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Evaluation and characterization of locally developed maize inbred lines for agro-morphological traits in Sri Lanka

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Abstract

Hybrid maize is widely grown in Sri Lanka due to its higher yield potential gained through the heterosis breeding. The evaluation and characterization of maize inbred lines is primarily essential for selecting inbred parents with desirable agro-morphological traits to develop new hybrids with higher hybrid vigor. A study was conducted to evaluate and characterize 44 locally developed maize inbred lines for 17 agro-morphological traits at Field Crops Research and Development Institute, Mahailluppallama. A higher coefficient of variation was observed in grain yield, grain weight per cob and number of tassel branches. Inbred lines were significantly different for all agro-morphological traits. Grain yield showed significant and positive correlation with ear length, number of seeds per row, plant height, height to upper most ear, leaf length and tassel length. The grain yield was negatively correlated with anthesis to silking interval and days to silking. The cluster analysis based on quantitative traits, grouped 38% inbred lines into large cluster (C-3), three similar size clusters (C-1, C-2, and C-4) and two inbred lines formed distinct cluster (C-5). The evaluated maize inbred lines, MI2008-35, MI2008-13 and MI2008-7 consisted of more than one desirable traits such as high yield (4.0-4.6 t/ha), high 100 grain weight (28 -34 g), high seed number per row (30-32), low ASI (-2 – 2), long ears (16 cm). The information on cluster analysis can be used to identify the male and female parents from different clusters to develop maize hybrids with higher heterotic effect.

Key words: Agro-morphological traits, Cluster analysis, Heterosis breeding, Hybrid vigor, Maize inbred lines

Introduction

Maize (*Zea mays* L) is a highly cross pollinated cereal crop that occupies one fourth of the cultivated cereal extent in the world. It is the second most important cereal crop in Sri Lanka where the cultivated extent is next to rice. The maize grains mainly used to formulate animal feeds and 10-15% from the production is being used for human consumption as boiled green cobs and as a supplementary diet. In 2004, the first local maize hybrid "Sampath" was released and exotic maize hybrids were also introduced. Subsequently MI Maize Hybrid 01 and MI Maize Hybrid 02 were released in 2013 and 2016, respectively (Bentota 2013; Kumari *et al*., 2018). Farmers rapidly realized the advantages of hybrids and the national maize productivity increased two-fold from 1.5 to 3.7 t/ha during the last 8 -10 years (Agstat 2010; Agstat 2018).

Maize inbred lines are used as parents for hybrids or synthetic varieties. Shull (1908) reported a general deterioration of yield and vigor of maize inbred lines, but a cross between two inbred lines has immediately and completely recovered. Maize inbred lines are produced through inbreeding by a series of self-pollination for 8-10 generations. However, self-fertilization is the most extreme form of inbreeding resulting in rapid approach to homozygosity. There are less restrictive forms of inbreeding as half-sib, full-sib and back crossing (Hallauer and Miranda, 1988). Apart from conventional breeding techniques, double haploid techniques are used to produce inbred lines for commercial maize hybrid breeding programs in the recent past. It enables to produce completely homozygous lines within a year compared to conventional methods which takes 4-5 years (Aliyi, 2017).

The morphological characterization of developed maize inbred lines is the initial step of selecting parents for a hybrid development program. The characterization and grouping of germplasm facilitates the breeders to identify genetically diverse parents, which can contribute for high heterotic effect, and to avoid duplications in germplasm. Cluster analysis is a convenient method for organizing data sets so that the information can be retrieved more efficiently and be easily understood without the need for complicated mathematical techniques. Further, it enables the breeders and geneticists to identify subsets of accessions which have potential utility for specific breeding or genetic purposes (Rincon *et al*., 1996). Hence, the maize inbred lines developed at the Field

Crops Research and Development Institute (FCRDI) at Mahailluppallama, Sri Lanka were evaluated for agro-morphological traits with the objective of identifying the genetic diversity and to incorporate them to hybrid maize breeding program.

Materials and methods

Development of maize inbred lines

A total of 44 Maize inbred lines were developed at FCRDI using pedigree method during 2008-2015 and 2012-2016. Three exotic source populations (2008-series lines, 2012-P lines and 2012-NK lines) were used to isolate inbred lines. Eight to nine generations were advanced and homozygous promising inbred lines were selected.

