed number were monomorphic and therefore not discriminative between low and high values.

The results obtained by the use of three SSR primer pairs associated with pulp color (mPgCIR079, mPgCIR137, and mPgCIR161) showed only mPgCIR161 was polymorphic for white and cream pulps, whereas primer pairs mPgCIR099137 and mPgCIR099 showed no amplification products (Figure 1C).

Finally, few guava accessions were characterized for vitamin C content and their DNA was not pooled. The primer pairs mPgCIR007 and mPgCIR094 showed being informative for selection of individuals with higher vitamin C content, but mPgCIR208 proved to be monomorphic for the feature (Figure 1D).

Based on the amplification patterns, the mPgCIR161 primer pair was finally selected as a marker of white pulp and lower soluble solids content, the mPgCIR136 primer pair was used for individuals with thinner external pulp, and the mPgCIR131 primer pair was selected as a marker of internal pulp thickness.

Improvement genetic in guava based in selection assisted by SSR markers

With the aim of validating the efficiency of the use of SRR primers in genetic improvement programs based on molecular markers, the different alleles amplified by each pair of primers assayed were analyzed. Alleles were classified in groups according to their size and homozygote (a single allele) and heterozygote (two alleles) types were discriminated. The assessed characteristics of commercial importance were internal and external pulp thicknesses, soluble solids, and pulp color.

Marker mPgCIR131 related to internal pulp thickness, showed presence of three amplification patterns in the analyzed guava accessions. Individuals from accessions LS3-A6, LS8-A2, and Selections-4-46-SEL-126 were homozygous with an allele sized at 148 bp, while individuals from other accessions were heterozygous with allele sizes of 141 and 148 bp. In the heterozygous alleles (141 and 148 bp), the calculated selection efficiency was 85%, indicating a value of internal pulp thickness of 3.84 mm. Homozygous alleles (148 bp) were unlinked to pulp thickness, being found in individuals with either thick or thin pulp.

In marker mPgCIR136 associated with external pulp thickness homozygous individuals with 110 or 120 bp alleles and heterozygous individuals with alleles of both sizes were observed. The selection efficiency calculated for the 110 bp allele was 100%, indicating a lower thickness of external pulp. The calculated average for each allelic type of external pulp thickness was 0.72, 1.3 and 0.90 mm for alleles 110, 110/120, and 120, respectively. Significant differences were also found between the homozygous individuals presenting the 110 or 120 bp alleles, suggesting the possibility that the 110 bp allele might be associated to thinner external pulp and that the 120 bp allele could be associated with thicker external pulp, heterozygous individuals having an intermediate value of external pulp thickness (1.3 mm).The marker mPgCIR 161 was associated with two features of agronomic interest, pulp color and soluble solid content. The results indicated homozygous individuals had allelic sizes of either 250 or 263 bp, and heterozygous individuals had alleles sized 246/263bp or 250/263bp. The selection efficiency calculated for the 250/263bp heterozygous allelic combination was 69% for selection of individuals with white pulp and 75% for selection of individuals with lower soluble solid content. Few homozygous or heterozygous individuals presented the alleles sized 250, 263, and 246/263bp, respectively, and they did not share pulp color or percent value of solid content, because of which the selection efficiency of these alleles could not be evaluated.

In the case of white pulp, the selection efficiency was 70%. All individuals homozygous for the 263 bp allele had white pulp, while one half of the homozygous individuals for the 250 bp allele had white pulp, and the remaining 50% of these individuals had pink pulp. Heterozygous individuals with both allelic combinations (246/263bp and 250/263bp) had a 1:2 a proportion of white pulp and pink pulp, suggesting that selection for white pulp might be based on the 263 bp allele (Figure 4A).

For the same mPgCIR-161 marker, the proportion of individuals having low values of soluble solid content appeared to be higher in presence of the 263 bp allele, which allows for concluding that this allele can be used for early selection of low soluble solid content and white pulp (Figure 4B).

The codominant nature of microsatellites facilitates the identification of homozygous and heterozygous individuals in early generations, which in turn allows for obtaining pure lines in the shortest time and discarding heterozygote lines from the beginning of the improvement process; an advantage over the traditional selection process (Zhou et al., 2003; Liu et al., 2007).

According to the information generated here, the SSR markers mPgCIR131, mPgCIR136, and mPgCIR161 could be linked to QTL related to internal and external pulp thickness, pulp color, and soluble solid contents. The variation observed in the selection efficiency values of each SSR allele associated with fruit characteristics was probably due to the different distances between the markers and the QTL (as closer the marker is to the QTL,

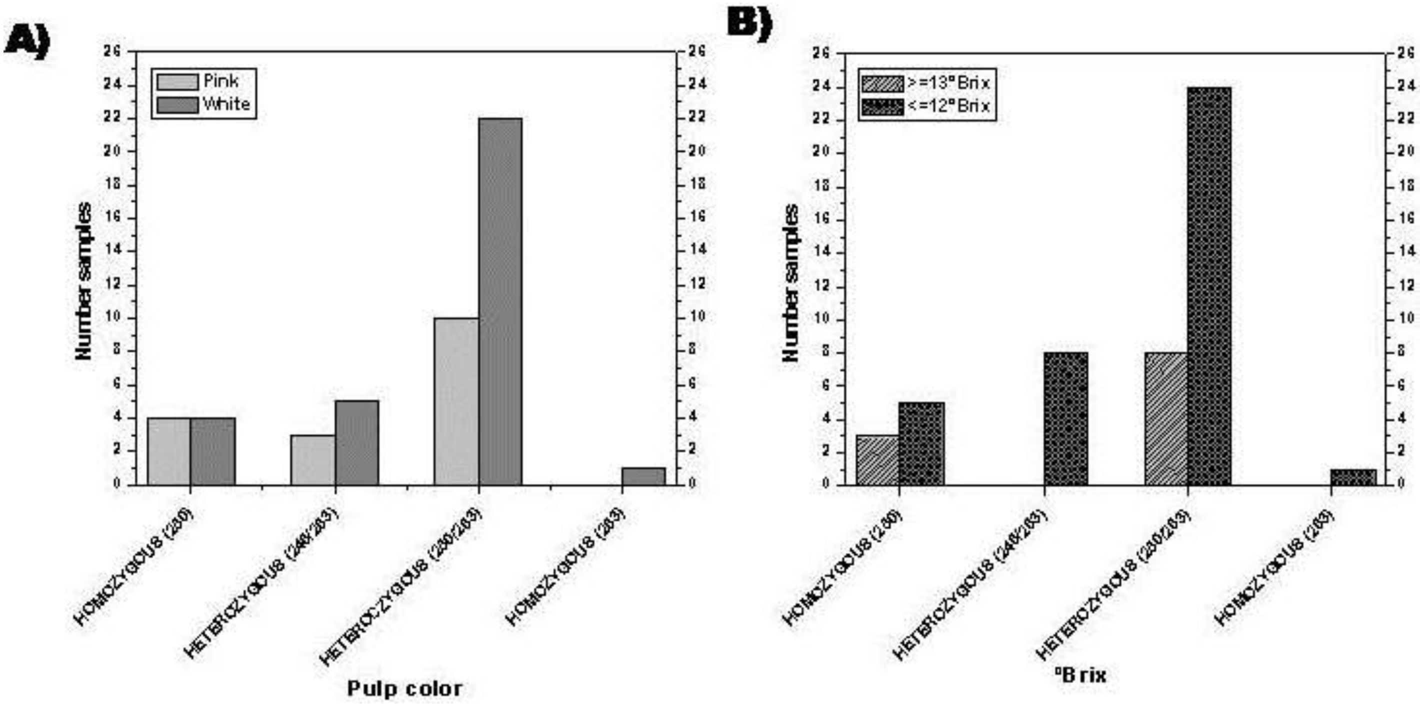

Figure 4. Number of homozygous and heterozygous individuals with different pulp color (A), and number of individuals with different soluble solid contents (°Bx).

the greater will be their association, which would increase efficiency) and to the observed variation in allele size for a same marker linked to a specified fruit property, which may be explained as the effect of the QTL on the feature. If the effect is less likely to be observed, it cannot be known if a given allele is responsible for a particular physiological characteristic (Maliepaard et al., 2001). These results indicated that the mPgCIR131 marker is linked to QTL related to internal pulp thickness and shows 46% of the variance of the character. This marker also presented selection efficiency of 85% and, according to data reported by Ritter et al. (2010), the distance to the QTL is 5.5 cM (centimorgans). It is well known that selection efficiency is closely related to the distance between the marker and the QTL. The recommended distance to QTL for successful selection based on linked markers has been reported to be of nearly 5 cM (Collard and Mackill, 2008).

In addition, the mPgCIR136 marker linked to the QTL associated with external pulp thickness explained 59% of the variance in the character and, also, it had a very high selection efficiency (100%), and was closer (5 cM) to the QTL related to this fruit characteristic (Risterucci et al., 2005; Ritter et al., 2010).

The mPgCIR161 marker related to pulp color and soluble solid content explained 81% of the variance and a selection efficiency of 75% of these characters of interest. The distance between mPgCIR161 and the QTL related to pulp color and soluble solid content was 13.2 cM (Ritter et al., 2010). According to the results obtained, it is possible that the use of the mPgCIR161 marker allows discriminating between individuals with pink and white pulp.

The low selection efficiency reported for the mPgCIR161 marker may have been influenced by genetic changes, such as deletions, insertions, transpositions, and DNA recombination, that may be affecting the association of the marker with the QTL specific to fruit color (Barton and Keightley, 2002; Juenger et al., 2005; Birchler and Veitia, 2010). Butelli et al. (2012) reported in other species that the movement of retroelements in the genome may be associated with change in coloration and that DNA recombination may be affecting the association of the marker with the QTL specific to this character; in some cases, recombination occurs between the QTL and the marker, theoretically, even if the distance between them is small (<5 cM). One centimorgan indicates that the probability of segregating separately is only 1% (Sharp et al., 2001; Thomas, 2003; Collard and Mackill, 2008; Rafalski, 2010).

In Mexico most guava crops were initially obtained through uncontrolled crosses, which increase the probability that genetic changes and recombination events resultant of hybridization of plants can affect the associations between markers and the QTL. By obtaining pure lines it is possible that the selection efficiency of marker associated to specific features will be increased (Anderson et al., 2001); which may be a future target for the improvement of guava.

There are few studies validating the use of markers for selection of fruit quantitative characteristics such as size and shape, softness, texture, and content of sugars. Other crops of commercial importance in which the SSR markers were shown to be associated to quantitative features of interest include papaya (Blas et al., 2011), potato (Li et al., 2013), and apple (Longhi et al., 2013), crops in which the use of these markers in improvement programs has been successful.

Furthermore, early selection would allow for obtaining pure lines with desired fruit characteristics in less time for satisfying the needs of producers, consumers, and the industry (Darwin-Robbins and Staub-Jack, 2009; Li et al., 2013). Currently desired guava phenotypes are white pulp, thick external and internal pulp, and high soluble solid content. The use of the SSR markers analyzed can be used for early selection of guava individuals with specific features and molecular marker assisted breeding can possibly improve this crop (Padmakar et al., 2015; Tuler et al., 2015).

Conclusions

Conventional breeding methods to improve woody species as guava are limited and molecular markers have been useful for characterizing the genetic diversity among different guava cultivars and for identifying genes of commercial interest in the guava species. In this research, PCA considering eight morphological characteristics of Mexican guava indicated that the two first components explain 79% of the morphological variability observed among the accessions. Accessions grouped in five main groups in which pulp color showed not being determinant for discrimination of PCA groups. However, clustering of S-SAP results suggests that the chosen primers hold great potential for their use in selection in guava of crops with specific pulp color, and could be used in early selection of elite material according to the agronomic characteristics of interest. Also, according to the information generated in this study, the SSR markers mPgCIR131, mPgCIR136, and mPgCIR161 could be linked to QTL related to internal and external pulp thickness, pulp color, and soluble solid contents. These results indicate that both molecular markers, S-SAP and SRR could be used in a future for the improvement of guava cultivars.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Variability evaluation of castor seeds (*Ricinus communis*) by multivariate analysis of local accessions from Mexico

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Castor oil is an important raw material used in a diverse range of chemical products with high added value. From a collection of Mexican grown seeds from the castor plant (*Ricinus communis*), 18 accessions with a high potential for oil production and panicle indehiscent were selected and cultivated. The variability was studied from three aspects: seed yield, seed composition and the oil quality, through 22 descriptors measured experimentally. Two hybrid seeds cultivated under the same conditions were used as a control. The variability was studied by descriptive statistics and the conformation of clusters via multivariate techniques. It was found that the average coefficient of variation in the descriptors was 32.5%. The seed and oil yields together with content of minor fatty acids corresponded to the descriptors with the greatest dispersion. The correlation coefficients between the descriptors were low. The clustering methods used were capable of identifying and isolating the various hybrids of the local accessions. The principal components analysis identified a group of 6 local seeds with an average seed yield but high oil content.

Key words: Fatty acids, yield, *Ricinus communis*.

INTRODUCTION

The castor plant belongs to the family of Euphorbiaceae and is developed in tropical and semi-tropical regions (Severino et al., 2012). Given its huge, adaptive capacity it can currently be found practically all over Mexico (Rodríguez and Zamarripa, 2012); however, its cultivation and commercial use has not been developed to the same extent as Brazil, Nigeria, India and China (Salimon et al., 2010). Castor oil has a wide range of uses and is used as a raw material in the synthesis of high added value products such as oleorresines, polymers and synthetic fibres, as well as chemical products such as Undecylenic acid and Hydroxystearic acid (Ogunniyi, 2006; Perdomo et al., 2013; Van der Steen et al., 2011). The main characteristic of castor o is the high content of ricinoleic acid ($C_{18}H_{32}O_3$ (R)-12-Hydroxy-cis-9-octadecenoic acid) that, possessing a hydroxyl group and unsaturation can contribute to reactions to obtain products with a high added value (Yusuf et al., 2015).

In Mexico, during the decade of the 1960's, over 12,000 ha were grown whereas in the year 2000 that had reduced to only 1,800 ha and today there is practically no agricultural production of this plant (FAOSTAT, 2017); Mexico currently imports castor oil. Government authorities promote production of the castor seeds (SAGARPA, 2009), however, the value chain for the utilization of the seed and castor oil has not been consolidated, and there is a need to substantially increase the growth area together with agricultural productivity and the planning of the processing industry and marketing channels.

Deeper knowledge about and an ability to identify those varieties of local castor plants with high oil production potential would make their cultivation attractive. Furthermore, the study on variability of the seeds via their descriptors would enable the most apt accessions to be chosen for their growth and industrial use (Lima et al., 2014). In this last decade, the seed has been grown in a marginal way in Mexico leading to a preference for the introduction of foreign varieties which have raised the farming costs. Nevertheless, there is a diversity of local varieties of the castor plant that have been little studied yet offer an interesting potential and are better adapted to the region. This signifies the importance of evaluating these varieties for the quality of their oil that could be competitive with commercial varieties.

In this work a variability study of 18 local accessions of seeds collected in Mexico from castor plants was carried out through 22 descriptors of their agronomic yield, proximate composition of the seed and the quality of the oil. This study was carried out using descriptive statistics and multivariate analysis to implement clustering algorithms to identify and select the seeds for their attributes and yields.

MATERIALS AND METHODS

The collection of castor seeds used in this study came from a national harvest that was made up of 120 local accessions that was carried out by INIFAP-Bajio (Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias Unidad Bajío) en 2010. The accessions considered in this study came from Guanajuato, a state located in the centre of Mexico, and were selected for their high agricultural yield and oil content. These seeds are safeguarded and preserved in facilities INIFAP-BAJIO.

The seeds were grown on plots located in the Campo Experimental de INIFAP-Bajío under the same agro-climatic conditions (20°34´47” N and 100°49´14” W) and agricultural technologies (Hernández-Martinez et al., 2013). The commercial varieties k75B and k93B were used as controls along with the seeds sown and harvested from varieties known as k75G and k93G. The seeds were stored in hermetically sealed plastic vessels at a temperature of between 4 and 5ºC for their subsequent study (Valero and Díaz, 2014).

Fatty acid profiles

Oil from the seeds was extracted with hexane using a Soxhlet apparatus and underwent transesterification reactions to convert the fatty acids into methyl esters (FAMEs, Kirk et al., 2004). The FAMES were dissolved in heptane to be analyzed by gas chromatography (Perkin Elmer; GC-Clarus 500). An AT-Wax capillary column 30 m x 0.25 mm x 0.5 µm (Alltech Heliflex®) was used. The operational temperatures were 230, 230 and 250°C in an injector, oven and FID detector, respectively. Chromatographic grade N_2 was used as a carrier gas at a pressure of 14 psig with a 15:1 split , the injected sample volume was 1 µL. Analytical standard methyl heptadecanoate, (Sigma) was used as an internal standard.

Chemical analysis of the oils

The following values were determined: acidity (Horwitz, 2002), peroxide (Crowe and White, 2001), iodine (Canesin et al., 2014) and saponification (Canesin et al., 2014). The values were determined in triplicate using the oils extracted from seeds of the castor plant considered in this study as raw materials.

Proximate composition

The proximate composition of the seed samples taken from each accession: humidity (Horwitz, 2002), fixed and volatile solids (sf, sv: Horwitz, 2002), protein (Horwitz, 2002), fat percentage content (Xu, 2007), crude fiber (Horwitz, 2002) and carbohydrates (Lima et al., 2014) were determined. The weight of 100 seeds were also determined (Goytia et al., 2011).

Statistical analysis

The experimental details for 22 selected castor plant seeds were analyzed by descriptive statistics: Mean, standard deviation, maximum and minimum values, range and coefficient of variation. The matrix of correlation coefficients between the descriptor studies was also constructed. The Turkey test (α= 0.5) was carried out to determine the similarity between the measurements of the different descriptors of the accessions of the seeds studied. In order to study variability, multivariate analysis was used, implementing

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Table 1. Average of the variables evaluated in the eighteen local seeds of the castor plant in Mexico and the commercial hybrid varieties.

