Genetic diversity of maize accessions for maize lethal necrosis disease resistance

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ABSTRACT

Maize is among the most preferred crop in Tanzania and other parts of the world. However, its production has been facing a number of challenges. Maize Lethal Necrosis Disease (MLND) is a new challenge in Eastern Africa. The control of MLND is said to be complicated as it is caused by a combination of more than one virus *viz. Maize Chlorotic Mottle Virus* (MCMV) and *Sugarcane Mosaic Virus* (SCMV). Stakeholders agree that the priority is to identify MLND resistant maize varieties. Genetic diversity provides the source of traits required against maize production challenges such as MLND. The study of genetic diversity in maize accessions often involves characterizing morphological plant characteristics as well as molecular marker techniques to study variation at DNA level. This review explores different literatures that address the importance of genetic diversity and the possibility of generating information towards obtaining potential materials against maize challenges and MLND in particular.

Key words: Genetic diversity, Landraces, Maize, MLND, Molecular markers, Morphological characterization.

Maize (*Zea mays* L.) stands to be among the most important crops in the world, because it is one of the main sources of human food, animal feed, and raw materials for industrial processes (Romay *et al.*, 2013). In Tanzania, maize is the major food and cash crop where its supply is normally equated to the national food security (Katinila *et al.*, 1998). However, despite its importance, the general average yields are still very low with 1.2 metric tonnes per hectare as compared with the estimated potential yields of 4 to 5 metric tonnes per hectare (Moshi *et al.*, 1990; Otunge *et al.*, 2010). The cultivation of maize has been limited by several diseases which cause serious grain loss (Anjichi, 2005; Pechanova, 2015). Recently, East Africa has been hit by another deadly disease called Maize Lethal Necrosis (MLN) which was not there before while potyviruses that form one of the causing pathogens used to exist (Wangai *et al.*, 2012a; Wangai *et al.*, 2012b; Adams *et al.*, 2014; Gowda *et al.*, 2015; Mahuku *et al.*, 2015). The disease is caused by a combination of *Maize Chlorotic Mottle Virus* (MCMV) and other Potyviruses such as *Sugar cane Mosaic Virus* (SCMV), *Maize Dwarf Mosaic Virus* or *Wheat Streak Mosaic Virus* (Scheets, 1998; De Groote *et al.*, 2016; Isabirye and Rwomushana, 2016). There is a need to screen a wide range of accessions for MLN disease tolerant or resistant materials. Maize landraces (accessions) are usually genetically heterogeneous populations (Ignjatovic *et al.*, 2013) which are typically selected by farmers for better adaptation to specific environments as well as resistance to biotic and abiotic stresses (Aci *et al.*, 2013). Characterization of those genetically heterogeneous populations using conventional and molecular tools has been the most efficient ways of establishing diversities for different important traits including disease resistance (Anumalla *et al.*, 2015; Prasanna, 2012). That means, developing improved varieties with required traits through plant breeding would very much depend on the availability of a wide and reliable crop genetic diversity (Abraha *et al.*, 2014; Sharma *et al.*, 2015).

Maize Lethal Necrosis Disease (MLND): MLND was first observed in areas of South Rift Valley region of Kenya in 2011 and spread to several other places of Kenya (Wangai *et al.*, 2012b). Later, the disease was identified as MLN after serological and molecular tests were carried out on infected maize plants from Bomet County and Nakuru District in 2012 (Wangai *et al.*, 2012b). The same year, the disease was also reported to spread to neighbouring countries of Tanzania and Uganda (Makumbi and Wangai, 2013) and later it was found in Rwanda (Adams *et al.,* 2014). The control of the disease has been reported to be difficult due to the combination of more than one virus that do not separately result into any significant symptoms (Xia *et al.*, 2016 and DSMZ, 2014). MLND occurs as a result of a positive interaction between *Maize Chlorotic Mottle Virus* (MCMV)