Field management

A total of 44 locally developed homozygous maize inbred lines and one CIMMYT (International Maize and Wheat Improvement Center) maize line were evaluated in a Randomized Complete Block design with two replicates during *Maha* season (October to February) 2018/19. The experiment site was located at an altitude of 117 m, longitude of 80° 28" E and latitude of 80° 07" N. The plot size was 5 m x 0.6 m. The spacing between two plants within row was 0.3 m and 17 plants were maintained per experiment plot. An inorganic fertilizer mixture was applied at sowing (75 kg/ha of Urea, 50 kg/ha of Muriate of Potash and 100 kg/ha of Triple Super Phosphate) and 250 kg/ha of Urea was applied in two equal split as a top dressing at four to six weeks after sowing. Weeding was done manually and insect pests were controlled chemically.

Data collection and data analysis

A total of 16 qualitative traits were measured. Days to 50% tasseling and silking were recorded in each plot while plant characters such as plant height at flowering (cm), height to upper most ear (cm), ear leaf length (cm), ear leaf width (cm), tassel length (cm) and number of tassel branches were measured in three randomly selected plants in a plot. The ear characteristics such as ear length (cm), ear girth (cm), number of seed rows, number of seeds per row, grain weight per ear (g), 100 grain weight (g) and shelling percentage (%) were measured from three randomly selected ears from each plot after harvesting (IPBGR, 1991). The grain weight was adjusted to 14.5% moisture level.

Quantitative traits were subjected to Analysis of Variance. The major descriptive statistics were calculated. Correlation coefficient among quantitative characters was estimated by using the formulae of Snedecor and Cochran (1980). Principal Component Analysis (PCA) was done using standardized quantitative variables by employing MINITAB 14 software (MINITAB, 2004). The scores of first five principal components, which accounted for 77% of the total variability, were used for the cluster analysis. The similarity matrix was calculated using Euclidean distance and germplasm accessions were grouped using Wards linkage method (Ward, 1963). Dendrogram was obtained from MINITAB 14 software.

Results and discussion

Descriptive statistics

Table 1 shows the important descriptive statistics calculated for local maize inbred lines. A substantially higher variability was observed in most of the agronomic traits. Higher coefficient of variation was observed for grain yield, grain weight per cob, anthesis-silking interval (ASI) and number of tassel branches. Among the traits, the days to 50% flowering (tasseling and silking) and shelling percentage showed the lowest variability. The observed variability of morphological traits in locally developed maize inbred lines was supported by many diversity analysis studies on maize inbred lines (Iqbal *et al*., 2015; Natalija *et al*., 2016; Rocha *et al*., 2019; Sokolove and Guzhva, 1997). The morphological variation is mainly due to genetic factors and also subjected to environmental factors. Even though these 44 inbred lines were derived from three exotic populations, it showed significant variability and coefficient of variation of measured morphological traits.

Analysis of variation of maize inbred lines showed significant differences ($p<0.05$) in all measured agro-morphological traits (Table 2). The highest grain yield (4.2-4.6 t/ha) was observed in inbred lines MI2008-7, MI2008-35, MI2008-13, MI2012-P1 and MI2012-P3. The highest number of seed rows (16 rows) were observed in MI2008-13, MI2008-18 and MI2008-23. The longest ears (16 cm) were observed in MI2012-NK-9, MI2008-7, MI2008-35, and MI2008-2. The lowest ASI (-2 to 1) days was observed in MI2008-30, MI2008-2, MI2012-NK9, MI2008-37, MI2012-NK8, MI2012-P-1, MI2008-25, MI2008-17 and MI008-13. The inbred lines, MI2008-35, MI2008-13 and MI2008-7 were elite lines and consisted of two or more economically important traits such as higher yield, longer ears, lowest ASI, highest number of seed rows and higher seeds per row.