Accession	Agronomic yield	Oil Yield	Weight of 100 seeds (g)	Fatty Acid Profiles (% w/w)						
				Ricinoleic	Linoleic	Oleic	Stearic	Palmític	Linolenic	Arachidonic
Nomenclature	Re[+] kg seed ha^{-1}	Ra kg oil ha^{-1}	P	Oil1	Oil2	Oil3	Oil4	Oil5	Oil6	Oil7
A553	1,190	660.80	44±0.66^{ab}	82.11	15.15	7.12	1.11	0.44	0.26	0.12
A559	2,120	1,011.66	44±0.66^{ab}	82.19	15.33	3.6	0.88	0.74	0.65	0.16
A1	2,169	984.07	79.98±0.66^{ab}	83.21	16.12	4.64	1.67	1.39	0.23	0.12
A2	967	445.20	48.02±0.66^{ab}	85.65	14.23	3.56	1.87	1.03	0.33	0.23
A3	2,403	1,098.89	78.81±0.66^{ab}	85.83	16.83	4.86	1.32	0.52	0.25	0.25
A4	1,856	886.79	86.83±0.66^{ab}	81.01	15.47	5.23	2.1	1.11	0.69	0.1
A5	1,308	610.96	43.09±0.66^{ab}	86.78	14.66	4.22	1.9	1.01	0.6	0.21
A6	752	363.59	50.78±0.66^{ab}	85.6	15.82	4.95	1.52	1.28	0.5	0.3
A7	572	268.84	31.08±0.66^{ab}	88.43	14.94	4.11	1.73	1.08	0.22	0.21
A8	780	379.002	21.13±0.66^{b}	85.32	15.98	4.3	1.06	1	0.32	0.2
A9	1,248	599.91	40.94±0.66^{ab}	88.74	14.59	3.65	1.32	1.03	0.39	0.24
A10	452	215.87	33.42±0.66^{ab}	89.06	14.45	3.66	1	0.93	0.44	0.42
A11	969	450.77	36.65±0.66^{ab}	84.31	15.48	5.3	2.69	1.09	0.32	0.12
A12	670	319.38	27.74±0.66^{ab}	81.23	15.84	6.31	1.19	0.86	0.51	0.32
A13	2,023	980.54	31.76±0.66^{ab}	85.78	15.4	4.5	1.13	1.06	0.53	0.39
A14	1,196	554.22	38.65±0.66^{ab}	87.06	15.1	4.57	1.09	1.07	0.57	0.51
A15	2,023	945.95	91.65±0.66^{a}	89.35	14.81	3.3	0.84	0.8	0.53	0.33
A16	1,196	559.8476	86.95±0.66^{a}	82.13	15.89	3.5	1.4	1	0.98	0.25
k75G*	2,200	1,347.621	39.90±0.66^{ab}	60.676	17.582	11.151	4.145	3.495	1.417	1.533
k93G*	2,350	1,361.2472	39.80±0.66^{ab}	67.180	14.253	9.998	3.385	3.034	0.961	1.187
k75B**	3,201$^{\Delta}$	1,145.1	32.7±0.66^{ab}	81.98	15.9	4.2	1.12	0.99	0.43	0.31
k93B**	3,352$^{\Delta}$	1,171.11	30.2±0.66^{ab}	82.9	15.44	5.98	0.9	0.77	0.65	0.18
Average	1,590.77	743.70	48.09	83.02	15.42	5.12	1.61	1.17	0.54	0.35
Minimum	452.00	215.88	21.13	60.68	14.23	3.30	0.84	0.44	0.22	0.10
Maximum	3,352.00	1,361.25	91.65	89.35	17.58	11.15	4.15	3.50	1.42	1.53
Range	2,900.00	1,145.37	70.52	28.67	3.35	7.85	3.31	3.06	1.20	1.43
Standard deviation (DS)	826.62	359.53	21.61	6.78	0.82	2.02	0.84	0.71	0.29	0.35
Coefficient variation (CV %)	51.96	48.34	44.94	8.17	5.30	39.43	52.30	61.11	53.77	99.34

Accession	Proximal (% Mean±Standard deviation)							Chemical Properties (Mean± Standard deviation)				
	Ashes	Volatile solids	Fat content	Protein	Humidity	Fiber	Carbohydrates	Oil ashes (%)	Acidity value	Peroxide value	Saponification value	Iodine value
Nomenclature	sf	sv	PA	Pro	H	Fi	CH	Cea	IA (%)	IP (meq O_2/kg)	IS (mg KOH/g)	IY (g I_2/100 g)
A553	3.37±0.42^{efg}	91.55±1.16^{a}	55.53+0.26^{a}	25.37±0.64^{def}	4.01±0.01^{bc}	14.49±0.54^{ij}	11.68±0.05^{h}	0.030	1.20±0.00^{a}	1.02±0.72^{ef}	110.26±27.8^{a}	84.66±0.57^{c}
A559	4.74±0.05^{cde}	91.15±0.77^{a}	47.72+0.95^{cdef}	27.18±0.03^{cd}	3.48±0.42^{c}	17.48±0.56^{cde}	19.05±0.04^{d}	0.040	1.26±0.05^{a}	1.13±0.56^{def}	131.72±1.55^{a}	78.45±1.05^{c}
A1	5.71±0.84^{abc}	90.85±0.03^{a}	45.37+1.28g	27.12±0.84^{cd}	7.26±0.15^{a}	16.67±1.61^{efgh}	16.87±0.65^{ef}	0.011	1.23±0.05^{a}	1.08±0.77^{cdef}	128.72±5.67^{a}	84.13±3.9^{c}
A2	3.07±0.07^{g}	92.58±0.83^{a}	46.04+2.68fg	25.57±2.64^{def}	4.5±0.5^{bc}	17.05±2.68^{defg}	20.96±0.58^{c}	0.002	1.23±0.05^{a}	1.25±1.10^{bcdef}	131.9±11.05^{a}	85.96±5^{bc}
A3	5.27±0.65^{bc}	91.01±0.67^{a}	45.73+0.98g	24.69±0.17^{fg}	5.05±0.31^{bc}	17.56±3.26^{cde}	12.82±0.71^{h}	0.050	1.13±0.11^{a}	1.28±0.56^{abcdef}	135.32±4.25^{a}	87.8±0.67^{bc}
A4	3.51±0.04^{defg}	93.12±0.02^{a}	47.78+0.5^{cdef}	27.03±2.07^{cd}	5.28±0.25^{bc}	15.13±2.8^{hij}	16.27±0.06^{fg}	0.003	1.20±0.1^{a}	2.24±1.07^{abc}	131.33±2.18^{a}	78.33±0.96^{c}
A5	4.89±0.07^{cd}	90.66±1^{a}	46.71±0.6^{cdefg}	25.74±1.74^{def}	5.73±0.58^{ab}	13.47±1.79^{j}	18.65±1.01^{de}	0.050	1.06±0.05^{a}	2.21±0.80^{a}	120.30±10.16^{a}	79.08±0.46^{c}
A6	4.91±0.02^{cd}	91.30±1.13^{a}	48.35±0.82^{cd}	26.15±0.66^{def}	5.16±1.04^{bc}	15.33±4.48^{ghij}	16.13±0.28^{fg}	0.011	1.1±0.1^{a}	1.27±0.21^{abcdef}	133.2±0.38^{a}	78.42±0.38^{c}
A7	4.63±0.2^{cdef}	92.94±0.60^{a}	47±1.57^{cdefg}	22.86±1.46^{gh}	5.63±2.17^{ab}	18.9±1.45^{bcd}	21.43±0.92^{c}	0.020	1.46±0.5^{a}	2.30±0.55^{a}	132.87±0.33^{a}	76.9±0.56^{c}
A8	5.10±0.1^{bc}	94.58±0.05^{a}	48.59±0.14^{c}	28.63±4.96^{bc}	4.63±0.5^{bc}	17.23±5.62^{def}	14.78±0.39^{g}	0.010	1.46±0.51^{a}	2.11±1.6^{abcd}	126.25±0.35^{a}	77.51±1.08^{c}
A9	5.06±0.48^{bc}	96.38±1.72^{a}	48.07±0.63^{cde}	31.04±0.05^{a}	5.5±1.88^{ab}	16.93±6.70^{efgh}	11.29±0.17^{h}	0.014	1.1±0.1^{a}	1.81±0.42^{abcdef}	103.37±0.58^{a}	78.29±0.54^{c}
A10	5.00±0.09^{bc}	96.53±1.03^{a}	47.76±0.14^{cdef}	24.95±0.35^{ef}	5.46±0.64^{ab}	17.67±9.21^{cde}	18.72±0.38^{de}	0.015	1.43±0.37^{a}	1.98±0.3^{abcdef}	114.48±0.81^{a}	75.30±0.81^{c}
A11	5.03±0.47^{bc}	96.39±0.81^{a}	46.52±2.73^{defg}	26.62±0.45^{de}	5.30±0.44^{bc}	14.53±3.05^{ij}	16.25±0.5^{fg}	0.003	1.33±0.15^{a}	2.27±0.57^{abcdef}	113.96±0.03^{a}	75.91±1.52^{c}
A12	4.42±0.32^{cdefg}	95.64±1.06^{a}	47.67±0.33^{cdef}	28.62±0.31^{bc}	4.36±0.47^{bc}	15.83±3.78^{efghi}	16.25±0.57^{fg}	0.020	1.16±0.2^{a}	2.10±0.20^{a}	114.31±0.31^{a}	77.27±0.28^{c}
A13	3.21±0.01^{fg}	94.18±0.55^{a}	48.47±0.22^{c}	29.92±6.01^{ab}	4.6±0.69^{bc}	19.33±2.30^{bc}	12.87±0.06^{h}	0.030	1.36±0.28^{a}	2.30±1.00^{abc}	129.31±5.45^{a}	100.42±20.04^{abc}
A14	7.01±0.01^{a}	92.83±9.16^{a}	46.34±0.36^{efg}	29.52±0.12^{ab}	4.83±0.83^{bc}	19.9±5.23^{b}	15.66±0.05^{fg}	0.002	1.10±0.1^{a}	2.00±1.00^{ab}	134.04±1.02^{a}	110.46±10.02^{ab}
A15	4.33±0.07^{cdefg}	98.22±1.15^{a}	46.76±1.09^{cdefg}	27.26±0.46^{cd}	4.67±0.54^{bc}	21.9±5.23^{a}	16.12±0.07^{fg}	0.001	1.16±0.15^{a}	2.23±0.57^{abcde}	134.55±14.2^{a}	90.4±10.02^{abc}
A16	5.76±1.36^{abc}	89.13±0.52^{a}	46.81±1.41^{cdefg}	22.47±1.33^{h}	5.06±0.3^{bc}	16.88±0.59^{efgh}	25.73±0.17^{b}	0.013	1.30±0.17^{a}	2.20±1.9^{abc}	136.75±14.2^{a}	113.78±5.78^{a}
k75G*	5.38±0.56^{bc}	98.40±1.04^{a}	42.1±1.01^{h}	20.10±0.16^{ij}	4.66±0.12^{bc}	15.22±1.13^{ghij}	30.02±0.47^{a}	0.010	1.03±0.05^{a}	1.01±0.05^{f}	134.42±14.2^{a}	91.03±0.05^{abc}
k93G*	5.21±0.17^{bc}	97.53±0.06^{a}	40.61±2.20^{h}	21.21±1.01^{hi}	4.17±0.21^{bc}	15.56±1.54^{fghi}	29.31±0.31^{a}	0.012	1.4±0.6^{a}	1.01±0.60^{f}	136.62±14.2^{a}	91.4±0.60^{abc}
k75B**	5.28±017^{bc}	96.74±2.22^{a}	52.05±0.23^{b}	19.17±0.04^{j}	5.56±0.23^{ab}	15.15±1.77^{hij}	20.19±0.69^{cd}	0.010	1.2±0.17^{a}	2.20±1.95^{abcdef}	135.17±8.68^{a}	110.774±19.54^{ab}
k93B**	6.40±0.87^{ab}	97.88±1.99^{a}	49.86±0.10^{ij}	19.86±0.10^{ij}	5.26±0.30^{bc}	16.66±1.37^{efgh}	21.57±0.25^{c}	0.013	1.7±0.3^{a}	1.01±0.3^{f}	133.91±1.02^{a}	94.9±5.55^{abc}
Average	4.88	94.07	47.36	25.50	5.01	16.77	18.30	0.02	1.25	1.73	127.40	87.33
Minimum	3.07	89.13	40.61	19.17	3.48	13.47	11.29	0.00	1.03	1.01	103.37	75.30
Maximum	7.01	98.40	55.53	31.04	7.26	21.90	30.02	0.05	1.70	2.30	136.75	113.78
Range	3.94	9.27	14.92	11.87	3.78	8.43	18.73	0.05	0.67	1.29	33.38	38.48
Standard deviation (DS)	0.97	2.87	2.96	3.35	0.76	1.98	5.08	0.01	0.16	5.36	9.87	12.04
Coefficient variation (CV %)	19.97	3.05	6.26	13.12	15.26	11.84	27.75	86.55	12.98	3.09	7.75	13.78

*Commercial hybrid varieties under study conditions, ** Commercial hybrid varieties (initial reference), Δ Yields based on seed datasheet in kg ha^{-1}.

clustering algorithms. Two methodologies were used: the Un-weighted Pair Group Method with Arithmetic Mean (UPGMA), to represent the categorization of accessions via a dendrogram (Cuadra, 2016) and the Principal Component Analysis, PCA methodology to describe and explain the variability between the characteristics of the seeds using the JMP Software (JMP-Statistical Discovery Software from SAS) ver. 5.0.1 as a statistical tool.

RESULTS

Table 1 shows the characterization of the different accessions of the seeds studied, indicating the average value, standard variation, maximum and minimum values, and the range and coefficient of variation of each of the descriptors that were experimentally measured. The Turkey tests of means are indicated by superscripts; same letters indicate means with no significant statistical difference (α= 0.5), the variables of volatile solids, acidity values and iodine values show similar means in all the accessions. Figure 1 shows the variation coefficients (CV) of each one of the descriptors studied. As can be seen, the amount of araquidonic acid (Oil7) and the ashes content in the oil (Cea) are the variables that exhibit the greatest variability among the accessions. The agronomic yields of the seed (Re) and the oil (Ra) are also sources of important variability. The variables that describe the proximal seed composition and the chemical properties of the oils show lower CV.

One of the variables of greatest interest is the yield of the castor plant seed (Re). The average world yield is 1.1 t ha^{-1}; under favorable conditions yields of up to 4-5 t ha^{-1} have been reported (Scholz and da Silva, 2008). In the results obtained in this work, the values reported in the technical datasheets of the hybrid seeds are the

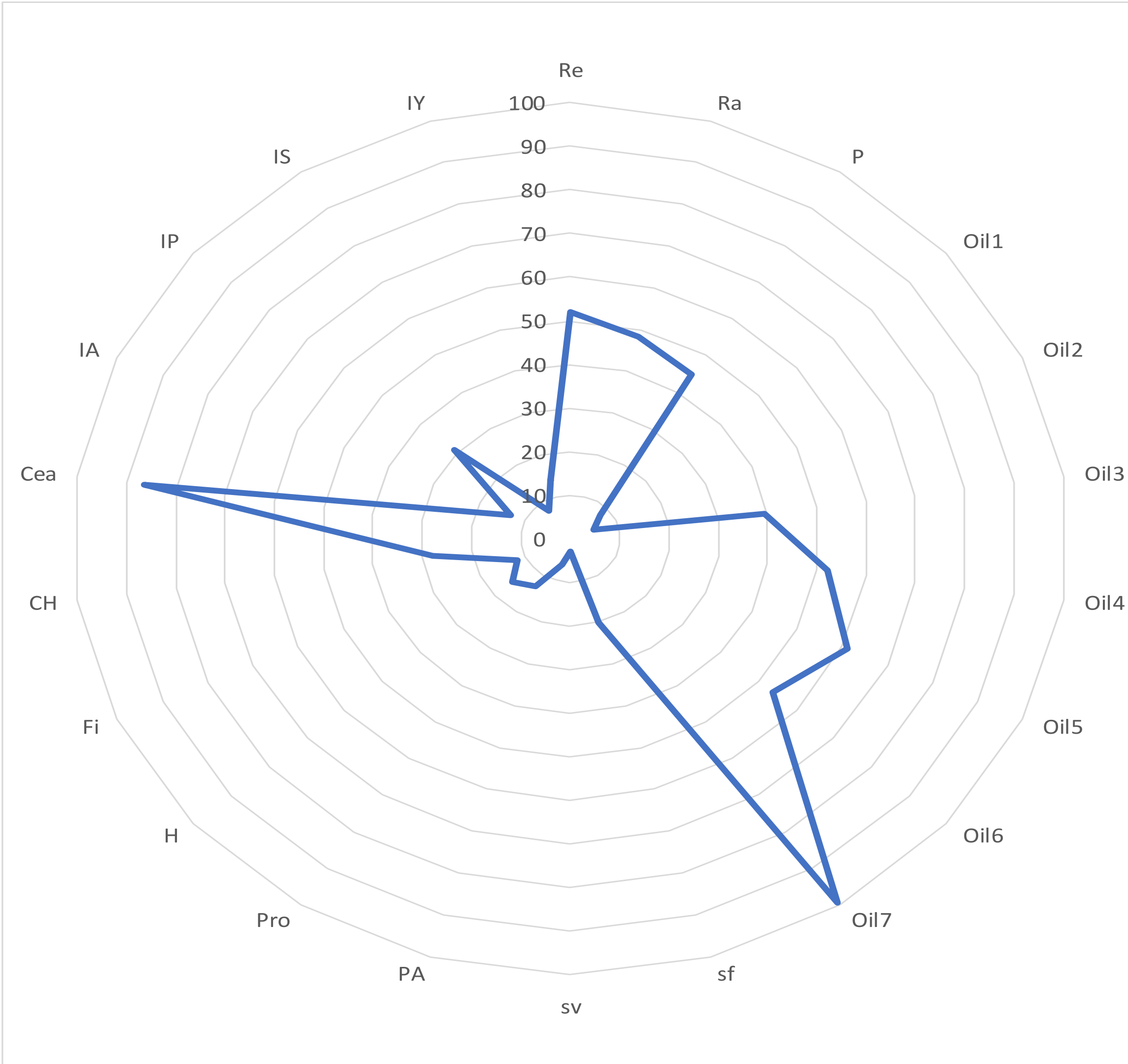

Figure 1. Variation coefficients of the descriptors of the castor plant seed accessions studied.

greatest (K75B: 3,201 kg ha^{-1} and K93B: 3,352 kg ha^{-1}). However, in agricultural testing in Mexico these reduced to (K75G: 2,200 kg ha^{-1} and K93G 2,350 kg ha^{-1}). Local accessions with attractive yields such as A3 with 2,402 kg ha^{-1} were found.

In a collection of castor plant seeds in the United States, the weight of 100 seeds (P) between 10.1g and 73.3 g with an average of 28.3 g were determined (Wang et al., 2010). A collection of seeds in Chiapas State, southeastern Mexico has a P of between 7 and 123.9 g with an average of 48.72 g (Goytia-Jiménez et al., 2011). In this study, that varies between 21.13 and 91.65 g with an average of 48.55 g. In landrace collections, seed oil contents, PA, of 40–55% have been reported (Wang et al., 2010), in selected seeds, 54.47% (Chen et al., 2016) and hybrid seeds, 45.5-52.1% (Alexopoulou et al., 2015). The accessions studied in this work, showed a content range of 40.51-55.53% and an average of 47.47%. Local variety A553 is the highest content.