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and any of the cereal viruses in the family, Potyvirideae, such as *Sugarcane Mosaic Virus* (SCMV), *Maize Dwarf Mosaic Virus* (MDMV), or *Wheat Streak Mosaic Virus* (WSMV) (Adams *et al*., 2014; Makone *et al*., 2014; Liu *et al*., 2016). In eastern Africa, the disease has been reported to be caused by a combination of MCMV and SCMV infection (Gowda *et al.,* 2015; Mezzalama *et al*., 2015; Kiruwa *et al*., 2016). The two viruses together inflict serious damage or even completely kill infected plants (Scheets, 1998; CGIAR, 2012) and farmers in the affected areas have been reported to experience extensive to total crop loss (Wangai *et al*., 2012b). The disease causing viruses are mainly transmitted by insects (Makone *et al*., 2014) from plant to plant or by wind from field to field over long distances (CGIAR, 2012; Mezzalama *et al*., 2015). MCMV is normally transmitted by thrips and beetles while SCMV is transmitted by aphids (CGIAR, 2012; Kiruwa *et al*., 2016). The viruses can as well be transmitted from one generation to another through seed which are infected, this enhance the possibility for wide spread of MLND (Zhang *et al*., 2011). Infected plants show mild to severe mottling on the leaves (Gowda *et al*., 2015; De Groote *et al*., 2016; Kagoda *et al*., 2016), usually starting from the base of young leaves in the whorl and extending upwards toward the leaf tips (Wangai *et al*., 2012b). Also, stunting and premature aging of the plants, dying (necrosis) of the leaf margins that progresses to the mid-rib and eventually the entire leaf (Wangai *et al*., 2012b; Gowda *et al*., 2015). Necrosis of young leaves in the whorl before expansion, leads to a symptom known as "dead heart" and eventually plant death (Kagoda *et al*., 2016). In addition, infected plants often bear barren ears which are small and deformed with little or no seed set (CGIAR, 2012; Gowda *et al*., 2015; Kagoda *et al*., 2016).

Genetic diversity of maize accessions: Genetic diversity refers to the heritable genetic variation that occurs within and between populations of particular organisms (Rao and Hodgkin, 2002). The diversity in plants provide an opportunity for developing new varieties and improved cultivars with desirable characteristics (Govindaraj *et al*., 2015; Saleh *et al*., 2016). A number of methodologies exist for the assessment of genetic diversity in maize, those are (i) morphological characterization (Ristic *et al*., 2014) (ii) biochemical characterization that uses electrophoresis to detect allelic variants of enzymes at gene level (Govindaraj *et al*., 2015) (iii) pedigree that employ the extraction of genealogical information (Drinic *et al.*, 2012) and (iv) DNA molecular analyses (Sao *et al.*, 2015) such as Simple Sequence Repeats (SSR), Restriction Fragment Length Polymorphism (RFLP) (Mondini *et al*., 2009).

Morphological diversity: The morphological characteristics (phenotype) express the genetic constitution (genotype) of a given organism, while in other words genetic constitution give rise to what we see (Liao *et al*., 2010; Uphoff *et al*., 2015). However, the expression of phenotype (morphological characteristics) is always affected by the impact of environment (Anumalla *et al*., 2015). On the other hand, the impact by environmental interaction coupled with the expression of genetic constitution presents the advantage that can only be obtained with morphological markers (Durga *et al.*, 2015). The following equation show the interaction; $P = f(G + E + (G \times E))$, where P stands for phenotype, G for genotype and E for environmental influences, and the interaction term $G \times E$ refers to their joint effects (Uphoff *et al*., 2015). Genetic variation (diversity) can be evaluated using morphological characterization (Mondini *et al*., 2009). Morphological characterization of maize is being conducted through assessing plant characteristics that are given as the list of descriptors provided by the International Board for Plant Genetic Resources (IBPGR, 1991). The morphological traits that are used to evaluate maize genetic diversity include i. Vegetative data ii. Ear data and iii. Kernel data (Table 1). Collected data from the parameters (descriptors) as shown in Table 1 are further subjected to statistical programs which generate informative results that explain the behaviour of each crop accession as well as how accessions relate to each other. The results also help to identify promising accessions through potential traits evaluation. Therefore, analysis of variance (ANOVA) can be performed for all measured traits in order to test the significance of variation among accessions (Beyene *et al*., 2005). The standardized traits mean values can be used to perform principal component (PC) and cluster analyses (to calculate similarities or dissimilarities between accessions) using softwares such as NCSS 2000 (Jerry, 2000) or NTSYS pc 2.1 (Rahman *et al*., 2008). Further cluster analysis can be conducted on the Euclidean distance matrix with the unweighted pair group method based on arithmetic averages (UPGMA) (Beyene *et al*., 2005).

