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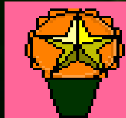

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Full Length Research Paper

In vitro protocol optimization for micropropagation of elite Lemmon verbena (*Aloysia triphylla*)

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The family *Verbenaceae* includes 36 genera and 1035 species. Among them lemon verbena (*Aloysia triphylla*) is known to have high medicinal value. Therefore, development of fast and new *in vitro* micropropagation protocol will have a high importance in lemon verbena mass-propagation. This research study targeted to develop rapid *in vitro* micropropagation protocol for lemon verbena. Up to 88% of clean survived plantlets were obtained after treating the nodal explants with 0.5% berekina (NaClO) for 10 min. Shoot initiation and multiplication was achieved using node as explant planted on MS medium supplemented with different strength of 6-benzyladeninepourine (BAP) and kinetin (Kin) individually and in combination. Plants were put on root induction ½ strength MS medium fortified with different strength of indole-3-butyric acid (IBA) alone. The best treatment for shoot initiation was 6-benzyladeninepourine (1.5 mg/L) with 84.1% of initiation. The best treatment for shoot multiplication was 6-benzyladeninepourine (2.0 mg/L) with 9.23 shoots per explant. Best rooting (100%) and maximum root number per shoot (14.4) were found at 1.0 mg/L indole-3-butyric acid (IBA). The longest root (3.1 cm) was achieved without supplementing the media with plant growth regulators. The plantlets were hardened and acclimatized in fully automated greenhouse and survival percentage was greater than 70% planting on a combination of sterilized river sand, top forest soil and animal manure in a 1:2:1 (v/v/v) ratio. This *in vitro* micropropagation protocol can be used instead of conventional propagation techniques, as a fast and economically cheap method to propagate a wide range of similar plants.

Key words: Acclimatization, auxin, cytokinin, micropropagation, lemon verbena.

INTRODUCTION

Lemon verbena (*Aloysia triphylla* L.) is a perennial shrub that belongs to the family *Verbenaceae* (Gomes et al., 2006; Rotman and Mulgura, 1999). It has got its name due to the fact that it has whorls of three (tri) leaves (phylla) at each node. Lemon verbena is locally known as Lominat (Beemnet et al., 2013) and is native to South American countries; Argentina, Paraguay, Brazil, Uruguay, Chile, Bolivia and Peru (Carnat et al., 1999; Vogel et al., 1999; Armada and Barra, 1992; Botta, 1979).

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The leaves of lemon verbena are the most economical part of the plant that can be used to add a lemony taste in salads, tea, milk, ice creams and jellies (Hanna et al*.,* 2011; Beemnet et al*.,* 2010). It makes one of the best beverage teas, especially when blended with mint (Hanna et al*.,* 2011). Likewise, the essential oil obtained through distillation of the leaves is used in fragrance industries, food flavoring industries, soft drink industries and folk medicine. Traditionally, it is used for treatments of spasms, cold and fever as folk remedy (Carnat et al*.,* 2004), asthma, flatulence, colic, diarrhoea, indigestion, insomnia and anxiety (Durat and Chritina, 2005; van Hellemon, 1986; Newal et al*.,* 1996; Cowan, 1999; Graca et al*.,* 1996).

Essential oil of lemon verbena has anti-oxidant, anti-bacterial and anti-fungal properties (Hanna et al*.,* 2011) as well as being used in tea and tinctures (Bilia et al*.,* 2008; Cowan, 1999). Due to its diverse uses and applications, lemon verbena has got open and large market potential for herbal preparation and extraction of essential oils (Beemnet et al*.,* 2010). Despite its high importance in food, pharmaceutical, soft drink and stimulant-processing industries introduced to the country long time ago, continuous interest of producers and investors for its production and application in Ethiopia (EIAR, 2009) and the presence of different agro-ecological conditions in the country (NMSA, 1996; Andargachew, 2007), there exist limited information on the propagation, processing and utilization technologies in Ethiopia. Thus, lack of such information is the major problem to exploit the potential of the plant (Beemnet et al*.,* 2013).

Lemon verbena is best propagated by cutting, taken in summer by keeping it in a shade and well-watered conditions, which otherwise will wilt readily. It is highly susceptible to pests like that of spider mites and white flies and also requires long time to grow into full plantlets. Since it is difficult to obtain seeds of *A. triphilla* owing to Ethiopian climate, *in vitro* micropropagation of this plant to produce high quantity of genetically homozygous planting material will be a better solution.