Acidity value (IA) based on the ricinoleic acid of the wild varieties A7 and A8 showed the highest values (1.46%), while variety A5 showed the lowest, 1.06%. Commercial seed K93B exhibited an index greater than 1.7%. Wesoøowski and ErecinÂska (1998) reported an IA of 1.59% (oleic acid) in rapeseed oil. The oils of local varieties A1, A12, A2, A3, A553, A559 and A6, as well as hybrids k75G, k93B and k93G have peroxide value (IP) of around 10 meq O_2 kg^{-1}, indicating greater chemical stability of the oil to oxidation reactions that give rise to rancidity.

A14 and A16 exhibited the highest iodine value (IY); 110.46 and 113.78 g I_2/100 g respectively. Vegetable oils generally have an IY between 30 and 60 g I_2/100 g (Meshram et al., 2013). Akpan et al. (2006) and Yusuf et al. (2015) reported that IY values between 81 and 91g I_2/100 g, similar to the results presented in this work.

Table 2. Correlation coefficients among the descriptors of the seeds and oil of the castor plant.

	Re	P	H	Fi	Pro	sf	sv	PA	CH	IA	IP	IY	IS	Cea	ra	oil1	oil2	oil3	oil4	oil5	oil6	oil7
Re	1.000																					
P	0.192	1.000																				
H	0.045	0.213	1.000																			
Fi	-0.017	0.187	-0.104	1.000																		
Pro	-0.485	0.045	-0.054	0.318	1.000																	
sf	0.135	0.465	-0.005	0.289	-0.030	1.000																
sv	-0.432	0.038	0.023	-0.104	0.627*	-0.289	1.000															
PA	0.048	-0.198	-0.043	-0.102	0.037	-0.136	-0.159	1.000														
CH	0.236	-0.045	-0.086	-0.174	-0.746*	0.099	-0.497	-0.532*	1.000													
IA	0.117	-0.332	0.005	0.159	-0.284	0.100	-0.387	0.219	0.177	1.000												
IP	-0.352	-0.034	0.185	0.238	0.313	0.123	0.237	0.092	-0.251	0.003	1.000											
IY	0.473	0.140	-0.044	0.251	-0.338	0.422	-0.497	0.048	0.302	-0.014	0.072	1.000										
IS	0.486	0.334	-0.057	0.302	-0.476	0.239	-0.347	-0.326	0.524*	0.113	-0.157	0.532*	1.000									
Cea	0.088	-0.040	-0.146	-0.177	0.038	-0.257	0.390	0.113	-0.223	-0.136	-0.132	-0.172	-0.129	1.000								
ra	0.860*	0.238	-0.104	-0.048	-0.446	0.187	-0.295	-0.356	0.436	-0.050	-0.481	0.362	0.497	0.091	1.000							
oil1	0.895*	0.240	0.041	-0.012	-0.375	0.201	-0.375	0.316	-0.027	0.059	-0.339	0.466	0.289	0.128	0.720*	1.000						
oil2	0.867*	0.161	-0.034	-0.013	-0.429	0.152	-0.401	0.400	-0.026	0.060	-0.313	0.508*	0.303	0.188	0.656*	0.973*	1.000					
oil3	0.791*	-0.012	0.031	-0.252	-0.505*	0.035	-0.435	0.495	0.007	0.161	-0.314	0.432	0.178	0.076	0.551*	0.905*	0.904*	1.000				
oil4	0.782*	0.182	0.353	-0.317	-0.437	-0.004	-0.276	0.378	-0.018	0.092	-0.137	0.355	0.213	-0.009	0.491	0.826*	0.804*	0.863*	1.000			
oil5	0.563*	0.465	0.392	0.025	0.043	0.180	0.035	-0.186	-0.007	-0.263	-0.112	0.317	0.280	-0.108	0.612*	0.570*	0.439	0.363	0.528*	1.000		
oil6	0.777*	0.284	-0.133	-0.031	-0.400	0.369	-0.401	0.115	0.279	-0.007	0.032	0.657*	0.470	-0.024	0.664*	0.709*	0.693*	0.653*	0.622*	0.495	1.000	
oil7	0.722*	-0.080	0.045	0.139	-0.402	0.082	-0.443	0.289	0.067	-0.026	0.063	0.696*	0.366	-0.043	0.466	0.708*	0.767*	0.719*	0.643*	0.340	0.699*	1.000

Comparing with local varieties for IS, accession A14 was the highest (110.46 mg KOH g^{-1}) and A9 (103.37 mg KOH g^{-1}) the lowest. Amongst the hybrids, k93B (133.91 mg KOH g^{-1}) exhibited a smaller IS than k93G (136.62 mg KOH g^{-1}). Variety k93B, being a commercial variety, had been harvested for a longer time than the k93G; however data presented in this work do not coincide with those reported by Wesoøowski and Erecin Âska (1998), given that they reported values of 173 and 191.3 mg KOH g^{-1}; the first in castor plant oil and the second in mangrove oil whereas lower values were found in this work.

Table 2 shows the correlation coefficients among the variables that describe the seeds studied. It can be observed that the majority of coefficients differed from the unit, indicating that the variables are mainly independent of each other. The castor seed (Re) and oil yield (ra) possess a correlation coefficient of 0.860, which, if all the seeds had the same oil content (PA) this coefficient would approach unity. It is for this reason that the oil content (PA) has a low correlation with respect to Re and ra. Coefficients with low variation imply problems in the description of variability of the castor plant accessions in function of their original descriptors.

Clustering analysis

Clustering is based on dissimilitude analysis of objects that make up a population, seeking to group objects with more homogenous properties. Dissimilitude is measured mainly by the difference in the attributes of the objects, in this case,

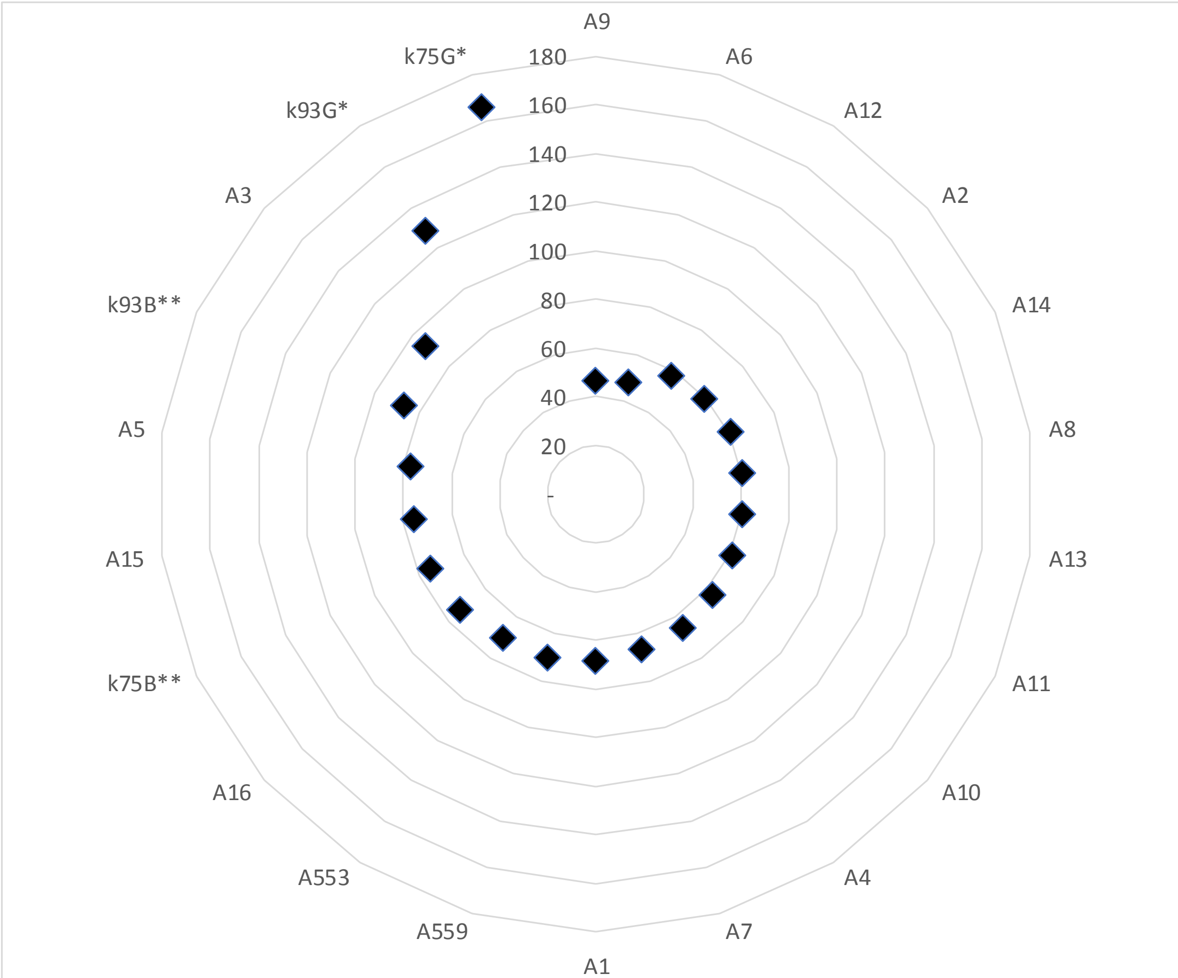

Figure 2. Euclidean distance with respect to the averages of the normalized descriptors for the accessions of the castor plant.

euclidean distance. Figure 2 shows the modules of the distance vectors for each accession in an ordered way. The variables were normalized with respect to their maximum value, in percentage units. The components of each distance vector, as the difference between the measured value and the average of each variable studied were calculated. It can be clearly observed that the commercially grown hybrid varieties k93G and k75G show an important distancing from the rest of the seeds. The majority of the local accessions are found between polygons 40 and 80. Some local seeds and the commercial varieties k75B and k93B are found between polygons 80 and 100.

The results of the clustering algorithm using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) methodology are shown by a dendogram in Figure 3. In this method, the distance vectors are conformed by the differences in descriptors between two accessions; the pairs of closest accessions are associated in a cluster with average properties. In the next stage of clustering the new distances are calculated and new clusters are created. It is interesting to observe that the original hybrid seeds and the cultivated hybrids grouped in two different clusters separate from the local seeds. The seeds with the greatest differences are the local varieties A1 and A553 which make up unitary clusters. The local vanity A553 is associated with the great majority of the local varieties has the average attributes similar to the set of seeds involved in this category.

The seed accessions show high dispersion in their descriptors, the average CV was 32.45% and the correlation coefficients among variables, were in general, low. PCA found that 11 Principal Components are necessary to represent 95% of the total variation. For the variability study of the seeds, components PC1 and PC2 were used representing 33.4% and 13.6% of the total variation respectively. Table 3 shows the components of each eigenvector. These coefficients are used to transform the values of the characteristics of each seed into one single value for each main component. In this

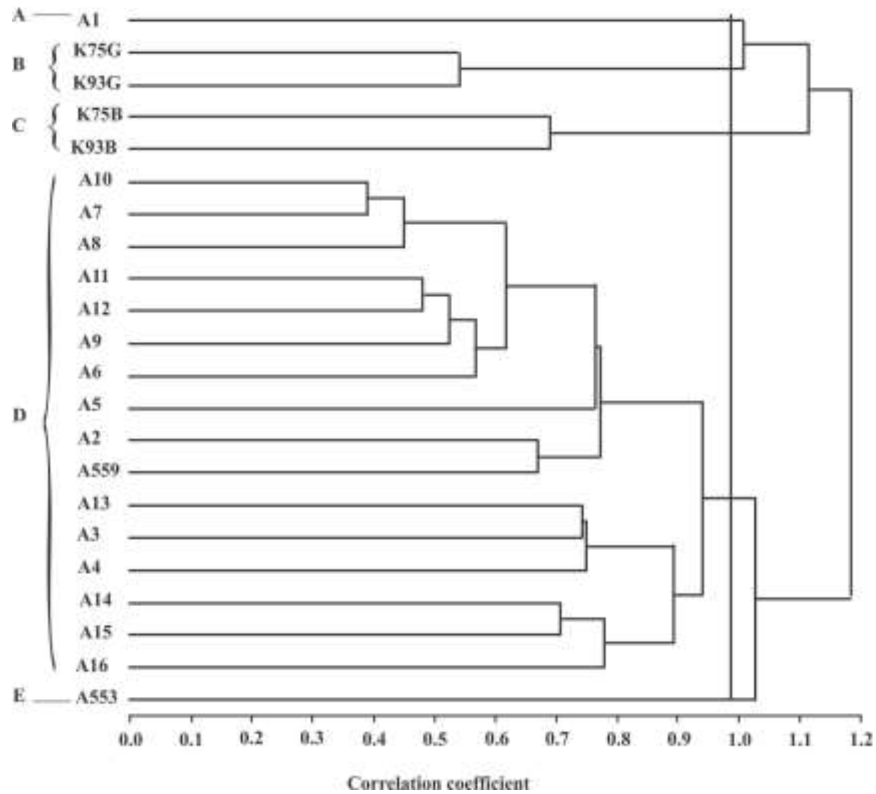

Figure 3. Dendogram obtained from clustering analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on euclidic distances.

Table 3. Principal components analysis of the correlation coefficient matrix.

Variable	PC1	PC2
Agronomic Yield (g ha^{-1})	0.334	0.077
Weight of 100 seeds (g)	0.086	0.065
Humidity (%)	-0.168	0.380
Fiber (%)	0.183	-0.165
Protein (%)	0.015	0.088
Fixed solids (%)	-0.046	0.066
Volatile solids (%)	-0.227	0.231
Fat content (%)	0.078	-0.007
Carbohydrates (%)	-0.192	0.143
Acidity value (%)	0.010	0.414
Peroxide value (meq O_2 kg^{-1})	0.136	-0.470
Iodine value (g I_2/100 g);	0.030	-0.042
Saponification value (mg KOH g^{-1})	-0.174	0.197
Ashes in oil (%)	0.201	0.022
Oil Yield (g ha^{-1})	0.186	-0.189
Ricinoleic Acid	0.311	-0.110
Linoleic Acid	0.299	-0.006
Oleic Acid	0.324	0.229
Stearic Acid	0.312	0.241
Palmitic Acid	0.300	0.219
Linolenic Acid	0.276	0.243
Arachidonic Acid	0.207	0.116

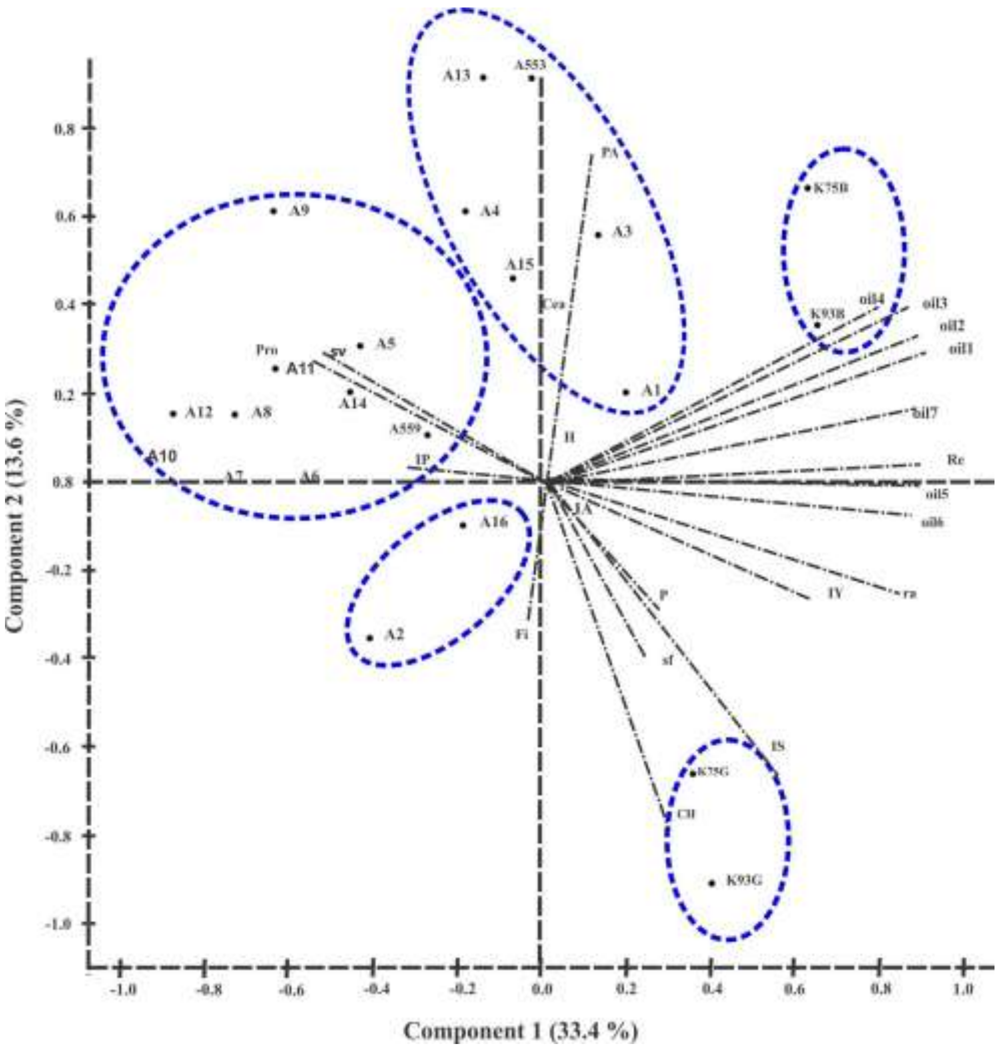

Figure 4. Distribution and grouping of local and commercial hybrid seeds of *Ricinus communis* as a function of the main components of their descriptors.

way, each seed was represented in function of components PC1 and PC2 in Figure 4; this graph, known as biplot, also shows the correlation between the original variables with each main component using dotted lines.

As can be seen, the highest amount of correlation lines coincides around the positive axis of PC1. The variables with the highest positive correlation with PC1 are Re, ra and oil1-oil7. The seed accessions from PC1 are ordered from lowest to highest, left to right, in accordance principally with their agronomic yield of oil and seed. In contrast, PC2 exhibits a high positive correlation with PA and a high negative correlation with CH. It can be seen in Figure 4 that the accessions with the greatest seed and oil yield are k93B and k75B whereas the local accession A10 is the lowest. Moreover, accessions A13 and A553 produce seeds with the highest amount of oil and hybrids k75G and k93G, commercially grown in Mexico, show the lowest.

A cluster analysis of the seed accessions, based on their representation in function of PC1 and PC2 was carried out. The resulting clusters are represented in Figure 4 as ellipses. In Figures 3 and 4 the two methods of multi-variant analysis groupings were able to discriminate and separate the accessions of commercial hybrid seeds from the local accessions. The local seeds in both methods group together in different heterogenic groups. In particular, A1 and A553 were unique and distant from other accessions using UPGMA, these accessions may be desirable parents for cross breeding in castor plant. In the dendogram, A5 had been identified associating the average properties of a group of local accessions. However, in the PCA, A5 is again found located close to the centroid of the clustering on the second quadrant between PC1 and PC2. Accessions A6, A7, A8, A9, A10, A11 and A12 can again be observed in this cluster. Both grouping techniques show that the accessions of castor plant seeds exhibit high dispersion in their descriptors.