Diversity at molecular level: In the past few decades, analyzing genetic diversity based on phenotypic traits (morphological characterization) has been enhanced with the use of molecular (DNA) markers (Ristic *et al*., 2014). This is due to the fact that discrepancies encountered with morphological markers are checked by the use of molecular markers for the purpose of obtaining variation at the DNA level (Dubreuil *et al*., 2006). Evaluating genetic diversity of genetic resources at molecular level is essential in the fact that morphological differences themselves are usually determined by a small number of genes and may not be representative of genetic divergence in the entire genome (Brown-Guedira *et al.*, 2000). However, the importance of these genetic resources and their potentials for selection has been constrained due to limited amount of important traits being characterized at molecular level (Rao and Hodgkin, 2002; Drinic *et al*., 2012; Prasanna, 2012; Sood *et al*., 2014). A number of reports on maize populations have been showing a considerable amount of variability on morphological and agronomic traits (Ihsan *et al.*, 2005) as well as at molecular

Table 1: Some of the descriptors that are used to evaluate genetic diversity through morphological characterization (IBPGR, 1991).

Descriptor No.	Item	Description
	Vegetative	
4.1.1	Days to tasseling	Days from sowing to when 50% of plants shed pollen
4.1.2	Days to silking	Days from sowing to 50% of plants having silks
4.1.4	Plant height [cm]	From ground level to the base of the tassel. After milk stage
4.1.5	Ear height [cm	From ground level to the node bearing the uppermost ear. After milk stage
4.1.6	Foliage	Rating of total leaf surface. After milk stage. Observed on at least 20 representative plants
4.1.7	Number of leaves above the uppermost ear including ear leaf	Counted on at least 20 representative plants. After milk stage
4.1.9	Stem colour	Indicate up to three stem colours in the order of frequency. Observed between the two topmost ears. At flowering
4.1.12	Sheath pubescence	
6.1.2	Leaf length	From ligule to apex. Measure the leaf which subtends the uppermost ear. After flowering
6.1.3	Leaf width [cm]	Mid-way along its length. Measured on the same leaf as 6.1.2
6.1.5	Leaf orientation	After flowering
6.1.6	Presence of leaf ligule	After flowering
	EAR DATA	
6.2.2	Ear length [cm]	Length
6.2.4	Ear diameter [cm]	Measured at the central part of the uppermost ear
4.2.3	Kernel row arrangement	Use the uppermost ear
4.2.4	Number of kernel rows	Count number of kernel rows in the central part of the uppermost
6.2.5	Cob diameter [cm]	
6.2.6	Rachis diameter [cm]	
6.2.8	Number of kernels per row	
6.2.9	Cob colour	
6.2.10	Shape of uppermost ear	
	KERNEL DATA	
4.3.1	Kernel type	Indicate up to three kernel types in the order of frequency
4.3.2	Kernel colour	Indicate up to three kernel colours in the order of frequency
4.3.3	1000 kernel weight [g]	Adjusted to 10% moisture content
6.3.1	Kernel length [mm] measured	Average of 10 consecutive kernels from one row in the middle of the uppermost ear, with calliper
6.3.2	Kernel width [mm]	Measured on the same 10 kernels as 6.3.1
6.3.3	Kernel thickness [mm]	Measured on the same 10 kernels as 6.3.1
6.3.4	Shape of upper surface of kernel	
6.3.5	Pericarp colour	
6.3.6	Aleurone colour	
6.3.7	Endosperm colour	

level (Legesse *et al.*, 2006). This calls for the efforts of ensuring that potential useful traits through a wide range of plant genetic resources are made available to plant breeders to enhance required crop improvement (Frese *et al.*, 2012; Maxted *et al.*, 2013).