MATERIALS AND METHODS

This *in vitro* protocol optimization for micropropagation of lemon verbena research was conducted in the Plant Tissue Culture Laboratory of National Agricultural Biotechnology Research Center at Ethiopian Institute of Agricultural Research from September 2014 to June 2015.

Media composition

The nutrient medium used for growth of lemon verbena was semi solid (Murashige and Skoog, 1962) which contains macro, micro elements and vitamins. To prepare the nutrient medium we used, individually prepared stock solutions were mixed with the required concentrations of plant growth regulators and sucrose (3%). The solution was mixed completely using magnetic stirrer. The pH of the media was adjusted at 5.75 using 0.1 N NaOH or 0.1 N HCl before the addition of 0.4% agar, and then boiled until the agar melts completely. Around 10 mL (for test tubes) and 50 mL (for Jars) of the media were added in each culture test tubes (150 mm long and 25 mm diameter) and culture jar (250 mL). The test tubes and culture jars containing the nutrient medium were plugged tightly with non-absorbent cotton and autoclavable lids prior to sterilization at 121°C with 0.15 KPa pressure for 20 min.

Mother plant establishment

The mother/donor plants, brought from Wondo Genet Agricultural Research Center, were further established by cutting in greenhouse at the National Agricultural Biotechnology Research Center, Holetta, Ethiopia (Figure 1).

Surface sterilization

Healthy and young shoots (3 to 4 cm length), having axillary buds (3rd, 4th and 5th nodes; from top tip), were taken from lemon verbena plant by excising with clean scissor/surgical blade and taken as explant. Young parts (juvenile plants) were used as they give good response to shoot initiation and multiplication than explant sources from old/adult forms (Naghmouchi et al*.,* 2008). The shoots were washed with clean water three to five times followed by liquid soap for 15 min with continuous agitation to remove contaminants from the surface, and sterilized with 70% ethanol for 30 s and then in NaClO (0.5% and 1% w/v) containing two drops of 'Tween 20' per 45 mL solution for 5, 10 and 15 min respectively and thoroughly washed with sterile double-distilled water. They were aseptically cultured for four weeks in 40-ml glass tubes containing 10 ml of semi-solid Murashige and Skoog (MS) (1962) medium. Number of clean and survived plants was recorded and percentage of contaminated plants was computed.

Shoot initiation

Fully cleaned nodal explants were planted in test tubes containing MS (Murashige and Skoog) (1962) nutrient medium supplemented with 3% sucrose, 0.4% agar (Agar-Agar, Type I) and different level of BAP (0.5, 1.0, 1.5, 2, 2.5, 3, 3.5 and 4 mg/L) alone. Medium without the addition of hormone was used as control. For each shoot induction treatment, 15 glass tubes were ordered randomly in completely randomized design (CRD) in three (3) replications. All culture tubes were properly sealed with non-absorbent cotton and parafilm and placed in the growth room at standard conditions (25 ± 1°C and 16/8 h light/dark and relative humidity (RH) of 70 to 80%). After four weeks of culturing, number of explants initiated was recorded and shoot initiation percentage was computed.

Shoot multiplication

Fully initiated shoots were placed on a medium without plant growth hormone for two weeks. Cleanly initiated 1.5 to 2.5 cm long shoots with a number of nodes were cut at both ends and planted vertically in 250-ml culture jars with 50-ml nutrient medium fortified with 3% sucrose, 0.4% agar and different concentration of BAP (0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/L) alone and in combination with 0.5, 1.0, 1.5 and 2.0 mg/L Kn. MS medium without plant growth hormones was used as control. For each treatment, 15 jars (five shoots per jar) were cultured randomly in CRD with five replications. All plants were allowed to grow for one and half month, thereafter number of shoots per explant was recorded.

Rooting of shoots

Multiplied shoots were planted on ½ strength MS medium fortified

Figure 1. Greenhouse established mother/donor plants of Lemon verbena (*Aloysia triphylla*).

Table 1. The effect of different concentrations of Berekina (NaClO) and length of exposure time on survival percentage.

Number of treatments	Berekina (NaClO) concentration (%)	Time of exposure (min)	Clean and Survived plants (%)	Contaminated plants (%)
1	0.5	5	82.4[b]	17.38[a]
2	0.5	10	87.9[a]	10[b]
3	0.5	15	74.8[c]	8.04[d]
4	1	5	78.5[c]	9.04[c]
5	1	10	63.4[d]	6.0[e]
6	1	15	40.0[e]	4.1[f]
CV			4.12	6.89

*Means followed by the same small letters are not different according to Tukey's test at 5% of probability. *CV: Coefficient of variation.

with 3% sucrose, 0.4% agar and different concentrations of IBA (0, 0.5, 1.0, 2.0 and 3.0 mg/L). Nutrient medium without plant growth hormones were used as control. For each treatment, 15 jars, each with five plantlets, were cultured randomly in CRD with five replications. Percentage of shoots with root, number of roots per shoot, and average root length (cm) were recorded after the shoots were planted on the root induction media for a month.