Table 1. Descriptive statistics of quantitative traits of maize inbred lines evaluated at Mahailluppallama *Maha* 2018/19

Quantitative trait		Mean	SE	CV%	Minimum	Maximum
Grain yield (t/ha)	GY	2.6	0.13	40	1.0	4.6
Grain weight per ear(g)	EGW	63	3.10	33	25	111
Shelling percentage (%)	SP	86	0.72	6	74	97
No. of seed rows	SR	12	0.19	10	10	16
Ear Length (cm)	EL	13	0.24	12	10	16
Ear girth (cm)	EG	11	0.17	10	9	13
No. of Seeds per row	SN	24	0.74	20	13	35
Weight of 100 seeds (g)	100SW	24	0.69	19	16	34
Plant height (cm)	PH	113	2.74	16	78	150
Ear height(cm)	EH	42	1.77	28	20	69
Ear Leaf length (cm)	LL	70	1.22	12	51	90
Ear leaf width (cm)	LW	8	0.16	14	6	10
Days to 50% Tasselling	DT	65	0.45	5	59	73
Days to 50% silking	DS	67	0.51	5	61	76
Anthesis silking interval	ASI	2.1	0.21	65	-1.5	5.5
Tassel Length (cm)	TL	33	0.64	13	24	43
No. tassel branches	TB	5	0.31	39	1	9

SE – Standard Error, CV – Coefficient of variation

Table 2. Mean sums of square and probability of F test of quantitative traits of maize inbred lines

Variable	GY	EGW	SR	EL	SN	100 SW	PH	EH	LL	LW	DT	DS	TL	TB
MSS	3.2*	4.0*	2.9*	1.3*	4.7*	2.1*	7.3*	5.7*	6.5*	2.6*	9.1*	6.6*	6.0*	3.6*
CV%	30	27	10	25	16	17	8.2	15	6.8	12	2.4	2.9	7.6	29

**maize inbred lines are significantly different at p=0.05*

GY - Grain yield (t/ha), EGW - Grain weight per ear (g), SP - Shelling percentage (%), SR - No. of seed rows, EL - Ear length (cm), EW - Ear width (cm), SN - No. of Seeds per row, 100 SW - Weight of 100 seeds (g), PH - Plant height (cm), EH - Ear height (cm), LL - Leaves length (cm), LW - Leaves width (cm), DT - Days to 50% Tasselling, DS - Days to 50% silking, ASI - Anthesis silking interval, TL - Tassel

Correlation analysis of quantitative traits

The correlation analysis of morphological traits are given in Table 3. The grain yield was positively and significantly correlated with ear length, number of seeds per row, plant height, height to upper most ear, leaf length and tassel length. The grain yield was significantly ($p<0.05$) but negatively correlated with anthesis silking interval and days to silking.

Table 3. Pearson's correlation coefficient and probability among the 12 qualitative traits of 45 maize inbred lines at FCRDI, Sri Lanka, *Maha* 2018/19

GY	EGW	EL	SN	PH	EH	LL	LW	DT	DS	ASI	TL	
EGW	0.98*											
	0											
EL	0.35*	0.35*										
	0.02	0.02										
SN	0.64*	0.64*	0.42									
	0	0	0.01									
PH	0.35*	0.35*	0.19	0.39*								
	0.02	0.02	0.2	0.01								
EH	0.36*	0.36*	0.06	0.29	0.84*							
	0.02	0.02	0.69	0.06	0							
LL	0.42*	0.42*	0.24	0.43*	0.49*	0.43*						
	0	0	0.11	0	0	0						
LW	0.22	0.22	0.01	0.15	0.35	0.46	0.39					
	0.16	0.16	0.93	0.32	0.02	0	0.01					
DT	-0.17	-0.17	-0.07	-0.26	-0.47*	-0.52*	0.09	-0.1				
	0.27	0.27	0.64	0.08	0	0	0.57	0.53				
DS	-0.30*	-0.3	-0.15	-0.36*	-0.46*	-0.47*	0.09	-0.05	0.91*			
	0.05	0.05	0.34	0.02	0	0	0.58	0.73	0			
ASI	-0.37*	-0.37*	-0.2	-0.31*	-0.13	-0.04	0.02	0.07	0.09	0.49*		
	0.01	0.01	0.19	0.04	0.39	0.79	0.88	0.63	0.55	0		
TL	0.50*	0.50*	0.28	0.40*	0.38*	0.31	0.64*	0.31*	0.05	0	-0.1	
TB	0.2	0.2	0.14	0.13	0.36*	0.28	0.37*	0.50*	0.15	0.22	0.22	0.34*
	0.19	0.19	0.35	0.38	0.02	0.06	0.01	0	0.32	0.14	0.14	0.02

GY - Grain yield (t/ha), EGW - Grain weight per ear (g), (%),EL - Ear length (cm), EG - Ear girth (cm), SN - No. of Seeds per row, , PH - Plant height (cm), EH - Ear height(cm), LL - Leaves length (cm), LW- ear leaf width (cm), Tasselling, DS - Days to 50% silking, DT- Days to tasseling, ASI - Anthesis silking interval, TL - Tassel Length (cm), TB - No. tassel branches