Molecular marker techniques: Molecular marker techniques presents the ability to detect variation at the DNA level through breeding program and plant biotechnology (Anumalla *et al.*, 2015). PCR based molecular marker techniques have made it possible for breeders and other scientists to make genetic diversity estimates as generated through different molecular markers (Arif *et al.*, 2010; Poczai *et al.*, 2013). Genetic diversity results from the genetic variation among individuals and may be expressed in DNA sequences (Bindroo and Moorthy, 2014; Osawaru *et al.*, 2015). It can be categorized in terms of the number of

different alleles existing in different populations, distribution of those alleles in the chromosomes, the impact they have on performance and the general variability among different populations under various environmental conditions (Rao and Hodgkin, 2002; Mondini *et al.*, 2009; Bindroo and Moorthy, 2014). Some of the techniques that have been applied in molecular studies include RFLPs (Mondini *et al.*, 2009), RAPDs (Brown-Guedira *et al.*, 2000; Mondini *et al.*, 2009), AFLPs (Mondini *et al.*, 2009), SSRs (Kumari *et al.*, 2005; Beyene *et al.*, 2005; Mondini *et al.*, 2009; Aci *et al.*, 2013; Abraha *et al.*, 2014). However, these different marker techniques emphasize on different features (Abdel-Mawgood, 2012) and different aspects of genetic diversity (Matsuoka *et al.*, 2002; Mondini *et al.*, 2009). Therefore, different marker techniques may lead to different results and the range of variation produced can be different (Hodgkin

et al., 2001). Among those several marker techniques, microsatellites (SSR) have been exploited in many ways (Ignjatovic *et al.,* 2013) and specifically for characterizing genetic diversity in Maize (Reif *et al.*, 2006).

SSR markers: These are microsatellites which are abundant and occur frequently and randomly (Ristic *et al.*, 2014) in eukaryotic genomes that are examined (Matsuoka *et al.*, 2002; Wan *et al.*, 2004). Regardless of microsatellites being time consuming and costly, they actually are advantageous in terms of ease of use, high levels of inherited variation, co-dominant, reliable and highly reproducible (Rao and Hodgkin, 2002; Mondini *et al.*, 2009; Ristic *et al.*, 2014). The following constitutes major steps towards executing molecular characterization procedures:

DNA extraction: Extraction of DNA from sample to be analyzed is the first step for molecular marker diversity study (Semagn *et al.*, 2006). DNA is extracted from the leaf samples taken from young seedlings (Legesse *et al.*, 2006; Semagn *et al.*, 2006) using the CTAB procedure such as that explained by Saghai-Maroof *et al.* (1984). Sometimes modifications can be made depending on circumstances. Primers of a specific marker type such as SSR are selected on the basis of their genomic locations (Kumari *et al.*, 2005).

Polymerase Chain reaction (PCR): The first step with PCR is denaturation process or melting step which separate the two DNA strands (template DNA), this step requires very high temp 94 - 98^{0}C (Kumari *et al.*, 2005; Legesse *et al.*, 2006). Annealing step follows that allow primers to bind to the complementary sequences on the template DNA and the temperature here ranges from 40-60^{0}C (Matsuoka *et al.*, 2002; Kumari *et al.*, 2005). The next step is elongation after the primers are bound, this requires a temperature of 72^{0}C (Matsuoka *et al.*, 2002; Kumari *et al.*, 2005).

Gel Electrophoresis: Finally, the DNA sample is loaded into wells of agarose gel and then the gel is ran and scanned under UV light on transilluminator for interpreting the results (Yilmaz *et al.*, 2012). From the gel electrophoresis, polymorphism (variation) is expected to be determined (Senior *et al.,* 1998). Also similarity matrix is analysed by NTSYS-pc analytical package to generate hierarchical classification by the use of Unweighted Pair Group Method using Arithmetic Averages (UPGMA) (Kumari *et al.*, 2005).