Conclusions

In this article, 22 accessions, 18 local seeds from a national collection in Mexico and 4 hybrid seeds from *Ricinus communis* were evaluated from three aspects: agronomic yield, seed composition and oil quality, evaluating 22 descriptors. In the descriptive statistical analysis, high variability was found; the variation coefficients of the descriptors ranged from 3.05 to 99.34% with an average of 32.45%. This explains why the grouping techniques form dissimilar sets of sedes. The two clustering algorithms used were able to identify and separate the commercial hybrid accessions from the local wild varieties. From both clustering methodologies, it was possible to identify 5 groups; the hybrid varieties were located in 2 identical groups. The UPGMA identified two notable accessions of local seeds, A1 and A553, whose properties situate them as extreme opposites of all the seeds studied.

Castor seeds were described and classified by PCA methodology, in two major components, PC1, correlated to crop yields of oil and seed and PC2, correlated to the oil content and seed composition. The hybrid seeds exhibit high crop yields but oil content decreases, a group of local accessions with medium agronomic yields and high seed oil content was identified, 1944 kg ha^{-1} and 48.27%: A1, A3, A4, A13, A15 y A553.

This methodology can be extended to a greater number of castor seed accessions to obtain groups with a higher number of individuals and more defined, homogeous characteristics. This analysis technique can be extended to correlate seed characteristics to their agricultural performance such as germination, adaptation to climatic conditions and resistence to pathogens.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Response of *Eleusine indica* to herbicides and N fertilizer in dry seeded rice

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Weed has become one of the most serious problems in dry seeded rice (DSR). To increase the competitiveness with weeds and achieve the optimum growth of rice, weed management in DSR needs an integration of herbicides and optimum nitrogen (N) fertilizer rates. In this study, the bioassay species were treated with a series of pre-emergence (pretilachlor and pendimethalin) and early post-emergence (propanil+thiobencarb) herbicide concentrations and N fertilizer. It was found that pretilachlor at an application rate of 0.25 and 0.50 kg ai ha^{-1} almost completely inhibit the emergence and shoot growth of *E. indica* by 100% inhibition, at 50, 100 and 150 kg ha^{-1} N, respectively, but it showed severe injury symptom to the root of rice seedlings. Meanwhile, pendimethalin and N fertilizer at moderate application rate gave a strong reduction of weed emergence (>50% inhibition) and shoot fresh weight (>85% inhibition), with negligible effect on the rice seedlings growth. Significant inhibitory effects on weed emergence (60% inhibition) and shoot growth (90% inhibition) also were noticed at the highest application rate of propanil + thiobencarb (3.6 kg ai ha^{-1}) and N fertilizer (150 kg ha^{-1} N), with stimulation effects on the growth of rice seedlings. This promising effect showed combination of propanil + thiobencarb and N fertilizer (3.60 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N) was the most efficient treatment for *E. indica* control in dry seeded rice.

Key words: *Eleusine indica*, herbicides, N fertilizer, dry seeded rice.

INTRODUCTION

The dry seeding (DSR) technique of rice cultivation is becoming popular to farmers and growers in many parts of Asia. DSR is primarily practiced as a response to the labour shortage, and is currently practiced in Malaysia, Thailand, Vietnam, the Philippines, and Sri Lanka (Nirmala et al., 2016). However, weeds become the major problems to the sustainability of DSR where it had caused high yield losses in rice production (Ahmed et al., 2015). It was found that estimated losses in rice yields from weeds are up to 90% in DSR systems (Mahajan et al., 2014). The major weeds in direct seeded rice were grasses, followed by broad leaved weeds and sedges

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(Singh et al., 2016). A wider range of weeds, especially those of grasses, are found infesting direct-seeded fields with *Eleusine indica* being among dominant species.

Weed infestation in DSR depends on several factors such as weed seed bank, tillage system, cultivar used, fertilizer, and water management (Mahajan and Chauhan, 2013). Under such circumstances, weed management in DSR is very crucial. Various means can be used to reduce weed infestations, which include mechanical, chemical, and biological control. However, the use of herbicide is considered the best tool to manage weeds in rice field since it is a very effective, reliable and inexpensive method compared to hand weeding (Rashid et al., 2012). Although using herbicides is an economical weed control method, reliance on herbicides alone is not sustainable in achieving season-long weed-free crop (Chauhan and Opena, 2013). It was found that the application of pre and post-emergence herbicides was not enough to achieve optimum weed control in DSR (Chauhan et al., 2015). Therefore, an alternative weed control method is needed. One of the alternative strategies might be through banded application of herbicide and N fertilizer to increase the competitive ability of crops over the weeds.

Nitrogen (N) plays a significant role in crop-weed competition (Unruh, 2013). Previous studies found that the addition of N fertilizer reduces the competitive advantage of the crop while in other cases enhances the growth of weeds (Tosti et al., 2016). The response of crop and weed to N level depends on several factors such as species and density of the crop or weed (Mick Assani, 2016). Studies also reported that a N fertilizer application is able to break weed seed dormancy and influence weed structure and densities (Nichols et al., 2015). According to a study conducted by Nader et al. (2012), N has been used as carrier for enhancing herbicide efficacy, however, N function was not fully understood and was thought to enhance herbicide absorption by the target species. Therefore, this study was conducted to assess the herbicidal activity of pre and early post-emergence herbicides with N fertilizer on *E. indica* and to determine tolerance levels of local rice variety to herbicides and N application.

MATERIALS AND METHODS

Plant materials

Seeds of the bioassay species, *Eleusine indica* were collected from rice fields of Pasir Mas, Kelantan, Malaysia (6.07704°N, 102.2384°E), and propagated in a glasshouse; meanwhile local rice seeds (MR263) were provided by Kelantan Agricultural Development Authority (KADA), Kelantan, Malaysia.

Chemicals

Commercial herbicides were purchased from Agricultural Chemicals (M) Sdn. Bhd. (ACM), Penang, Malaysia, while the N fertilizer was purchased from Golden Rengat Enterprise, Temerloh Pahang.

Soil bioassay

Herbicidal activity of pre and early post-emergence herbicides with N fertilizer was carried out by using the MR263 local rice variety and *E. indica* as the bioassay species. Moist silty loam soil was filled into a plastic pot (8 cm diameter by 9 cm height) with holes at the bottom. Through the direct seeding method, the rice seed was covered with soil immediately after sowing (Zheng et al., 2016). The pots were placed in an 80- by 60- by 5-cm tray and water was applied from the bottom of pots for proper growth of rice seedlings. The trays were immediately placed in a glasshouse and maintained at relative humidity of 75 to 80% and a temperature range of 25 to 30°C, with a 12-h photoperiod. The herbicides and N fertilizer were prepared in three application rates; pretilachlor (0.125, 0.25 and 0.50 kg ai ha^{-1}), pendimethalin (0.25, 0.50 and 1.00 kg ai ha^{-1}) propanil + thiobencarb (0.9, 1.8 and 3.6 kg ai ha^{-1}) and N fertilizer (50, 100 and 150 kg ha^{-1}). N fertilizer at each application rate was applied onto the soil surface with different growth stage of rice seedlings in the pots to provide different level of N in the soil. Then, 30 seeds of *E. indica* were sown on the soil surface when the rice seedlings were at the growth stages of 0, 10 and 15 days after being sowed in the soil. After one day, herbicides with different rates were applied onto the soil surface with a micropipette. Non-treated rice plant and *E. indica* seeds were used as control treatments.

Observations

The number of emerged *E. indica* seedlings was counted and recorded after 30 days of treatment. Meanwhile, the shoot fresh weight of *E. indica* and rice seedlings was determined by harvesting and weighing aboveground living tissues remaining for each seedling. Root lengths and shoot heights of rice seedlings were then measured, and the data were expressed as percentages of the respective controls.

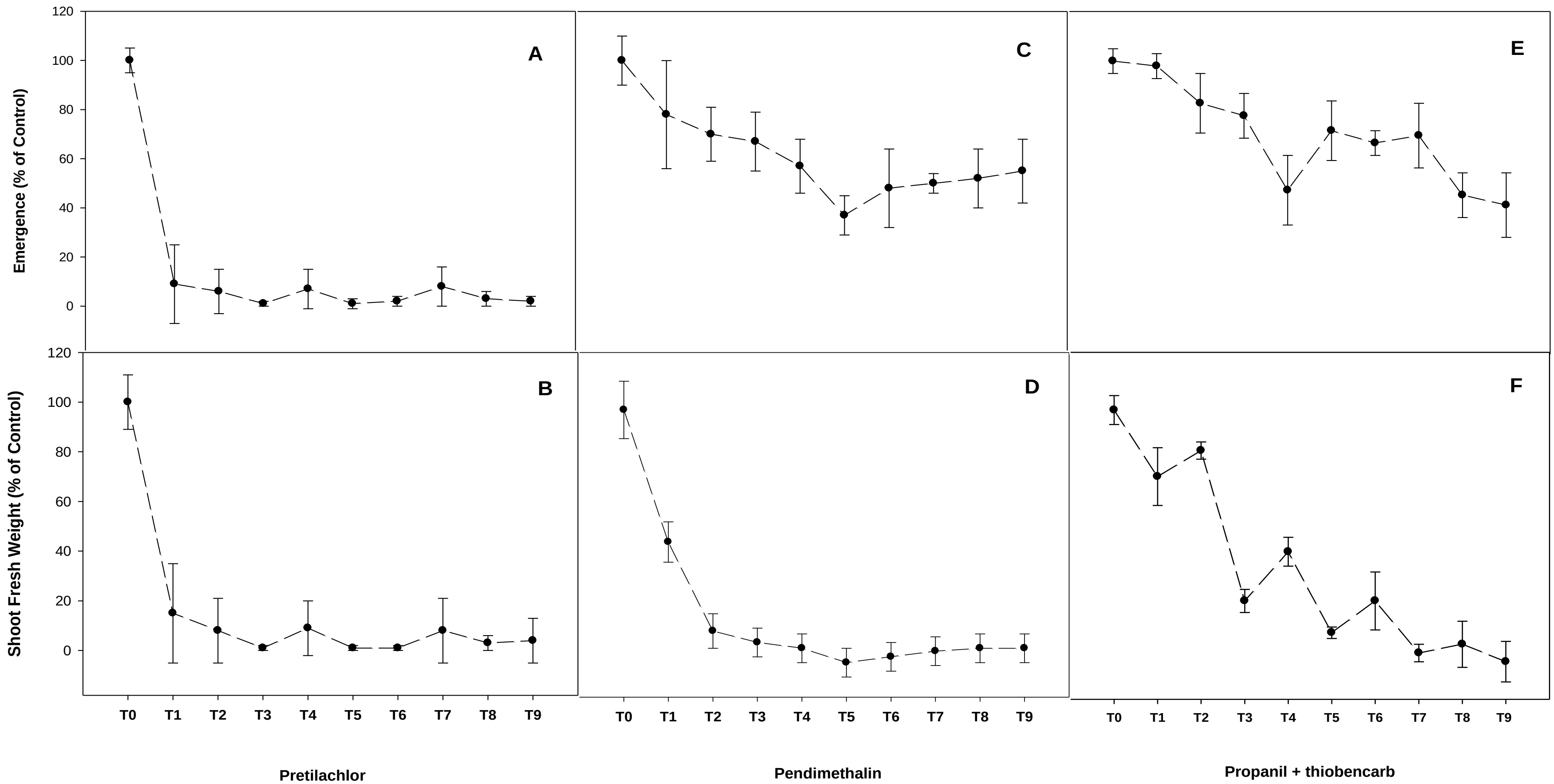
Statistical analysis

Each experiment was arranged in a completely randomized design (CRD) with five replications. Each experiment was repeated twice. All data were subjected to two-way ANOVA and excluded non-treated control data. The Tukey HSD was used to compare the mean among the treatments. Differences were regarded as significant when the p-values were less than 0.05 ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of herbicides and N fertilizer on weed

The effects of commercial herbicides and N fertilizer treatment on *E. indica* seeds are presented in Figure 1. Since there was no significant difference in phytotoxic effects of commercial herbicides and N fertilizer to *E. indica* sown at different growth stages of rice plants, all data were pooled for analysis. It was found that pretilachlor at an application rate of 0.25 and 0.50 kg ai ha^{-1} almost completely inhibited the emergence and shoot growth of *E. indica* by 100% inhibition, at 50, 100 and 150 kg ha^{-1} N, respectively (Figure 1A and B). These



T0 - Control
T1 – 0.125 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N
T2 – 0.25 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N
T3 - 0.50 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N
T4 – 0.125 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N
T5 – 0.25 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N
T6 – 0.50 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N
T7 – 0.125 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N
T8 – 0.25 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N
T9 – 0.50 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N

T0 - Control
T1 – 0.25 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N
T2 – 0.50 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N
T3 – 1.00 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N
T4 – 0.25 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N
T5 – 0.50 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N
T6 – 1.00 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N
T7 – 0.25 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N
T8 – 0.50 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N
T9 – 1.00 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N

T0 - Control
T1 – 0.90 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N
T2 – 1.80 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N
T3 – 3.60 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N
T4 – 0.90 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N
T5 – 1.80 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N
T6 – 3.60 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N
T7 – 0.90 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N
T8 – 1.80 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N
T9 – 3.60 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N

Figure 1. Effects of commercial herbicides and N fertilizer application on weed emergence (A, C, E) and shoot fresh weight (B, D, F) of *Eleusine indica*. Vertical bar represents standard deviation (SD) of the mean.

Table 1. Effects of pretilachlor + N fertilizer rates and growth stage at application on rice root length, shoot height and shoot fresh weight 30 day after application.

Main effect	Root length	Shoot height	Shoot fresh weight
		(% of Control)	
Treatment			
T1 - 0.125 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N	95^{cd}	84^{b}	92^{cd}
T2 - 0.25 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N	100de	80^{ab}	66^{a}
T3 - 0.50 kg ai ha^{-1} pretilachlor + 50 kg ha^{-1} N	85^{b}	74^{ab}	76^{ab}
T4 - 0.125 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N	94^{cd}	80^{ab}	73^{ab}
T5 - 0.25 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N	74^{a}	86^{b}	94^{cd}
T6 - 0.50 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N	94^{cd}	77^{ab}	78^{ab}
T7 - 0.125 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N	88^{cd}	81^{ab}	74^{ab}
T8 - 0.25 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N	101^{e}	71^{a}	77^{ab}
T9 - 0.50 kg ai ha^{-1} pretilachlor + 150 kg ha^{-1} N	75^{ab}	85^{b}	83^{b}
Application timing (DAS)*			
0	83^{a}	74^{a}	73^{a}
5	97^{b}	83^{b}	85^{b}
10	97^{b}	93^{c}	88^{b}
Non-treated control (T0)	100	100	100

* The rice seeds were treated with pretilachlor + N fertilizer at 0, 5 or 10 days after sowing (DAS). Main effect mean within the same column followed by the same letter has no significant difference at *P*<0.05 after determined by a Tukey test.

treatments were highly toxic to the bioassay species, where the seedling injury was severe. Conversely, pendimethalin and N fertilizer at a moderate application rate gave >50% inhibition on the emergence of *E. indica* (Figure 1C). Strong reduction in shoot fresh weight of weeds also was noticed at this application rate with >85% inhibition (Figure 1D).

Early post-emergence herbicide, propanil + thiobencarb was found to have the greatest inhibitory effects on seedling emergence of *E. indica* at the highest application rates of 3.6 kg ai ha^{-1} propanil + thiobencarb and 150 kg ha^{-1} N, with 60% inhibition (Figure 1E). It was noticed that this treatment also was highly toxic to the bioassay species, where it reduced the weed shoot fresh weight by > 90% with symptoms of leaf wilting and discoloration (Figure 1F). Similar symptoms were also found at moderate to low concentration where the seedling injury was severe. By contrast, untreated seedlings for all herbicide types remained healthy 30 days after treatment.

Weed control is a major problem in direct-seeded rice (DSR) because the weed seeds germinate simultaneously with the rice seeds (Kumar, 2017). According to Singh et al. (2014), the density of grasses, sedges and broad leaf weeds in direct seeded rice increased with increased nitrogen rates from 120 to 150 kg ha^{-1}, but a further increase in nitrogen rate (180 kg N ha^{-1}) leads to a decrease in weed density. Later on, Awan et al. (2014) found that added N favoured rice biomass production more than it did the weed where weed biomass increased by 82 to 160%, whereas rice biomass increased by 92 to 229%, with the application of 50 to 150 kg N ha^{-1}. Meanwhile, Morshed et al. (2015) reported that *Scirpus maritimus* L. in Boro rice showed the maximum visual abundance (58%) after treated with N up to 202.4 kg ha^{-1} under selected pre-emergence and post-emergence herbicides; suggesting that availability of nitrogen in soil favoured the luxuriant growth of weeds. In this study, it was noticed that the emergence and shoot fresh weight of *E. indica* showed a different response along with herbicides and N rates (Figure 1). Therefore, manipulating the N environment could be effective in enhance herbicide efficacy.

Effect of herbicides and N fertilizer on rice

The degree of susceptibility of rice seedlings toward herbicides and N fertilizer was evaluated in this study. Since herbicides and N fertilizer rate-by-growth-stage interaction was observed, data were pooled over and main effects are presented (Tables 1 to 3). Moderate application rates of pretilachlor and N fertilizer (0.25 kg ai ha^{-1} pretilachlor + 100 kg ha^{-1} N) had an adverse impact on the rice root length (Table 1). Averaged across pretilachlor and N fertilizer rates, rice seedlings growth at 0 DAS were 70 to 80% of the non-treated control. It was observed that starting at 5 DAS, the growth of rice seedlings were less affected by the pretilachlor and N fertilizer treatment, where the rice seedlings growth exhibited at least 80% of non-treated control. In contrast, the injury of rice seedlings was likely negligible when

Table 2. Effects of pendimethalin + N fertilizer rates and growth stage at application on rice root length, shoot height and shoot fresh weight 30 day after application.