The phenotypic correlations are influenced by genetic nature (pleiotropic effects, linked genes) and environmental factors and hence, they are often assumed to reflect genotypic correlation. Therefore, correlations also give reliable and useful information on nature, extent and direction of selection (Zeeshan *et al*., 2013). Correlation between yield and other triats were observed in many previous studies (Iqbal *et al.*, 2015; Shazia *et al*., 2017; Pavlov *et al*., 2015; Rocha *et al*., 2019). The tassel length and number of tassel branches showed a significant positive correlation ($p<0.05$) with plant height, ear leaf length and width. The taller lines with longer leaves, longer tassel and longer ears indicated the higher vigor of inbred lines. Naturally, inbreeding leads to loss of vigor. However, comparative higher vigor of inbred lines with higher yield is important to improve the yield of single cross hybrids and increase the hybrid seed yield.

Principal componenet analysis

Principal Component Analysis (PCA) assist in finding a new set of uncorrelated variables (principal components) from original correlated variables. Hence, principal components are the linear combinations of the original variables. The PCA showed that the first five PCs contributed to 77% of total variability of 45 maize inbred lines for the 17 traits (Table 4). The PC-1 separated the accessions based on grain yield per ha, grain weight per ear, ear length, ear girth and number of seeds per row. The traits, 100 grain weight, days to 50% flowering and number of tassel branching contributed for PC-2. First two components together captured 51% of total variability. Iqbal *et al*. (2015) and Natalija *et al*. (2016) reported that ear parameters, plant height and days to flowering and grain yield contributed to the first two PCs.

Table 4. Principal component coefficients of first five principlal componenets, their eigen valuaes and commulative percentage of variability expained for quantitative traits

Variable	PC-1	PC-2	PC-3	PC-4	PC-5
GY	**0.33**	0.09	0.25	0.14	-0.03
EGW	**0.33**	0.09	0.25	0.14	-0.03
SP	0.06	-0.24	-0.01	0.61	-0.29
SR	0.11	0.01	0.00	**-0.58**	**-0.58**
EL	**0.31**	0.11	0.30	0.01	-0.04
EG	**0.31**	-0.17	0.07	-0.33	0.04
SN	**0.31**	0.05	0.21	0.20	-0.28
100SW	0.20	**-0.29**	-0.02	-0.19	0.51
PH	0.29	0.02	**-0.38**	0.01	0.19
EH	0.28	0.00	**-0.44**	-0.05	-0.01
LL	0.23	**0.33**	-0.07	0.01	0.16
LW	0.15	0.26	**-0.34**	-0.07	-0.28
DT	-0.20	**0.38**	0.31	-0.14	0.09
DS	-0.25	**0.42**	0.14	-0.05	0.07
ASI	-0.17	0.19	**-0.33**	0.18	-0.02
TL	0.24	0.30	0.06	0.05	0.29
TB	0.09	**0.41**	-0.23	0.09	-0.04
Eigenvalue	5.86	2.88	1.98	1.40	1.01
Proportion	0.35	0.17	0.12	0.08	0.06
Cumulative	0.35	0.51	0.63	0.71	0.77

GY - Grain yield (t/ha), EGW - Grain weight per ear (g), SP - Shelling percentage (%), SR - No. of seed rows, EL - Ear length (cm), EG - Ear girth (cm), SN - No. of Seed rows per ear, 100 SW - Weight of 100 seeds (g), PH - Plant height (cm), EH - Ear height(cm), LL - Leaves length (cm), LW - Leaves width (cm), DT - Days to 50% Tasselling, DS - Days to 50% silking, ASI - Anthesis silking interval, TL - Tassel Length (cm), TB - No. tassel branches

Cluster analysis

The 45 maize inbred lines clustered into two main groups based on grain yield at higher level of distance. Then the main groups were divided into sub groups based on grain yeild, shelling percentage, days to flowering, ASI and number of tassel branches. Finally, five groups were formed at Eculeadian distance 11.25 (Figure 1). About 38% (17 lines) of inbred lines formed large cluster C-3. Then almost similar clusters i.e, C-1 (9 lines), C-2 (10 lines) and C-4 (7 lines) and the smallest size cluster C-5 consisted of 2 lines (Figure 1). Cluster C-1 and C-2 consisted higher yeilding, i.e. 2.9 - 3.5 t/ha, inbred lines. However, some of the inbred lines of C-4 also showed a high range for yeild i.e. 1.0 - 4.1 t/ha, but all the inbred lines in C-4 showed longer days to 50% tasseling (70 days) and 50% silking (73 days) compared to the rest (Table 5).