Importance of landraces

Landraces are plant populations which are cultivated by local farmers that have historic origin, unique identity with no any formal crop improvement (Prasanna, 2012; Hagenblad *et al.*, 2016). They are often endowed with diverse genetic inheritance (Zeven, 1998) as well as local adaption and strong connection to traditional farming systems (Camacho-Villa *et al.*, 2005). They are typically selected by farmers for better adaptation to specific environment (Ristic *et al.*, 204), yield potential, nutritional qualities and resistance/tolerance to biotic and abiotic stresses (Prasanna, 2012). However, domestication and selection of maize are said to cause reduced genetic diversity in the maize genome as compared with its progenitor population (Pineda-Hidalgo *et al.*, 2013). The reduction in the genetic diversity of crops represents an increase in vulnerability to new pests and diseases (De Jaramillo, 2009; Ogwu *et al.*, 2014). It has been reported that about 80% of African farmers grow only landraces since they are able to reuse the seeds in many seasons while on the other hand only 20% grow improved varieties together with landraces (Anjichi *et al.*, 2005). In Mexico, landraces occupy more than 80% of the area under maize production (Mercer and Wainwright, 2007). In Tanzania, landraces and traditional cultivars attract more attention than commercial improved cultivars (Mitawa and Marandu, 1996). In addition, since 1974 to 2000, maize researchers under maize research program in Tanzania have managed to utilize some potentials from maize germplasm sourced from in and outside the country to release a number of varieties (Kirway *et al.*, 2000). Some of those were able to be adopted because they possessed some traits prefered by the community as well as being connected to the existing farming practices (Westengen *et al.,* 2014). The characteristics of those released varieties extends from plant characteristics, yield performance, disease resistance/ tolerance etc; this gives an indication that maize germplasm present an opportunity for making genetic enhancement against maize production challenges. Landraces in Mexico attract great attention of both farmers and researchers (Rodriguez *et al.*, 1998). Tuxpeño maize for instance, is a Mexican landrace that was domesticated in the Oaxaca-Chiapas region (Rodriguez *et al.*, 1998; Prasanna, 2010) and it is a cultivar which is very productive in the fertile lowland and has been very much used in the breeding programmes (Rodriguez *et al.*, 1998; Prasanna, 2010). Another example is Tuxpeño crema, a Mexican cultivar which is characterized to be a late maturing cultivar and resistant to tropical foliar diseases, also with white kernels, short and has strong stalk (Rodriguez *et al.*, 1998). Therefore, landraces are expected to be a very important source of new and unique alleles which have not yet been well exploited (Mercer and Wainwright, 2007; Olson *et al.*, 2012). However, limited characterization data of landraces have caused difficulties to use, manage and conserve them (Rao and Hodgkin, 2002; Drinic *et al.*, 2012; Prasanna, 2012; Sood *et al.*, 2014). Another challenge with maize landraces is that many plant breeders limit their breeding research by using germplasm that contain narrow genetic base (Prasanna, 2012). Thus, there is an urgent need to establish efficient and well organized characterization of maize germplasm for creating comprehensive information that would be useful to generate strong maize varieties against maize production challenges (Saad and Rao, 2001; Drinic *et al.*, 2012; Prasanna, 2012) including MLND in particular.

CONCLUSION

Maize continues to be the most preferred staple food and cash crop in Tanzania as well as in other parts of the world. However, maize is affected by many pathogens and some of them cause big impacts to its productivity. The outbreak of a new disease in East Africa called Maize Lethal Necrosis Disease (MLND) presents immediate concerns as well as uncertain long-term consequences. MLND infection rate reaches 100% and yields severely affected up to a complete loss of the crop. Stakeholders in maize research and development admit that the identification of resistant maize varieties is mostly prefered as the priority for controlling MLND. Genetic diversity (morphological and molecular) study provides key information that may help in identifying important traits against several production constraints. Maize genome harbors potential amount of morphological and molecular diversity that can be sourced and invested for maize crop improvements. Therefore, good understanding of genetic diversity within and among maize accessions ensures effective utilization of the genetic resource available for the fight against the current major challenge (MLND) in maize production.

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