Main effect	Root length	Shoot height	Shoot fresh weight
	(% of Control)		
Treatment			
T1 - 0.25 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N	76^{ab}	90^{d}	72^{ab}
T2 - 0.50 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N	78^{ab}	84^{c}	78^{abc}
T3 - 1.00 kg ai ha^{-1} pendimethalin + 50 kg ha^{-1} N	71^{a}	78^{a}	69^{a}
T4 - 0.25 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N	90^{bcd}	86^{c}	83^{cde}
T5 - 0.50 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N	95^{d}	97^{d}	94^{e}
T6 - 1.00 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N	80^{ab}	80^{ab}	75^{abc}
T7 - 0.25 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N	95^{d}	90^{d}	89de
T8 - 0.50 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N	94^{cd}	82^{bc}	85^{cde}
T9 - 1.00 kg ai ha^{-1} pendimethalin + 150 kg ha^{-1} N	88^{bcd}	78^{a}	80^{bcd}
Application timing (DAS)*			
0	81^{a}	79^{a}	79^{a}
5	84^{a}	89^{b}	79^{a}
10	90^{b}	93^{b}	89^{b}
Non-treated control (T0)	100	100	100

* The rice seeds were treated with pendimethalin + N fertilizer at 0, 5 or 10 days after sowing (DAS). Main effect mean within the same column followed by the same letter has no significant difference at *P*<0.05 after determined by a Tukey test.

Table 3. Effects of propanil+thiobencarb + N fertilizer rates and growth stage at application on rice root length, shoot height and shoot fresh weight 30 day after application.

Main effect	Root length	Shoot height	Shoot fresh weight
	(% of Control)		
Treatment			
T1 - 0.90 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N	87^{a}	72^{a}	68^{b}
T2 - 1.80 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N	93^{ab}	67^{ab}	47^{a}
T3 - 3.60 kg ai ha^{-1} propanil + thiobencarb + 50 kg ha^{-1} N	94^{ab}	83^{ab}	30^{a}
T4 - 0.90 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N	97^{ab}	111^{cd}	95^{cd}
T5 - 1.80 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N	96^{ab}	109^{cd}	80^{bc}
T6 - 3.60 kg ai ha^{-1} propanil + thiobencarb + 100 kg ha^{-1} N	107^{ab}	109^{cd}	77^{b}
T7 - 0.90 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N	111^{b}	133^{def}	100^{d}
T8 - 1.80 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N	112^{b}	138^{ef}	111^{d}
T9 - 3.60 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N	182^{c}	147^{f}	146^{e}
Application timing (DAS)*			
0	89^{a}	97^{a}	85^{a}
10	110^{b}	106^{a}	98^{b}
15	127^{b}	136^{b}	104^{b}
Non-treated control (T0)	100	100	100

*The rice seeds were treated with propanil+thiobecarb + N fertilizer at 0, 5 or 10 days after sowing (DAS). Main effect mean within the same column followed by the same letter has no significant difference at *P*<0.05 after determined by a Tukey test.

treated with moderate application rate of pendimethalin and N fertilizer (0.50 kg ai ha^{-1} pendimethalin + 100 kg ha^{-1} N), and it also found that the growth of rice seedlings started to increase at 10 DAS (Table 2). It was interesting to note that the rice seedlings growth was greatly stimulated at the highest application rate of propanil + thiobencarb and N fertilizer (3.60 kg ai ha^{-1} propanil + thiobencarb + 150 kg ha^{-1} N). Averaged across propanil + thiobencarb and N fertilizer rate, a small reduction on rice root length, shoot height, and shoot fresh weight was noticed at 0 DAS (85 to 95% of the non-treated control). It was observed that starting at 10 DAS, the growth of rice seedlings were stimulated by propanil + thiobencarb with N treatment, where both root length and shoot height exhibited 106 to 110% of non-treated control (Table 3).

According to Knezevic et al. (2013) the timing of application was important in determining the effectiveness of a particular herbicide. This finding was in line with our study where weed control with early application of pretilachlor and pendimethalin at 0 and 5 DAS was toxic to rice crop. However, late application of pre and early post-emergence herbicides at 10 and 15 DAS respectively, was good for crop growth. Under DSR, highest application rates of propanil + thiobencarb and N fertilizer could reduce the emergence and shoot fresh weight of *E. indica* by 60 to 90%, respectively (Figure 1E and F). Interestingly, the rice root and seedling growth were highly stimulated at this application rate (Table 3). Thus, the study results suggest that this combination rate was efficient in control *E. indica* without injuring the rice seedlings. The period between 10 and 15 DAS was found suitable for effective weed control for this application rate with respect to toxicity to rice plants.

Conclusions

Early post-emergence herbicide mixture of propanil + thiobencarb (3.60 kg ai ha^{-1}) possesses promising herbicidal activity for control of *E. indica* along with N application (150 kg ha^{-1} N), thus highlighting its potential for controlling many weed species in rice fields. However, some additional experiments in the field should be undertaken to study the synergistic or antagonistic effect of herbicide and N fertilizer on rice-weed competition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Financial profitability and resource use efficiency of boro rice cultivation in some selected area of Bangladesh

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The paper analysed the profitability and resource use efficiency of boro rice cultivation in Bogra district of Bangladesh using farm level survey data of April-May, 2016. In total 103 farmers were selected randomly from the study area. Result based on Farm Budgeting model showed that per hectare variable cost and total cost of production was BDT (Bangladeshi Taka) 57,583 and BDT 71,208 respectively. Average yield was found 4.112 ton which was more than the previous year's national average yield of 3.965 ton. The average gross return, gross margin, and net return were BDT 86,548, BDT 28,965 and BDT 15,340 respectively. Benefit-Cost ratio (BCR) was found 1.22 and 1.50 on full cost and variable cost basis. Cobb-Douglas production function analysis showed that the key production factors, that is, human labour, irrigation, insecticide, seed and fertilizer had statistically significant effect on yield. MVP and MFC ratio analysis showed that growers allocated most of their resources in the rational stage of production.

Key words: Benefit-Cost ratio (BCR), Cobb-Douglas production function, elasticity and resource use efficiency.

INTRODUCTION

Rice is the staple food of the people of Bangladesh. About 160 million peoples in Bangladesh depend on rice as main food and about 75.1% of agricultural land use to grow rice of which 42.40 was employed on boro crops (BBS, 2015). Boro is the single most crops in Bangladesh in the context of total volume of production. It has been persistently contributing over last successive years (BBS, 2015). In 2015, the area under boro crop was 48,40,222 ha which was 1.04% higher than the previous year. In FY 2014-15, average yield rate was 3.965 metric tons husked rice per hectare and total boro production was 1, 91,92,164 metric tons which was 55.29% of total production of rice (BBS, 2015). HYV boro was found more efficient among all other main rice varieties (Local Aman, HYV Aman and HYV Boro) in Bangladesh (Regmi et al., 2016). Although rice was considered as the main

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crop in Bangladesh and the country was ranked as the fourth largest rice producer in the world (IRRI, 2009), it is not produced with full efficiency. Labor, fertilizer, seed as well as irrigation were the significant factors, which affect the level of technical efficiency of rice production (Hasnain et al*.,* 2015). Also, the ownership of key resources like land, labour, and draft animals significantly affect the efficiency of production (Rahman et al*.,* 2009). Besides land and labour, irrigation was also found as significant determinants of modern rice productivity where decreasing returns to scale prevailed (Rahman, 2011). At the time of considering individual inputs, human labour was found as the largest contributor of expenses of HYV boro rice production in Bhola district (Majumder et al., 2009). Irrigation played a positive and vital role in the productivity of both conventional and HYV of rice cultivation in Rajbari district of Bangladesh (Bapari, 2016). Profitability of boro rice production also depends on the cost of irrigation (Nargis et al., 2009). In northwest part of Bangladesh, defects have been found on the current irrigation water management system. Although the area irrigated for dry season rice (*boro*) has increased about three folds during 1981-2014 (Dey et al*.,* 2017). A study showed that 78.7% of the lifted water was important for boro rice production. This wastage of water increase irrigation as well as production cost (Dey et al*.,* 2013). Efficiency measurement shows that there were higher degrees of inefficiency in the cultivation of modern rice. These efficiency differences were depicted by soil fertility, infrastructure, experience, extension services, tenancy and share of non-agricultural income (Rahman, 2003). Some studies revealed that farmers who borrowed capital used more inputs and attained higher returns in contrast to non-borrower (Sarkar et al*.,* 2010). As efficiency and input use pattern varies with the socio-economic characteristics of farmer or manager (Islam and Sujan*,* 2016). For enhancing technical efficiency different strategies like ensuring better extension services and conducting farmers training programs, enhancing access to agricultural microcredit, reducing land fragmentation and raising awareness level of farmers were proposed by Backman et al*.* (2011). Efficient use of input through adaptation and spread of improved agricultural mechanization can be way of exploiting the full potential of technology (Nargis and Lee, 2013). Agricultural mechanization in cultivation was also poised to make a major difference in the future agriculture (Ahmed, 2001). Increased access to irrigation, tenurial reformation and assurance for higher price of rice can boost farm returns as well as offset the impact of uneven rise in labour wage. These will synergistically enhance the adoption of modern rice along with farm productivity (Rahman, 2011). For gaining higher profitability enhancement in labour productivity and formalization of the agricultural labour market should be more emphasized rather than mere input subsidization or price support (Selim, 2011). A review of existing literature reveals that so far the attention has been given by the researchers in investigating the efficiency of boro rice production in the study area are not adequate. Thus, the objective of the present study is to analyze the resource use efficiency of boro rice production in Bangladesh using data from boro rice farmers in April-May, 2016. Required data are collected from 103 boro rice producing farmers of Bogra district selected by using multistage sampling procedure. Note that, the weather condition for boro cultivation was favoruable in the growing stage in 2016.

MATERIALS AND METHODS

A micro-level empirical study based on primary cross-section data was designed to attain the purposes of the study. The methodology of the study is mainly about the sampling procedure, collection of data and analytical framework.

Sampling technique

In this research, study area and respondent were selected by multistage random sampling procedure. In first stage of sampling, Bogra district was selected purposively. In the second stage, four upazilas named Bogra sadar, Dhunat, Shibganj and Sonatala were selected purposively. After that, a complete list of boro rice farmers was collected from each upazila. At the final stage, a total of 103 rice farmers were selected by random sampling technique. Among the 103 rice farmers 25 numbers of farmers were included from each one of first three upazila and 28 were included from Sonatala upazila. Since the study focuses on resources use efficiency, attempt was made to choose respondent from that areas, which had an average level of agricultural performance in their respective sub-regions.

Method of data collection

As per the conventional survey techniques, primary information on available resources and their use, prices of farm product and different inputs were collected by direct interviewing of farmers using a designed and pre-tested questionnaire in April-May, 2016.

Analytical framework

Different parameters of costs and return were analyzed to measure the profitability of boro rice cultivation on the study area. The following algebraic equation was developed to assess the costs and returns of rice production.

$$GR_i = \sum_{i=1}^{n} Q_{mi}P_{mi} + \sum_{i=1}^{n} Q_{bi}P_{bi}$$

Where, GR_i = Gross return from ith product (BDT/ha)
Q_{mi} = Quantity of the ith main product (kg/ha)
P_{mi} = Average price of the ith main product (BDT/kg)
Q_{bi} = Quantity of the ith by product (kg/ha)
P_{bi} = Average price of the ith by product (BDT/kg)
i = 1,2,3,………n

BDT = Bangladeshi Taka

Net return was estimated by subtracting both variable and fixed costs from the gross return. Return from by products also included with net return. To calculate the net return of boro rice production the following formula was used on the study:

$$\pi = P_y Y - \sum_{i=0}^{n} P_{xi} X_i - TFC$$

Where, π = Net return (BDT/ha)
P_y = Per unit price of the product (BDT/kg)
Y = Quantity of the product per hectare (kg)
P_{xi} = Per unit price of i^{th} inputs (BDT)
X_i = Quantity of the i^{th} inputs per hectare (kg)
TFC = Total fixed cost (BDT)
i = 1,2,3,………., n (number of inputs).

Benefit-Cost Ratio (BCR) analysis

This ratio was measured in the study in two different ways:

$$BCR \ on \ TVC = \frac{GR}{TVC} BCR \ on \ TC = \frac{GR}{TC}$$

Where, GR = Gross return, TC = Total Cost, TVC = Total Variable Cost and, the decision rules are that, when
BCR>1, the return from boro rice is economically satisfactory;
BCR<1, the return from boro rice is not economically satisfactory; and
BCR=1, there is economic breakeven point of boro rice production.

Empirical model

For functional analysis of the data Cobb-Douglas production function is used. Logarithmic form of the function is linear and parsimonious which ease the estimation and interpretation of data (Beattie and Taylor, 1985). In general, the production of boro rice is mostly influenced by human labour, power tiller, seed, urea, TSP, MoP, insecticide and irrigation etc. The Cobb- Douglas regression function was as follows:

$$Y = AX_1^{\beta 1} X_2^{\beta 2} \text{--------------------------------} X_n^{\beta n} e^{ui}$$

The production function was converted to logarithmic form so that it could be solved by Ordinary Least Square (OLS) method, that is,

$$lnY = \alpha + \beta_1 lnX_1 + \beta_2 lnX_2 + \ldots\ldots + \beta_n lnX_n + U_i$$

The empirical production function was the following:

$$lnY = \alpha + \beta_1 lnX_1 + \beta_2 lnX_2 + \beta_3 lnX_3 + \beta_4 lnX_4 + \beta_5 lnX_5 + \beta_6 lnX_6 + \beta_7 lnX_7 + \beta_8 lnX_8 + U_i.$$

Where, Y = Yield (BDT/ha)
X_1 = Human Labor (BDT/ha)
X_2 = Power tiller (BDT/ha)
X_3 = Seed (BDT/ha)
X_4 = Urea (BDT/ha)
X_5 = TSP (BDT/ha)
X_6 = MoP (BDT/ha)
X_7 = Insecticide cost (BDT/ha)
X_8 = Irrigation cost (BDT/ha)
α = Intercept
β_1, β_2 ---- β_8 = Coefficients of the respective variables to be estimated; and
U_i = Error term.

In order to investigate the Resource Use Efficiency, the ratio of marginal value product (MVP) to the marginal factor cost (MFC) for each input was computed and tested for its equality to 1,

That is, $\frac{MVP}{MFC} = r$

Where, r = Efficiency ratio
MVP = value of change in output resulting from a unit change in variable input (BDT)
MFC = price paid for the unit of variable input (BDT)

Under this method, the decision rules are that, when:

r >1, the level of resource use is below the optimum level, implying under-utilization of resources. Increasing the rate of use of that resource will help increase productivity.
r <1, the level of resources use is above the optimum level, implying over utilization of resources. Reducing the rate of use of that resource will help improve productivity.
r = 1, the level of resource use is at optimum implying efficient resource utilization.

The most reliable, perhaps the most useful estimate of MVP is obtained by taking all input resources (X_i) and gross return (Y) at their geometric means (Dhawan and Bansal, 1977). All the variables of the fitted model were calculated in monetary value. As a result the slope co-efficient of those independent variables in the model represent the MVPs, which were estimated by multiplying the production co-efficient of given resources with the ratio of geometric mean (GM) of gross return to the geometric mean (GM) of the given resources, that is,

$$MVP\ (X_i) = \beta_i \frac{\bar{Y}(GM)}{\bar{X}i(GM)}$$

Where, $\bar{Y}(GM)$ = Geometric mean of gross return (BDT)
$\bar{X}_i(GM)$ = Geometric mean of different independent variables (BDT)
β_i = Co-efficients of parameter
i = 1, 2,………………..n

RESULTS AND DISCUSSION

Cost of cultivation

For determining the cost of boro rice cultivation, all the variable costs e.g. human labour, power tiller, seed, organic manures, fertilizers, insecticides and irrigation were calculated as per hectare. The fixed cost of boro rice cultivation comprised land use cost and interest on operating capital. The cost on human labor was calculated by considering different charge for male and female labour and also for different time of the season. The land use cost was determined on the basis of per hectare lease value of land. Actual land use values for boro rice cultivation were calculated as per its agronomic lifespan of the year.

The cost of boro rice production was approximated to be BDT 71,208 and BDT 57,583 per hectare on total cost and variable cost, respectively. The major share in total cost was human labour input cost (26.52%), followed by irrigation (17.43%), chemical fertilizers (17.36%) and land use cost (16.58%). On the 26.52% of labour cost 67% labours were family supplied and rest 33% were used on

Table 1. Production cost of boro rice in the study area *(in BDT/ha)*.

S/No.	Items	Amount (BDT)	Percentage of total cost
A.	**Variable Cost**	**57,583**	80.87
	Human labour	18,883	26.52
	Family labour	12,566 (67%)	17.65
	Hired labour	6,317 (33%)	8.87
	Power tiller	4,404	6.18
	Seed	2,401	3.37
	Organic manure	2,986	4.19
	Chemical fertilizers:	12,360	17.36
	Urea	4,440	6.24
	TSP	4,357	6.12
	MoP	1,590	2.23
	Zipsum	1,973	2.77
	Insecticides	4,139	5.81
	Irrigation	12,409	17.43
B.	**Fixed cost**	**13,625**	19.13
	Land use	11,806	16.58
	Interest on operating capital	1,819	2.55
	Total Cost (A+B)	**71,208**	100.00

Data Source: Author's calculation based on field survey (2016).

Table 2. Profitability of boro rice cultivation in the study area.

S/No.	Items	Formula	Unit	Amounts
01	Yield from main product	Y_1	kg/ha	4,112
02	Farm gate Price of main product	P_1	BDT/kg	19.65
03	Return from main product (R_1)	$Y_{1*} P_1$	BDT/kg	80,548
04	Yield from by product (R_2)		BDT/kg	6,000
05	Gross return ($R_{1+} R_2$)	*GR*	BDT/kg	86,548
06	Total variable cost	*TVC*	BDT/kg	57,583
07	Total cost	*TC*	BDT/kg	71,208
08	Gross margin	*GR-TVC*	BDT/kg	28,965
09	Net return	*GR-TC*	BDT/kg	15,340
10	Benefit cost ratio			
	Full cost basis	*GR/TC*		1.22
	Variable cost basis	*GR/TVC*		1.50

Data Source: Author's calculation based on field survey (2016).

hired basis. More or less same percentage of contribution by labour and fertilizer were found by Bapari (2016). Estimated result tabulated below on Table 1.

Profitability of rice cultivation

The yield of boro rice was 4.1 ton/ha which was higher than the previous year's average yield (3.62 ton/ha) (BBS, 2015). Prevailing congenial atmosphere throughout the season was the major cause of increasing yield. Including returns from both main product and by-product the gross return and gross margin of rice cultivation were BDT 86,548 and BDT 28,965 per hectare respectively. The net return of rice cultivation was BDT 15,340 per hectare which is significantly lower than the findings of Kazal et al. (2013). Prevailing lower price may be the cause of variant result. Estimated result tabulated on Table 2 showed that undiscounted benefit cost ratios (BCR) were 1.22 and 1.50 on full cost and variable cost

Table 3. Estimated parameters and other related statistics of boro rice production.