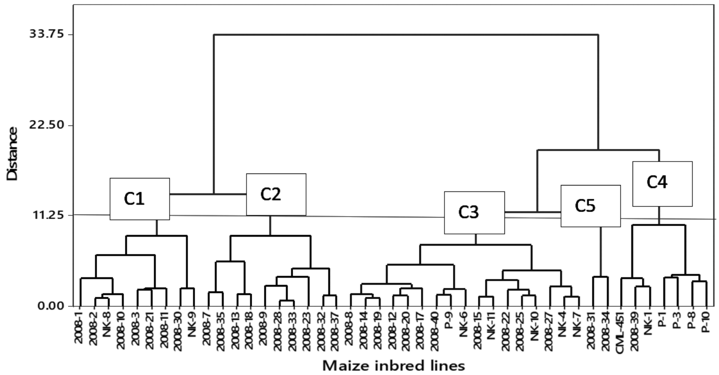

Figure 1. Dendrogram of 45 maize inbred lines based on first five Principal components of 16 qualitative traits using Ward method

Among the five clusters, C-5 consited of the lowest yielding lines with shorter plant height, small ears, shorter tassels and longer ASI lines. The C-2 consisted of higher yielding (3.5 t/ha) lines with taller plant height. However, cluster C-3 showed a higher range for all the traits as it is the largest cluster.

Cluster membership of inbred lines is given in Table 6. The inbred lines grouped together did not correspond to with their original source population that they were derived. Therefore, the possibiltity of genetically diversed inbred lines can be derived from the same source population was revealed. Wasala *et al*. (2013) also reported that grouping maize land races did not show specific correspondence with the geographical sites of collection of the accessions.

The selection of female parents from cluster C-1 or C-2 and male parents from C-4, C-3 or C-5 can be done as these two sets of groupes are diverse in morphological traits. This is because C-1 and C-2 consisted of higher yeilding, low ASI with comparetively vigourous growth habit and C-4, C-3 and C-5 are relatively lower yielding and less vigourous. However, some of the inbred lines of C-4 were as good as male parents as they were comparetively higher yeilding and possesed higher number of tassel branches that produces a high volume of pollen. Further, using inbred lines in C-5 can be resitricted due to their weak performance for most of agro-mophological triats. Endang *et al.* (1971) stated that the selection of parents from diverse clusters was likely to generate a higher heterosis and good amount of variability for effective selection of various economic traits. Similar studies have been reported to select the parents from different clusters to exploit higher heterosis in maize hybrids (Kumar *et al*., 2018; Jiban, 2013; Shazia *et al*., 2017).

Table 5. Mean valuaes of 17 qualitative triats of different clusters of maize inbred lines

Trait	Cluster No.	1	2	3	4	5
	No. of the line	**9**	**10**	**17**	**7**	**2**
GY- Grain yield (t/ha)	Mean	2.9	**3.5**	2.2	2.4	1.5
	SE	0.1	0.3	0.1	0.5	0.2
EGW - Grain weight per ear (g)	Mean	69.1	**84.6**	52.1	57	37.1
	SE	3.1	6.1	2	11.2	5.6
SP- Shelling percentage	Mean	86.6	87.6	85.7	82.4	**96.1**
	SE	1.4	1.1	1	2.2	0.2
SR- Number of seed rows	Mean	11.9	13	12.7	11.6	10.6
	SE	0.4	0.5	0.2	0.4	0.4
EL- Ear length (cm)	Mean	13.8	14.1	11.9	13	**10.4**
	SE	0.5	0.5	0.2	0.6	0.8
EG- Ear girth (cm	Mean	12.1	12.2	11.2	10.2	**9.2**
	SE	0.3	0.2	0.1	0.5	0.4
SN- Number of seeds per row	Mean	26.5	28.1	21.8	22.8	19.2
	SE	1.2	1.3	0.6	2.8	2.5
100SW -Weight of 100 seeds (g)	Mean	27.8	25	23.4	19	17.8
	SE	1.1	1.3	0.9	1.4	1.8
PH- Plant height (cm)	Mean	119.1	135.2	104.4	104.8	86.1
	SE	4.3	3.2	3.2	5.7	4.9
EH- Ear height(cm)	Mean	43	58.1	38	31.2	26.8
	SE	2.7	2.1	1.8	2.2	6.8
LL-Leaves length (cm)	Mean	67.9	76.4	67.2	74.1	52.6
	SE	2.7	1.9	1.4	2.6	2.1
LW - eaves width (cm)	Mean	7.1	8.9	7.5	8	6.8
	SE	0.3	0.2	0.2	0.2	1.2