Explanatory variable	Parameters	Co-efficient	Sd. Error	t-values	P-values
Intercept	β_0	3.91***	1.15	3.40	0.00
Human labor *(X_1)*	β_1	0.29**	0.13	2.14	0.03
Power tiller *(X_2)*	β_2	-0.04^{NS}	0.04	-0.96	0.34
Seed *(X_3)*	β_3	0.18**	0.07	2.47	0.02
Urea *(X_4)*	β_4	0.14*	0.08	1.76	0.08
TSP *(X_5)*	β_5	0.02 ^{NS}	0.03	0.60	0.55
MoP*(X_6)*	β_6	0.02 ^{NS}	0.09	0.21	0.83
Insecticide *(X_7)*	β_7	-0.10***	0.03	-2.80	0.01
Irrigation *(X_8)*	B_8	0.30***	0.06	4.87	0.00
R^2		0.8253			
F-value		61.22***			
Return to scale		0.808			

***, ** and * indicate significant at 1, 5 and 10% level respectively. Data Source: Author's calculation based on field survey (2016).

basis. Estimated undiscounted BCR on full cost basis was almost similar with the findings of Kazal et al*.* (2013). More or less same result was found by Bapari (2016), Suresh and Reddy (2006) and Khan (2004). This ratio was found slightly different (1.41) in Mymenshing region by Ahmed et al*.* (2009).

Factors affecting yield of rice

In order to assess the individual effects of different inputs of boro rice production Cobb-Douglas production function model was used. Estimated parameters and other related statistics have been presented in Table 3. The co-efficient of irrigation and insecticide were significant at 1% level of significance. Where irrigation had positive impact but insecticide had reversed. Implying that gross return of rice would increase 3.0% and decrease 1.0% by 10% increasing the use of irrigation and insecticide respectively. Result might indicate that boro rice cultivation in Bangladesh is highly sensitive to timely irrigation. Negative parameters of insecticide might indicate the inappropriate use of that input. Underlying causes might be improper knowledge about the doses and effects of insecticide on rice cultivation. Improper quality of insecticide could be another reason behind it. The parameters of human labour and seed were significant at 5% level of significance implying 10% increase in the use of those inputs would increase the gross return of rice by 2.9 and 1.8% respectively. Highly significant effect of these two variables was also found by Ahmed et al*.* (2009). Highly labour intensive boro rice cultivation might be explained by the higher parameter of labour (0.29). Different level of productivity of different variety of rice was described by the parameter of seed (0.18). If seed cost had increased the productivity as well as cost of rice cultivation would increase. Same result for seed also found by Majumder et al*.* (2009) and Ahmed et al*.* (2009). 1% increasing use of urea would increase the gross return of rice by 0.14% in facts its parameter was significant at 10% level of significance. Timely and properly application of urea had a positive effect on production might be revealed by the positive significant co-efficient of urea on gross return of boro rice cultivation. TSP and MoP had positive and power tiller had negative impact on the gross return of rice but the effects were insignificant. Insignificant effect annotated that these inputs had supportive not crucial effect on production.

Implication of R^2

The co-efficient of multiple determinations (R^2) were 0.8253, which indicate that about 82.53% of the variations in gross return of boro rice cultivation had been explained by the independent variables included in the model.

F-Statistics

The F-statistic of the model was found 61.22, which was highly significant at 1% level of significance. This result of F-statistic implies that all the independent variables included in the model were important for explaining the variations in gross returns of boro rice production.

Returns to scale

Returns to scale was calculated by summing up all the co-efficients of production. For boro rice production the rumination of the coefficients was 0.808 indicating the production functions exhibit decreasing returns to scale.

Table 4. Resource use efficiency of different inputs under rice cultivation.

Variable	Geometric mean *(GM)*	$\bar{Y}(GM)/\bar{X}_i(GM)$	Co-efficient	*MVP* (X_i)	*r=MVP/MFC*	Decision rule
Yield *(Y)*	79595.64					
Human labuor (X_1)	18762.09	4.242	0.287	1.220	1.220	Under-utilization
Power tiller (X_2)	4347.41	18.309	-0.038	-0.694	-0.694	Over-utilization
Seed (X_3)	2378.31	33.467	0.180	6.009	6.009	Under-utilization
Urea (X_4)	4397.79	18.099	0.139	2.522	2.522	Under-utilization
TSP (X_5)	4276.30	18.613	0.020	0.376	0.376	Over-utilization
MoP (X_6)	1584.33	50.239	0.020	0.984	0.984	Over-utilization
Insecticide (X_7)	4059.59	19.607	-0.097	-1.901	-1.901	Over-utilization
Irrigation (X_8)	12101.43	6.577	0.297	1.953	1.953	Under-utilization

MFC= BDT 1. Data Source: Author's calculation based on field survey (2016).

Almost same result was found by Majumder et al. (2009) and reversed result was found by Ahmed et al*.* (2009) for boro rice production.

Resource use efficiency

Resource use efficiency implies how efficiently the farmer can use their resources in production process. Efficient use of resource is deeply concerned for ensuring their sustainability. For determining resource use efficiency eight input factors namely human labor, power tiller, seed, urea, TSP, MoP, insecticide, and irrigation were considered.

Higher MVP indicates increasing productivity of resources whereas negative MVP implies unproductive use of it (Utamakili, 1992; Olayemi, 1998; Mbanasor and Obioha, 2003; Emokaro and Erhabor*,* 2006). The results are presented in Table 4. From the table it was found that the MVP for seed, urea, irrigation and human labour were 6.009, 2.522, 1.953 and 1.220 respectively. The MVP values for all the variables were greater than one which indicated that farmers had chances of increasing per hectare output by utilizing more seedling, urea fertilizers, irrigation and human labour. Majumder et al. (2009) reported a similar result for owners and tenant operators, the MVP of seedlings and insecticides were higher than one while for cash tenants MVP of seedlings, insecticides and fertilizers were greater than one. Greater than one MVP ratio to MFC for seed, chemical fertilizers, plant protection chemicals and human labour was also found by Parasar et al*.* (2016). Same ratios for fertilizer, labour and land were reported to be greater than one by Sani et al*.* (2010). Over-utilized labour and planting material were also found by Onyemauwa et al*.* (2013). Ratio analysis of MVP to MFC for TSP and MoP fertilizer showed that the MVP of all of these factors were found to be lower than their respective cost which suggest to decrease the use of these inputs. Same ratio for insecticide and power tiller was -1.901 and -0.694, respectively. Negative efficiency ratio for these input showed that additional input of these two factors bring no benefit but loss. So use of these two inputs must be decreased for maximizing outputs of rice production.

Elasticity of production

Percentage change in output due to the percentage change in input is defined by the elasticity of production. Elasticity concept can be applied to the production function for determining the stage in which farmers are allocating their resources (Table 5).

The summations of all the co-efficients of Cobb-Douglas production function express the direct measure of returns to scale which indicate the stage of production. Calculated elasticities for all farmers were less than one implying the allocation of resources on the *stage-II* of production functions where farmers get diminishing returns from their resources. Same result also found by Majumder et al. (2009) in similar study in Bhola district.

CONCLUSION AND POLICY RECOMMENDATIONS

The principal finding of the study is that the boro rice cultivation in Bogra district was a profitable venture. All of the factors namely human labour, seed, urea, insecticide and irrigation were very important for the cultivation. Function analysis implied that the farmers employed their scarce resources in boro rice production inefficiently. Resource use efficiency analysis also provided an alarm that most of the resources used in the production were not economically optimal. Improvement in irrigation system is the key recommendation for improving productivity of boro rice in the study area. Providing proper training to farmers for making them aware about different strategies of cultivation and taking necessary steps for increasing the quality of pesticide can also be policy implication. HYV boro seed should be made available to farmers in the production season within affordable cost. Agricultural labour should be made

Table 5. Elasticities of boro rice production.

S/No.	Input	Elasticity	Stage	Returns to scale
01.	Human labor *(X_1)*	0.29	*Stage-II*	Diminishing
02.	Power tiller *(X_2)*	-0.04	*Stage-III*	Diminishing
03.	Seed *(X_3)*	0.18	*Stage-II*	Diminishing
04.	Urea *(X_4)*	0.14	*Stage-II*	Diminishing
05.	TSP *(X_5)*	0.02	*Stage-II*	Diminishing
06.	MoP*(X_6)*	0.02	*Stage-II*	Diminishing
07.	Insecticide *(X_7)*	-0.10	*Stage-III*	Diminishing
	Irrigation *(X_8)*	0.30	*Stage-II*	Diminishing
	Total	0.81	*Stage-II*	Diminishing

Data Source: Author's calculation based on field survey (2016).

available when necessary and agricultural mechanization should be encouraged.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Medium-term effects of conservation agriculture on soil quality

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Inefficient management practices lead to soil organic matter depletion, structure breakdown and increased erosion. This has resulted into low crop yields of sub-Saharan Africa. Conservation agriculture (CA) is being considered as a potential system having the capability of improving soil quality and providing stable yields. A study was therefore conducted, at Chitedze Research Station-Malawi, to evaluate medium term effects of 5 years CA experiment potential in improving soil quality. The results indicated that chemical nutrient build up in CA is gradual and significant differences between treatments were realized from the 4th year of practicing CA. In the 5th year, CA treatments, on average registered 14 and 21% higher in pH and soil organic matter (SOM) respectively than in the common practice. A positive correlation (74%) between soil SOM and pH in the 5th year was observed. CA treatments had a range of 61.2 - 69.4% of the soil particles composed of soil aggregates greater than 2 mm in diameter compared to 30.1% under common practice by the 5th year. In the top 30 cm of the soil, 67 and 17 earthworm's' m^{-2} were recorded in CA and control, respectively. Maize yields were higher in the 5th year as compared to the 1st year. In all the parameters assessed, CA using maize - cowpea rotation treatment gave highest values. Conclusively, CA improves soil quality, especially when legumes are integrated.

Key words: Common practice, soil aggregate stability.

INTRODUCTION

Agricultural production in Malawi faces numerous problems which have resulted in the maize production being at less than 1.3 t ha^{-1} against the potential of 6 to 8 t ha^{-1} (Ellis et al., 2003). Soil degradation is one of the major constraints to maize production in Malawi, mainly due to poor soil management, deforestation and over grazing. Additionally, the current conventional agriculture techniques practiced by smallholder farmers are known

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to steadily deplete soil fertility, through erosion and loss of soil organic matter, thereby reducing the potential yield on the cultivated arable land (Joseph, 2008). Along with this, it is estimated that six hundred thousand tons of soil are moved manually each year to make ridges (FAO, 1993). Yearly movement of the soil through conventional crop production systems lead to declining productivity and soil degradation. Most operations following the initial ploughing tend to compact the soil, reduce water infiltration, soil aeration and organic matter content, and increase farming costs (Thierfelder and Wall, 2009). Consequently, soil movement leads into the disturbance of the habitat of soil micro flora and fauna and hence loss in microbial diversity (Ghabbour, 2010). This scenario has been worsened by climate change effects which have increased the occurrences of drought periods, combined with shifts and unpredictable rainfall patterns (IPCC, 2001).

A number of efforts have been initiated to address the problems of soil degradation particularly low soil fertility in Malawi. These included the studies which screened the potential of green manure crops as sources of green manure for maize (Gilbert, 1998). Other studies examined the suitability of these legumes in intercrops with maize (Ngwira et al., 2012). Maize pigeon pea intercropping has shown consistent N contribution of a minimum of 27 kg ha^{-1} from natural leaf fall without significant reduction in maize yield (Sakala, 1994). Crop rotation with velvet beans, soybean has also shown positive contribution to soil fertility (Kumwenda, 1997). Despite all these innovations, the current soil organic matter (SOM) inputs (from leguminous trees in fallows, tree leaf litter, cereal, legume crop residues, animal manures and composts) are insufficient to maintain SOM levels in most smallholder farm soils because it is not possible to grow and produce enough biomass to maintain SOM.

Conservation Agriculture (CA) is one of the climate smart agriculture systems which offer renewed hope for the smallholder production in Malawi. Through practicing minimum tillage, soil cover and integration of legumes, as CA principles, it would enhance build-up of both organic matter and soil microbes due to reduced erosion (Thierfelder and Wall, 2012). CA allows for improved water infiltration, water holding capacity and subsequent increased yields of maize (Thierfelder and Wall, 2009). Thus, CA is considered to be one of the agricultural systems with the potential of positively contributing to climate change adaptation and mitigation strategies. Based on this assumption, a study was carried out in a long term CA trial to understand the trends of soil quality improvement in maize based cropping systems in the first 5 years considered as a medium term period.

MATERIALS AND METHODS

Site description

The study was conducted in an ongoing CA trial that was planned for a long term study. The trial is situated at Chitedze Agriculture Research Station located at an altitude of 1145 m above sea level, 14°S latitude and 34°E (Thierfelder and Wall, 2012). Chitedze is a representative of the Lilongwe plain, which is a major maize producing region of Malawi. The terrain is flat to gently undulating and soils are ferruginous Latosols, which are deep and drain freely (Brown and Young, 1965). The soils have been described as having a well-developed structure containing dark to reddish brown top soil with pH ranging from 4.5 to 6.0. Mean annual temperature ranges from 18 to 21°C (Ngwira et al., 1989).

Chitedze Research station receives unimodal type of rainfall that normally starts in November and ends in March. The area receives on average 800 to 900 mm of rainfall, annually. The rainfall data for the first year (2007) and fifth (2011) year of trial implementation show that more annual rainfall was observed during 2007/2008 cropping season (1090.9 mm) and the least rain amount of 743.8 mm fell in the fifth cropping season - (2010/2011) (746.7 mm), with more rains received in the months of December and January (Figure 1).

Experimental treatments

The long term conservation agriculture experiment was laid out in a complete randomized block design with eight treatments (Table 1) replicated four times. Vegetable materials were consisted of maize (*Mays zea*), cowpea (*Vigna unguiculata*), pigeon pea (*Cajanus cajan*) and velvet bean (*Mucuna pruriens*).

The long term CA trial has a main plot size of 24 m x 13.5 m making a total of 18 rows with an area of 324 m^2. The net plot size is 20 m x 7.5 m (10 rows giving a total area of 150 m^2). All crop residues after the first year were retained in the plot of treatments 2 to 8, but removed in the Control plot (Treatment 1) following the common practice of farmers in Central Malawi where crop residues from the previous year's crops are either burned or grazed.

Soil sampling

Soil sampling was done at the end of each experimental cropping season, 2007 and 2011. Soil samples were taken in all the treatments and replicates. In each plot the soil was sampled at five soil depth points (0 – 10 cm, 10 – 20 cm, 20 – 30 cm, 30 – 60 cm and 60 - 90 cm) and at 3 randomly selected sampling points with an Eldeman soil auger. A composite sample from each depth was then obtained, giving rise to 5 samples, from respective depth, per plot. The soil samples were air dried and, except for the determination of aggregate stability, sieved to pass through a 2 mm sieve.

Soil pH and soil organic matter

Soil pH was determined in water (1:2.5 H_2O) (Wendt, 1996). Soil samples were analysed for total soil organic carbon using the Walkley and Black method as described by Anderson and Ingrams (1993) and the soil organic matter was derived from total organic carbon.

Soil aggregate stability

Soil aggregate stability was determined using soils from the 0 - 10 cm soil layer, in the 1st (2007) and the 5th year (2011) of the trial. Soil aggregate stability was determined using the Dry and Wet Sieving method as described by Kemper and Rosenau (1986). In the laboratory, 50 g of the soil with aggregates ranging between 10 mm to greater than 2 mm in diameter was placed on top of a set of 5 sieves (8, 4, 2, 1 and 0.5 mm), saturated with water for about

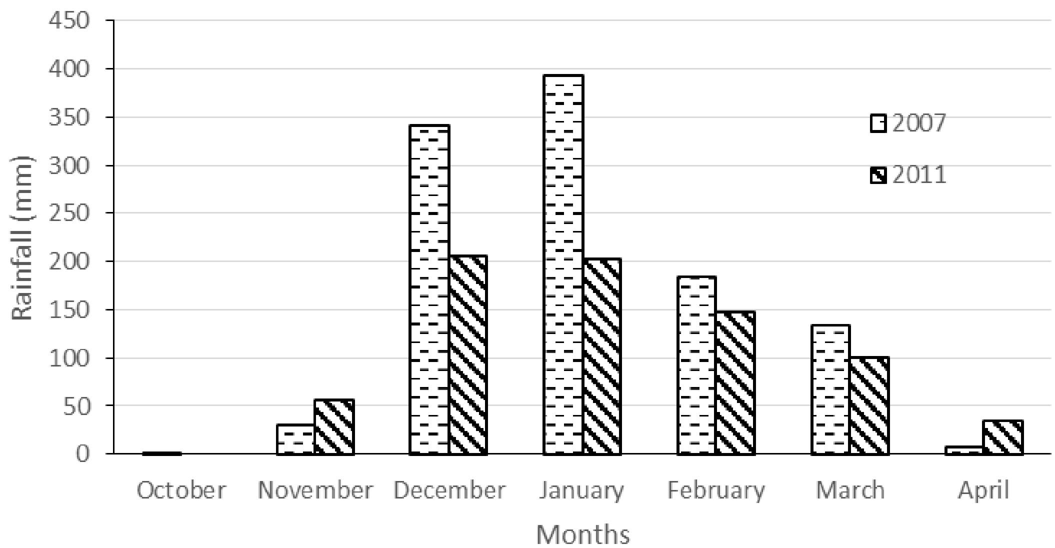

Figure 1. Rainfall distribution in the fist (2007) and fifth (2011) cropping seasons at Chitedze Research Station, Malawi.

Table 1. Treatment descriptions of long-term conservation agriculture trials at Chitedze Research Station, Malawi.

Treatment Number	Treatments Description
1	Control plot: traditional farmers practice using the hand hoe (ridge and furrow system), maize sole crop, no residues (Common practice)
2	CA sole cropping: Basin (0.15 m - length x 0.15 m - width x 0.15 m - depth), maize as a sole crop, residues retained
3	CA sole cropping: Direct seeding with dibble stick, maize as a sole crop, residues retained
4	CA crop rotation: direct seeding with dibble stick, maize-cowpea rotation, residues retained; (Cowpea – Maize – Cowpea)
5	CA crop rotation: direct seeding with dibble stick, maize-cowpea rotation, residues retained; (Maize-Cowpea – Maize)
6	CA intercropping: direct seeding with dibble stick, maize - pigeon pea intercropping, residues retained
7	CA intercropping: direct seeding with dibble stick, maize - cowpea intercropping, residues retained
8	CA intercropping: direct seeding with dibble stick, maize - velvet bean (*mucuna pruriens*) intercropping at 8 weeks after maize seeding, residues retained

20 min. The sieves were placed into a water vessel and a pulley allowing the sieve go up and down, ensuring an oscillation of 5 cm with 30 cycles per minute.

In the process of 10 min oscillation, unstable aggregates dissolved and passed through the sieves' meshes while stable ones stayed on top of the sieves. The sieves were then removed from the water and carefully separated from each other and soil fractions collected on the following sieves; >8, 4 - 8, 2 - 4, 1 - 2, 0.5 - 1 and <0.5 mm were collected into cups then dried for 48 h at 65°C. The dried soil samples were weighed and then percentage of each fraction was calculated.

Determination of earthworms

Earthworm counts were done when the soil moisture was estimated to be at field capacity in February 2012 (in the 5th year). Three sampling sites were selected at random per plot. Each soil sampling point had a dimension of 30 cm x 30 cm (width, length and depth). Soils were carefully collected using a spade at three depths (0 -10 cm, 10 - 20 cm and 20 - 30 cm), spread on a flat surface, and all the earthworms found in that particular soil depth of each sampling point were recorded.

Statistical data analysis

Data was statistically analyzed using GenStat 14th edition. Analysis of Variance, ANOVA, was used to determine treatment effects and their significances. The below statistical model was used:

$$Y = u + t_i + b_j + e_{ij}$$

Table 2. Soil pH values within the 0 - 10 cm depth of the soil profile in 2007 and 2011 at Chitedze Research Station, Malawi.

Treatment		Year	
		2007	**2011**
Common practice		5.19	4.68
CA Basin-sole maize		5.24	5.94
CA-Sole maize		5.28	5.89
CA-Cowpea after maize		5.25	5.89
CA-Maize after cowpea		5.18	5.98
CA-Maize + pigeon pea		5.20	5.91
CA-Maize + cowpea		5.13	5.90
CA-Maize + velvet beans		5.25	6.14
Mean		**5.05**	**5.82**
	P value	**LSD**	
Treatments (T)	<0.001	0.065	
Years (Y)	<0.001	0.032	
T x Y	<0.001	0.091	
CV (%)		**2.8**	

CV: Coefficient of variation, LSD: least significant difference.

Where Y = all variables under study
u = Overall mean
t_i= ith Treatment effect
b_j= jth Block effect
e_{ij}= Error term

Differences between and within treatments were separated using Least Significant Differences (LSD) tests at $p< 0.05$.

RESULTS

Soil pH

Table 2 represents results of soil pH in Year 1 (2007) and Year 5 (2011) of trial implementation. Lower pH values were observed in 2007 unlike in 2011 with an average of 5.04 and 5.82 respectively. The results revealed that by 5th year the control/common practice had decreased values of soil pH, while in the CA treatment plots there was an increase in soil pH values. Among the CA treatments, maize – velvet beans intercrop plot gave the highest pH value of 6.14 in the top soil.

Soil organic carbon (SOM)

SOM concentration steadily and gradually increased in the CA treatments from a mean value of 34.0 g kg^{-1} in the first year (2007) to 42.0 g kg^{-1} in the fifth year (2011) on the top soil of the CA treatments. Among conservation treatments, the cowpea - maize rotation had the largest SOM content of 45.0 g kg^{-1} % in the soil top layer, 0 - 10 cm, (Figure 2). Consequently, within a period of five years, common practice reduced SOM content from 35 g kg^{-1} in year 1 to 31 g kg^{-1} by the fifth year, giving a mean decrease in SOM of 4 g kg^{-1} (Figure 2). In both years, it was observed that the SOM content decreased with increase in soil depth. Results of SOM content also showed non-significant differences between soil depths, along the soil profile of study.

Correlation of soil organic matter and pH in the 5th year

Results on the relationship between soil organic matter and soil pH, (Figure 2), showed that there was a positive and strong correlation (74%) between soil organic matter and soil pH.

Soil aggregate stability

No significant differences were observed among treatment means of soil aggregates greater than 2 mm diameter, in the first year (2007) of trial implementation (Table 3). In contrary, significant differences (p=0.05) were observed in the fifth year (2011) for soil aggregates that were greater than 2 mm diameter among different treatments. In the fifth year, conservation agriculture treatments had larger percentage of soil aggregates that had a diameter of greater than 2 mm. The conservation agriculture soil aggregates, < 2 mm diameter, were in the range of 61.4 to 69.4% as compared to the conventional treatment that had 30.1% of its aggregates with diameter greater than 2 mm. Among the CA treatments, maize - cowpea rotation treatment recorded the highest percentage of soil aggregates (69.4%). greater than 2 mm diameter.

The results also revealed that practicing conservation agriculture for a period of five years contributed to the increase in the soil aggregates of >2 mm by 23.8 to 40.8% while in the control plot, aggregates >2 mm reduced by 0.3% on the top soil, 0 - 10 cm (Table 3).

Earthworms density

Earthworm counts per square meter of the soil in the fifth year (2011 – 2012) of the experiment showed significant differences among treatment means and at different soil depths (Table 4). There were more earthworms' populations in conservation agriculture than in the control treatments across the soil profiles under investigation. The highest population of earthworms was obtained in maize - cowpea rotation plots (Table 4).

Maize yield

Figure 4 represents results of maize grain yield in the

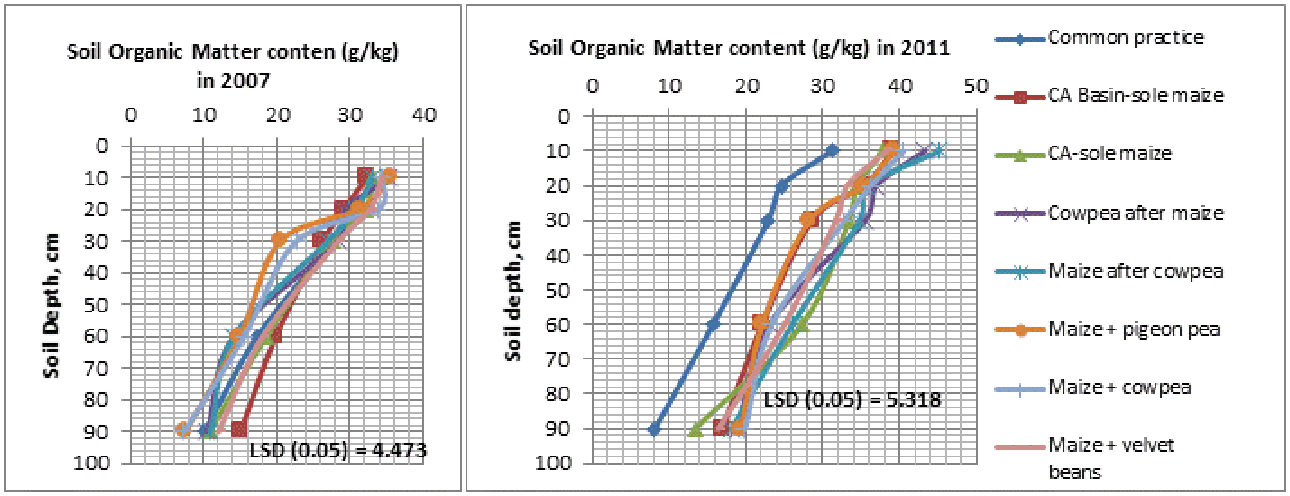

Figure 2. Soil organic matter content in Year 1 (2007) and 5 (2011) at five different soil depths at Chitedze Research Station, Malawi.

Table 3. Percentage of soil aggregates greater than 2 mm in the first year (2007) and fifth year (2011) at 0 - 10 cm soil depth at Chitedze Research Station, Malawi.

Treatment	% aggregates > 2 mm	
	2007	2011
Common practice	30.4	30.1
CA Basin-sole maize	21.5	61.8
CA-Sole maize	37.4	61.2
CA-Cowpea after maize	26.6	67.4
CA-Maize after cowpea	40.6	69.4
CA-Maize + pigeon pea	38.7	67.1
CA-Maize + cowpea	25.1	61.4
CA-Maize + velvet beans	26.5	63.6

	P value	LSD
Treatments (T)	0.004	11.6
Years (Y)	<0.001	5.8
T x Y	0.019	16.4
CV (%)		25.3

CV: Coefficient of variation, LSD: Least Significant Difference.

Year 1 (2007) and Year 5 (2011) of trial implementation. More grain yield in all treatments was observed in 2011 than in 2007. The rotation plot of maize after cowpea out yielded all treatments in both years.

DISCUSSION

Soil pH (H_2O)

The conservation agriculture treatments increased soil pH as compared with the one obtained from the control plot. This observation implies that, under common agricultural practices, there should be an increased solubility of the sesquioxides in form of aluminium (Al), Iron (Fe) and Manganese (Mn) due to depletion of the SOM as a result of yearly removal of crop residues (Bartoli et al., 1992). Accumulation of Al, Fe and Mn cause toxicity and impedes the availability of essential plant nutrients like phosphorus in the soil and to the growing plants. The high content of SOM in the CA plots influenced the increase in the pH resulting from a nutrient buffer effect (Duiker and Beegle, 2006). This is also evidenced by a positive correlation between the SOM and pH (Figure 3). The results from this study agrees with Ngwira et al. (2012) findings, who reported that soil pH was slightly higher under all conservation agriculture treatments than in the conventional after 4 years of practising CA. Sidiras and Pavan (1985) found less acidification and therefore higher pH values under zero tillage than conventional tillage at a depth of 60 cm in both oxisols and alfisols in Paraná, Brazil. Similarly, Govaerts et al. (2007) observed a significantly higher pH in the topsoil of the permanent raised beds with full residue retention compared to conventional raised beds with residue retention.

Soil organic matter

Increased content of soil organic matter, after the fifth year, in the CA treatments in the top soil, implies that the yearly residue retained in the field coupled with reduced tillage foster SOM build up through the slow decomposition rate taking place across the growing seasons (Karlen et al., 1994). This is as reported by (Bot and Benites, 2005) that the residues on the soil surface

Table 4. Earthworm counts per square meter of soil after five years of continuous cropping (2007 - 2011) at Chitedze Research Station, Malawi.

Treatment	Depth (cm)			Total
	0-10	10-20	20-30	
Common practice	9	7	1	17
CA Basin-sole maize	19	13	3	35
CA-sole maize	21	20	16	57
Cowpea after maize	43	27	11	80
Maize after cowpea	52	31	16	99
Maize + pigeon pea	30	13	7	50
Maize + cowpea	40	20	8	68
Maize + velvet beans	30	11	4	45

	P value	LSD
Treatments (T)	0.013	11.26
Depth (D)	<.001	6.9
T x D	NS	
CV (%)		39

CV: Coefficient of variation, LSD: Least Significant Difference, NS: Not significant.

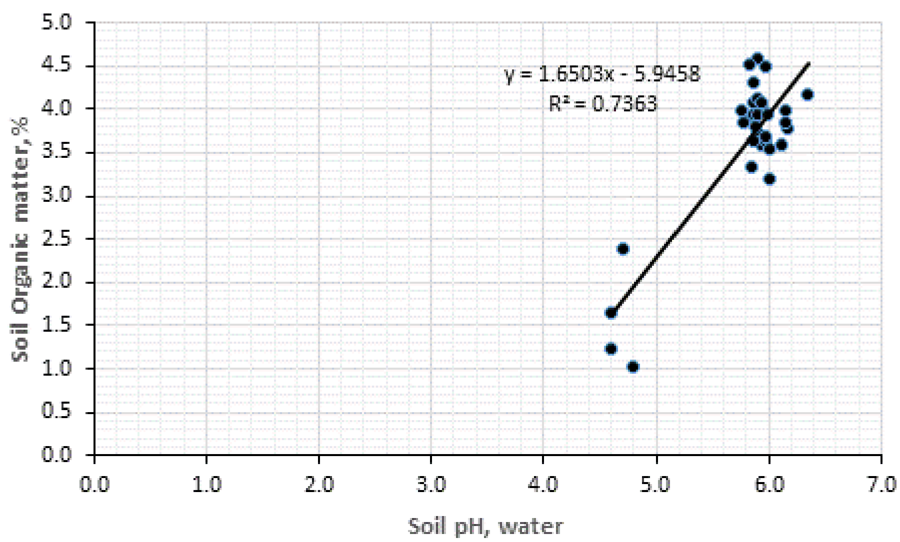

Figure 3. Correlation between soil organic matter and soil pH in the fifth year of practicing CA on the top soil, 0 – 10 cm soil depth.

slow the carbon cycle because they are exposed to fewer microorganisms and thus the decomposition is more slowly, resulting in the production of more stable humus. In the longer term, the slowly decomposing residue materials will lead to the accumulation of organic matter and availability of nutrients in the whole soil system under CA (Thierfelder and Wall, 2012). The low levels of SOM in the common practice plot are primarily due to crop residue removal and soil tillage that aerates the soil and speeds up the decomposition rate of the organic matter (Jackson, 1993). The level of organic matter present in the soil is a direct function of how much organic material is being produced or added to the soil versus the rate of decomposition (Flessa et al., 2000). Consequently, increased values of the SOM under conservation agriculture could create optimum conditions for plant growth as observed by Jackson (1993) who indicated that SOM maintains favourable conditions of moisture, temperature, nutrient status, pH and aeration for optimum plant growth.

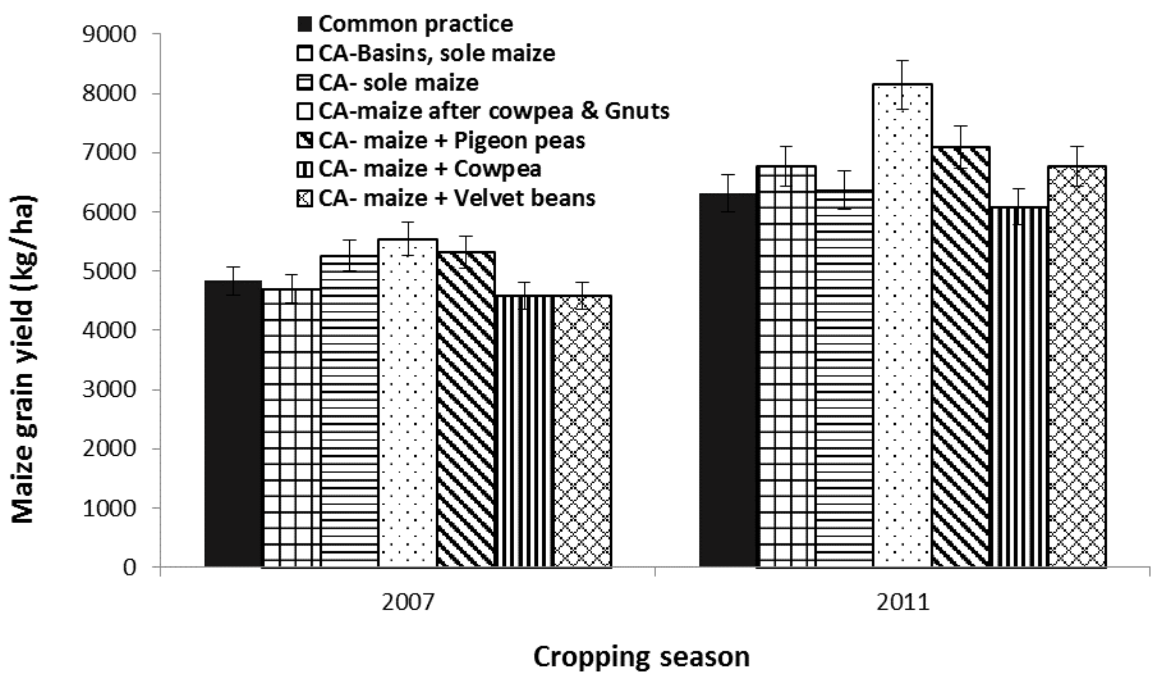

Figure 4. Maize grain yield (kg ha^{-1}) in year one, 2007 and year five, 2011 of trial implementation.

Correlation of soil organic matter and pH in the 5th year

A positive and strong correlation between soil organic matter and pH in the 5th year implies that the soil organic matter content has an influence on the acidity of the soil. When a soil has an increased SOM, the acidity decreases, because the carboxyl groups on the humus develop negative charge and suppress the positively charged H that reacts with the hydroxyl (OH^-) to form water (McCauley et al., 2017). Hence, with an increase in organic matter, the soil recovers its natural buffer capacity.

Soil aggregate stability

Larger percentage of soil aggregates greater than 2 mm diameter in the conservation agriculture treatments as compared to the control treatment was attributed to good soil structure brought in by minimum soil tillage and residue retention. Conservation agriculture practices would therefore promote increased water infiltration, increased aeration, and increased water-holding capacity (Boyle et al., 1989). The findings of this study are in agreement with Kladviko et al. (1986), Six et al. (2004) and Simpson et al. (2004) who noticed that reduced soil disturbance and higher soil organic matter contents contribute to the building up of a stable aggregated and porous structured soil matrix (Kladviko et al., 1986; Six et al., 2004; Simpson et al., 2004). Plant residues retention on the soil surface in conservation agriculture protect the soil from raindrop impact while no protection occurs when residues have been removed, causing further susceptible soil aggregate disruption (Six et al., 2000). The reduced aggregation in common tillage practice, again, is a result of direct and indirect effects of tillage on the soil aggregates (Beare et al., 1994). Physical disturbance of soil structure through tillage results in a direct breakdown of soil aggregates and an increased turnover of aggregates (Six et al., 2000). Tillage also increases fragmentation of roots and mycorrhizal hyphae, which are major binding agents for macro aggregates (Tisdall and Oades, 1982; Bronick and Lal, 2005).

Earthworm populations

The significant difference between earthworm's densities in conservation agriculture and common agricultural practice is a good indicator of improved soil health under CA cropping systems. Earthworms occur in warmer places with high content of SOM and soil N (Mando and Stroosnijder, 1999). Although tillage is the main factor that affects earthworm populations, mulched crop residues are also important in maintaining a good water potential of the surrounding soil media for increased growth in numbers of earthworms (Edwards and Bohlen, 1996). The vertical movements of earthworm into the soil aids air circulation deeper into the soil, stimulating microbial nutrient cycling at those deeper levels (Edwards and Bohlen, 1996). Earthworm tunnelling can increase the rate of water percolation into the ground 4 to 10 times higher than fields that lack worm tunnels (Edwards and Shipitalo, 1998). The earthworm counts obtained in this study supports the findings reported by Thierfelder and Wall (2010), from similar experiment in Zambia where earthworm populations were, on average, 450% higher in the conservation agriculture treatments than in the conventionally tilled treatment. Consequently, annual soil tillage constantly disturbs the earthworm habitat, whereby food and moisture available for earthworms and other organisms are acutely reduced. Additionally, tillage promotes soil aeration, enhancing rapid oxidation of the limited SOM which in return leads to reduced earthworm numbers (Edwards and Shipitalo, 1998; Thierfelder and Wall, 2012) in the conventionally tilled plot. Earthworms need oxygen, tapped just under the near soil surface in order to carry out their metabolic processes, hence larger earthworm population in the top soil than in subsoil. Increased number of earthworms in CA plots is as a result of the enhanced soil surface roughness brought in by soil cover that increased aeration, soil moisture and SOM content on the soil surface (Ghabbour, 2010).

Maize yield

Other than higher rainfall in the 5th year (Figure 1), the higher maize yields might have been attributed to improved soil quality trend observed in the 5th year as compared to the first year of trial implementation. This might be a reflection of the improved soil quality as identified by all the soil quality indicators (chemical, physical and biological) by the 5th year. The results are in line with the findings of Govaerts et al. (2007) who observed a direct and significant relation between the soil quality status and the crop yield under zero tillage with crop residue retention.