Table 5 (continued). Mean valuaes of 17 qualitative triats of different clusters of maize inbred lines

Trait	Cluster No.	1	2	3	4	5
	No. of the line	9	10	17	7	2
DT- Days to 50% Tasselling	Mean	63.8	62.5	64.5	**70.1**	63.3
	SE	0.4	0.7	0.4	0.7	2.8
DS- Days to 50% silking	Mean	65.3	64.4	66.6	**73**	67.3
	SE	0.6	0.9	0.4	0.7	2.8
ASI - Anthesis silking interval,	Mean	1.4	2	2.1	2.9	**4**
	SE	0.6	0.4	0.2	0.6	0
TL-Tassel Length (cm),	Mean	32.7	36.6	31.1	35	**24.4**
	SE	1.5	0.9	0.8	1	0.9
TB- No. tassel branches	Mean	4.4	6.8	4.3	**7.3**	3.5
	SE	0.6	0.5	0.4	0.5	0.7

Table 6. Cluster membership of maize inbred lines

Cluster No.	Maize inbred lines
1	MI2008-1, MI2008-2,MI2008-3, MI2008-10, MI2008-11, MI2008-21, MI2008-30, MI2012-NK-8, MI2012-NK-9
2	MI2008-7, MI2008-9, MI2008-13, MI2008-18, MI2008-23, MI2008-28, MI2008-32, MI2008-33, MI2008-35, MI2008-37
3	MI2008-8, MI2008-12, MI2008-14, MI2008-15, MI2008-17, MI2008-19, MI2008-20, MI2008-22, MI2008-25, MI2008-27, MI2008-40, MI2012- NK-4, MI2012-NK-6, MI2012-NK-7, MI2012-NK-10, MI2012-NK-11, MI2012-P-9
4	CML-451, MI2008-39, MI2012-NK-1, MI2012-P-1, MI2012-P-3, MI2012-P-8, MI2012-P-10,
5	2008-31, 2008-34

Conclusion

The locally developed maize lines derived from three different source populations showed a substantial diversity for 16 agro-morpphological traits. The inbred lines, MI2008-35, MI2008-13 and MI2008-7 were elite lines and consisted two or more economically important traits such as higher yield, longer ears, the lowest ASI, the highest number of seed rows, and higher seeds per row. Grouping of inbred lines into different heterotic groups can be used to make planned crossing schedule to develop new single cross maize hybrids. Hence, morphological evaluation, characterization and clustering of maize inbred lines will save time and resources to identify the parental line combinations those exhibit the highest heterotic effect rather than making all possible cross combinations.

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Manuscript preparation

Authors should kindly note that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

Manuscript should be submitted in the following format. Abstract, Introduction, Materials and methods, Results and discussion, Conclusions, Acknowledgements and References. Please use consistent style throughout the manuscript.

The manuscripts should be formatted double spaced on A4 papers with following page setup: Margins; Top, Bottom, Right and Left 3.75 cm; Header 2.5 cm; Footer 1.25 cm; Font: Times New Roman. Follow the formatting given in the Table below.

Section	Type Style	Alignment	Font Size	Type Case
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Abstract	Bold	Centre	12	Sentence
Abstract text	Bold	Justify	10	Sentence
Key Words	Bold	Justify	10	Sentence
Key word Text	Bold	Justify	10	Sentence
Introduction	Bold	Centre	12	Sentence
Introduction Text	Normal	Justify	11	Sentence
Materials and Methods	Bold	Centre	12	Sentence
Materials and Methods Text	Normal	Justify	11	Sentence
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Journal articles

Joachim, A., W.R.S. Kandiah and D.G. Pandithesekera. 1933. Studies on paddy cultivation 2. The effect of manures on the composition of paddy and soil. Tropical Agriculturist 81: 11-35.

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