Conservation agriculture fosters a gradual increase of soil pH, and SOM at medium term as compared to common tillage. Conservation agriculture cropping systems improves the physical structure of the soil (more soil aggregates >2 mm in diameter). Soil under conservation agriculture becomes more active biologically as compared to the soils under common tillage practice after a medium term period of five years and hence increased maize production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Dry matter production, chemical composition and nutrient accumulation in winter crops

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Some winter crops sown in no-tillage system can represent an important alternative to nutrient cycling. The objective of this work was to evaluate the production of dry matter (DM) and accumulation of nutrients for winter cultivation in the West of Paraná. The experimental design was a randomized block, with four treatments and six replications. The treatments were represented by four different winter crops (oat IPR 126, crambe FMS Brilhante, radish common cultivar and wheat BRS Taruma), and the DM, the contents of C, N, P, K, Ca, Mg and C/N ratio in DM and nutrients accumulation were determied The dry matter production was higher for radish with 4.929,14 kg ha^{-1}. The different winter crops used influenced the contents of C, N and C/N ratio. The other studied characteristics were not influenced. Among the four winter cultivation the radish presented larger production of dry matter. The chemical composition was influenced by the cultivations, the contents of C, N and C/N ratio, consequently in the contribution differentiated in the area. The winter cultivation in the studied conditions influences the accumulation of magnesium.

Key words: Nutrient cycling, decomposition, mineralization.

INTRODUCTION

The winter crops sowed in no-tillage system, have ability to absorb nutrients in subsurface layers, and, then releasing them in the surface layers through decomposition and mineralization of the residue (Torres et al., 2008), and contribute to the efficient use of fertilizers in annual crops succession (Calegari, 2004; Carvalho et al., 2004). These species help in soil conservation, the largest aggregation of particles and for

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Table 1. Chemical characteristics of the soil (0 - 10; 10 - 20 cm), in the experimental area, prior to deployment of winter crop, in Marechal Cândido Rondon.

Layer	P	MO	pH	H+Al	Al^{3+}	K^+	Ca^{2+}	MG^{2+}	SB	CTC	V
cm	mg dm^{-3}	g dm^{-3}	$CaCl_2$	------------------------$cmol_c$ dm^{-3}-----------------------------							%
0-10	24.49	32.64	4.55	9.40	0.46	0.53	4.56	1.54	6.63	16.02	41.66
10 - 20	25.86	32.64	4.65	8.62	0.34	0.44	5.32	1.67	7.42	16.04	46.32

the protection of the soil surface to the direct impact of the rains (Pacheco et al., 2011).

In addition, winter crops are recommended for prolonging the period of pasture use, as well as increasing the quality and increasing the contribution of biomass, allowing the pasture to supports a greater number of animals (Santos, 2003), promote recovery and the maintenance of soil quality, since they are used as cover crops with the objective of maintaining and increasing the organic matter contents (Kliemann et al., 2006). These can still provide grain production for human and animal consumption (Bortolini et al., 2004), increased sustainability of agricultural systems (Boer et al., 2007), and optimize land use, the infrastructure and workforce allow in diversifying and verticalizar in production (Mello et al., 2004).

The cultivations commonly cultivated are the white oats, oats, rye, the barley, triticale and wheat. These cultivations can also be used as dual-purpose species, producing fodder early and even grains, with low cost, contributing to greater stability in production (Bortolini et al., 2004). Crambe and radish cultivations as winter crops in the western region of Paraná are not yet frequent.

Magalhães et al. (2000) evaluated the relationship between dry mass production and the export of nutrients in soils situated in the cerrado vegetation with several years of use which observed that the production of dry matter, the nutrient contents of the aerial part, and the quantities exported varied with the amount of years of soil use by the forage. The amount of nutrients in plant biomass with high C/N ratio, which release slow and gradual nutrients over time, may reduce the cost of fertilizer use in the next crop for the best use of nutrients contained in decomposing biomass.

The presence of nutrient in the dry biomass of the plants results in less loss due to erosion and leaching, than being directly in the soil, thus, knowing the levels that is important for the management of these nutrients within the cycles of cultivation (Pittelkow et al., 2012). Therefore, the objective of this work was to evaluate the dry matter production and the nutrient contents in cover crops as the oats IPR 126, crambe FMS Brilliant, common radish and BRS Tarumã wheat, grown in Eutrophic Red Latosol (LVe).

MATERIALS AND METHODS

The study was conducted at the Experimental Farm "Professor Antonio Carlos dos Santos Person " (latitude 24°33 ' 22' ' S and longitude 54°03' 24 ' ' W , with an altitude of approximately 400 m) at the Universidade Estadual do Oeste do Paraná - *Campus* Marechal Cândido Rondon in Eutrophic Red Latosol (LVe) (Embrapa, 2013). Table 1 described the chemical and physical characteristics of the area before the experiment. Due to the low V% (percentage of saturation of bases) liming was performed 30 days before sowing at a dosage of 2 Mg ha $^{-1}$ (large 80 %) to raise up to 70%.

The area of conducting the experiment has a history in which for a period of four years, traditionally, the winter corn were grown (for silage production) in the off season and soybeans in the summer crop. These crops were always performed under the no-tillage system. The local climate, classified according to Koppen, is Cfa, subtropical humid mesothermal dry winter with rainfall were distributed throughout the year and in hot summers. The average temperatures of the quarter more cold vary between 17 and 18°C, and the quarter more hot between 28 and 29°C in its turn, the annual temperature ranged between 22 and 23°C. The total average annual precipitation normal pluvial for the region vary from 1600 to 1800 mm. with quarter, more humid presented totals which is between 400 to 500 mm (IAPAR, 2006). The climate data of the experimental period were obtained in automatic climatological station of the University of Paraná, distant approximately 100 m of the experimental area and are presented in Figure 1.

The experiment started in autumn-winter of 2012 and the area has been desiccated in 30 days before sowing, using glyphosate-isopropylamine salt in the dose of 3.0 L ha^{-1} with a volume of 250 L ha^{-1}. The experimental design used was a randomized block and the treatments consisted of 4 different winter crops (IPR 126 oats, Brillante FMS crambe, radish common cultivar and BRS Tarumã wheat) and 6 blocks. Winter crops were sown in the day 19/04/12, with drill seeder, coupled to the tractor on direct sowing system on maize straw. 60 kg ha^{-1} of oats` seed, 15 kg $ha^{-1}$ of crambe` seed, 15 kg ha^{-1} of radish` seeds and 90 kg $ha^{-1}$ of wheat` seeds, with 0.17 m between lines were used. The fertilizer for growing oats, f. radish, fodder wheat and radish, was performed according to CQFSRS/SC (2004). For the correction of soil fertility 200 kgha-1 formulated 8-20-20 (N, P_2O_5 and K_2O, respectively) were used. The fertilization in coverage was carried out using 90 kg ha^{-1} of N as urea.

Sampling for the determination of dry matter and nutrient contents by plants was performed 90 days after sowing of winter crops, in this period the crambe and the radish were at the maturation stage, the oats in the flowering stage and the wheat at the breeding stage. With the aid of a metallic square cast with a known area (0.25 m²) which was randomly released in each plot, all plant material contained inside was collected. After the collection, the material was submitted to drying in an oven with forced ventilation of air under a temperature of 55°C for 72 h, with subsequent weighing for determination of the dry matter.

After drying, the samples were crushed in the mill type Willey, with sieve of 20 meshes, for the determination of concentrations of total C, N, P, K, Ca and Mg. The C was obtained from the determination of organic matter in muffle as described by Silva and Queiroz (2006). To estimate the concentration of C in the samples the concentration of organic matter was divided by 1.72 as

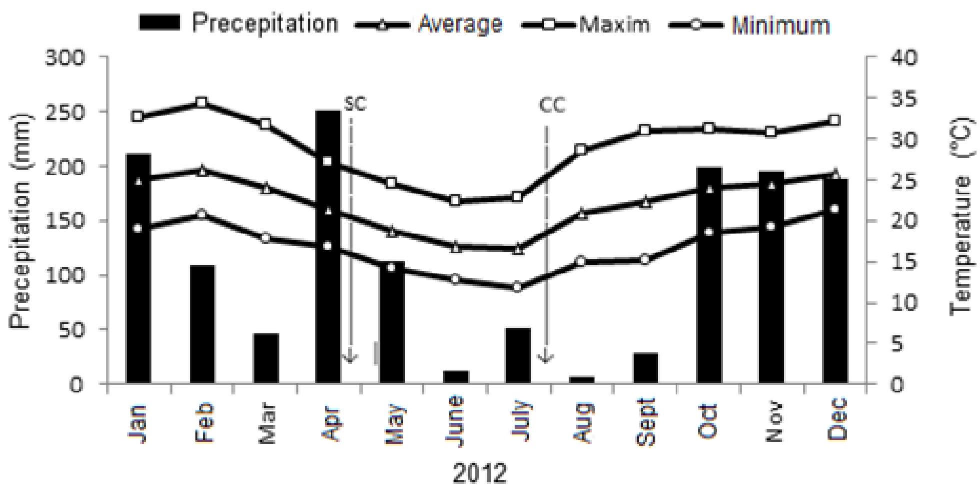

Figure 1. Monthly average temperatures maximum, minimum and average and precipitation accumulated during the months of the experimental period. SC: Seeding of winter crops. CC: collection of the winter crops. (Source: Automatic climatological station of the Nucleus of Experimental Stations of UNIOESTE, Marechal Cândido Rondon-PR).

Table 2. Calculated F values of the dry matter chemical composition of different winter crops, managed under no-tillage system

Source of variation	DF	Dry Matter	K	Ca	MG	P	C	N	Ratio C/N
Crops	3	5.71*	0.65	1.28	1.30	0.89	18.64*	69.20*	14.62*
Block	5	1.17	0.34	0.58	2.29	0.32	0.25	4.15	1.52
Error	15	-	-	-	-	-	-	-	-
CV (%)	-	21.71	35.22	49.68	42.39	37.25	11.20	5.75	14.45

*Significant at 5% probability by the F test, respectively. CV: Coefficient of variation.

recommended by Peixoto et al. (2007). The N was determined by sulfuric digestion and distillation in Kjeldal semi-micro system, while for macronutrient determination, nitroperchloric digestion was carried out, with subsequent reading in an atomic absorption spectrophotometer (Embrapa, 2009).

The accumulation values of N, P, K, Ca and Mg in the aerial part of the plant were obtained by multiplying their concentration in the tissue in the production of DM, being expressed in kg ha^{-1}. The data obtained were submitted to statistical analysis using the SISVAR program (Ferreira, 2011), and the averages compared by the Tukey test at 5% level of probability.

RESULTS AND DISCUSSION

According to the results, there was a significant difference ($p < 0.05$) in the dry matter production of winter crops, as well as changes in carbon (C), nitrogen (N) and relation C/N between the studied cultivation. However, the values of potassium (K), calcium (Ca), magnesium (MG) and phosphorus (P) were not influenced ($p > 0.05$) by treatments (Table 2).

Production of dry matter

For DM there was a significant difference between the studied crops, which radish provided higher dry matter yield than wheat, but similar to oats and crambe (Table 3). The results for the radish are very close to those found by Lima et al. (2007), evaluating the behavior of radish (*Raphanus sativus* L.), found that at the stage of flowering, this culture presented 5,480.5 kg ha^{-1} of dry matter, consisting of a desirable property for a green manure.

The highest production of radish in relation to the wheat due to the cycle of the crops is because the radish has a shorter cycle, presenting its peak production and accumulation of DM earlier in relation to the wheat of double purpose that has a longer cycle. Crusciol et al. (2005) verified the production of 2,938 kg ha^{-1} of dry matter in the aerial part of radish, cultivate Siletina, when the seeding density was 20 kg ha^{-1}. This value was lower than that found in the present study, in which the seeding density was 15 kg ha-1 of fodder radish.

According to Calegari (1998), the radish presents average yield of 3,000 kg ha^{-1} of shoot dry matter, and, even in areas without fertilization, this value may vary between 2,000 and 6,000 kg ha-1 of dry matter in the stage of flowering. Heinz et al. (2011) found the production of dry matter of the aerial part 5.586kg ha^{-1}

Table 3. Production and chemical composition of dry matter, of different winter crops, managed under no-tillage system.

Crops	DM (Kg ha^{-1})	C (g kg^{-1})	N (g kg^{-1})	Ratio C/N	K (g kg^{-1})	Ca (g kg^{-1})	MG (g kg^{-1})	P (g kg^{-1})
Oats	A	112.90^{b}	18.43d	6.16^{b}	20.54	5.23	1.40	0.54
Crambe	3951^{ab}	153.10^{ab}	26.19^{b}	5.86^{b}	22.29	7.08	1.52	0.56
Radish	4929^{a}	185.35^{a}	22.28c	8.46^{a}	16.83	6.17	1.71	0.41
Wheat BRS	2893^{b}	149.09^{ab}	29.23^{a}	5.11^{b}	21.06	4.06	1.04	0.57
Average	4014	150.11	24.03	6.4	20.18	5.64	1.42	0.52

*Medium followed by the same lowercase letter in the column do not differ statistically by the Turkey test (5%).

and 2.688kg ha^{-1} for the radish and the crambe, respectively, observing a 52% higher biomass production of the radish in relation to the crambe on the day of the management. The difference in the dry matter production observed between these works can be attributed to the climatic conditions, soil conditions, the cultivars used or the stage at which the plants were managed.

Nutrients concentration

The values obtained for the total concentration of N in the plants showed significant differences between the four studied crops, among which the N content of the wheat crop was higher (Table 3). The N content of the aerial part of this grass reached 29.23 g kg^{-1}, followed by the crambe with an accumulation of 26.19 g kg^{-1}, radish with 22.28 g kg^{-1} and finally the oat with 18, 43 g kg^{-1}. The oats, despite having a smaller amount of N than the other crops (Borket et al., 2003), may also contain reasonable quantities of N due to the amount of total N contained in biomass as well as the radish by efficiency in nutrient cycling of N in the soil (Aita et al., 2001).

Considering the levels of C found, there were significant differences between crops. The radish presented the highest values with 185.35 g kg^{-1}, differing from crambe and wheat, which presented intermediate values 153.10 and 149.09 g kg^{-1}, respectively and oats with 112.90 g kg $^{-1}$ (Table 3). The no-tillage system favors the sequestration of carbon by plants, since it increases the influx of C via organic material, which due to the minimal mobilization of the soil, shows slow and gradual decomposition, reducing the efflux of C from the ground to the atmosphere, determining the positive balance in the accumulation of C in the soil (Bayer et al., 2006).

For C/N ratio, the significant difference was found for radish in relation to other crops, and in 8.46 for the cultivation of radish. For the C/N ratio the significant difference was found for the radish in relation to the other crops, constituting in 8.46. The C/N ratio has been the most used feature in models to predict the availability of N in the soil during the decomposition of organic materials (Nicolardot et al., 2001). The leguminous have lower C/N ratio in the aerial part, forming a material that presents a C/N ratio lower than that of equilibrium (< 28/1), and is thus a material that during decomposition releases nitrogen to the crop deployed in succession on the crop residues (Diekow et al., 2005).

Radish, has a mean C/N ratio, in the range of 20 to 25 (Giacomoni et al., 2003), and, consequently, a high mineralization rate, comparable to that of leguminous (Amado et al., 2002). This species is also characterized by the behavior of a plant that recycles and provides nutrients, especially nitrogen (Aita and Giacomoni, 2003) and potassium (Giacomoni et al., 2003). In this study, the low value found for C/N ratio for the radish (8.46) is due to the time when the crop management was carried out.

The other chemical elements such as K, Ca, Mg and P studied, did not present significant differences among the crops. The highest concentration of potassium was obtained in the crambe crop with 22.29 g kg $^{-1}$, which also had the highest calcium concentration (7.08 g kg $^{-1}$). The magnesium was found in greater quantity in the culture of the radish that showed 1.71 g kg^{-1} of this nutrient. The phosphorus was found at higher concentrations in the wheat crop with 0.57 g kg^{-1} (Table 3).

Nutrient accumulation

For the accumulated values of the nutrients in the dry matter there was difference ($p < 0.05$) only for the values obtained of MG, already for the accumulation of C; N; K; Ca and P which has no difference (Table 4). In relation to magnesium the wheat crop accumulated a highest amount (9.15 kg ha^{-1}) when compared to the crambe (3.96 kg ha^{-1}) and the radish (4.31 kg ha^{-1}) and was similar to oats (5.50 kg ha^{-1}).

Kubo et al. (2007), with the objective of verifying the dry matter production and the nutrient accumulation by the crops of white oats, wheat and black oats verified that the greater accumulated amount of the nutrient phosphorus was in black oat with and without fertilization of N (9.71 and 10.95 kg ha^{-1}, respectively), which were higher significantly in wheat (0.76 kg ha^{-1}) and white oat (0.39 kg ha^{-1}). And for the potassium accumulated obtained the

Table 4. Accumulation of nutrients in dry matter, from different winter crops, managed under no-tillage system.

Crops	C (kg ha^{-1})	N (kg ha^{-1})	K (kg ha^{-1})	Ca (kg ha^{-1})	MG (kg ha^{-1})	P (kg ha^{-1})
Oats	614.62	114.74	80.11	24.99	5.50^{ab}	2.06
Crambe	595.54	81.10	78.60	17.87	3.96^{b}	1.71
Radish	571.26	92.24	80.59	20.14	4.31^{b}	2.13
Wheat BRS	630.08	91.11	81.23	30.46	9.15^{a}	2.34

*Medium followed by the same lowercase letter in the column does not differ statistically by the Tukey test (5%).

highest amount in black oat with N fertilizer (216 kg ha^{-1}) followed by black oat without fertilization of N (212 kg ha^{-1}) were significantly higher than in the white oats (48.38 kg ha^{-1}) and wheat (38.63 kg ha^{-1}).

The accumulation of N in this study although not significant, was higher in the culture of oats which presented 114.74 kg ha-1, differs from the results found by Monteiro et al., (2004), with the aim of assessing nutrient accumulation of species intercropped or grown alone which verified that in isolated cultivation, the amount of N accumulated by vetch in the three years was superior to that of oats and, in the second year alone, was superior to the radish. In the years evaluated, the accumulation of N by the aerial part of the leguminous reached 113, 91 and 63 kg ha^{-1} in 1998, 1999 and 2000, respectively, against 101, 67 and 63 kg ha $^{-1}$ in the radish and only 59, 57 e 42 kg ha^{-1} in oats.

Conclusions

Among the four winter crops the radish had higher dry matter production, crambe and oats showed intermediate production while wheat was the crop of lower production. The chemical composition was influenced by the cultures, in the C, N and C/N ratio, consequently in the differentiated contribution in the area.

Winter crops, under the conditions studied, influence the accumulation of magnesium.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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