Acclimatization

Shoots with well-developed roots were transferred and planted on a seedling tray having a mixture of sterilized river sand, top forest soil and animal manure in a 1:2:1 (v/v/v) ratio and taken to fully automated greenhouse for acclimatization. Plants were put in greenhouse and covered by polyethylene sheets and red cheese cloth for two weeks, in order to decrease light intensity and maintaining the moisture. They were watered 2 to 3 times per day using plastic spray bottle. After two weeks days, percentage of plantlets that were successfully acclimatized was recorded and successfully acclimatized plants were transferred to pot.

RESULTS AND DISCUSSION

Surface sterilization

From the six sterilization treatments, treatment 2 (0.5% berekina for 10 min) resulted in 87.9% of clean and survived plants and 10% of contamination. The second good result comes from 0.5% berekina for 5 min resulting in 87.4% clean and survived plants and 17.38% of contamination. Also, treatment 6 results in 40% of clean and survived plants (Table 1). Disinfection procedures showed high efficiency in preventing fungal and bacterial contamination (Figure 2). The use of 0.5% commercial bleach (berekina) in surface sterilization produced more than 75% of the cultures which are free from bacterial and fungal contaminations during the *in vitro* initiation of nodal cuttings (Braga et al., 2011).

Figure 2. Clean survived plants after sterilization treatment.

Table 2. Effect of different concentration of BAP on shoot initiation.

Number of treatments	BAP (mg/L)	Initiated plants (%)
1	0	73.3[c]
2	1	77.6[b]
3	1.5	84.1[a]
4	2	86.7[a]
5	2.5	66.7[d]
6	3	60.3[e]
7	3.5	50.3[f]
8	4	46.7[g]
CV		4.06

***Means with the same letter in the same column are non-significant at 5% significance level. *CV: Coefficient of variation.

Shoot initiation

Analysis of the CV indicated that supplement of different concentration of BAP alone had highly significant effect on time taken for shoots to initiate and percentage of usable shoots initiation (Table 2). On most of the treatments, explants started shoot initiation after one week of culture. There was shoot initiation in all treatments tested including the control treatment, medium without plant growth hormone (73.3%) indicating that lemon verbena has enough endogenous hormones for shoot induction. Nevertheless, length and number of usable shoots initiated differed with different treatments (Figure 3). Maximum percentage of shoot initiation were achieved on a media supplemented with 2 mg/L BAP alone and 86.7%, followed by 1.5 mg/L giving 84.1% of initiation. The shoot initiation percentage was increased up to 2 mg/L; and thereafter showed dramatic decrease in initiation along with increases in BAP concentration. The kind of explant used greatly influenced shoot induction and subsequent multiplication of the initiated shoots. Broadly speaking, the regeneration or shoot initiation frequencies were higher with nodal explants in micro propagation of *A. polystachya* (Burdyn et al., 2006).

Shoot multiplication

Analysis of CV showed that all the treatments have highly remarkable effect on mean number of shoots, length of shoots, and mean number of leaves during shoot multiplication (Table 3). The concentration of BAP alone was highly remarkable on resulting good shoot multiplication in comparison to BAP and Kin combination. Multiplication results indicated that the maximum number of shoots (7.42 and 9.23) per explant was achieved on media containing 3 and 2 mg/L BAP respectively (Table 3). There was no remarkable inutility on the number of shoots between those two treatments. These result is in agreement with lemon verbena (*Lippia citriodora*) micro propagation, in which the maximum number of shoots was found on a media supplemented with 3 mg/L BAP in combination with 0.1 mg/L IBA (Oladzad et al., 2012).

Combination of lower cytokinin with higher auxin showed an average of 2.0 shoots per explant and also revealed that IAA and Kn has effect on multiple shoot proliferation (Mosavi, 2012). BAP combined with NAA produced around five shoots per nodal explant in *Verbena litoralis* while applying NAA only decreased shoot multiplication, bolstering the effect of cytokinins for shoot multiplication (Braga et al., 2011). The addition of 0.23 µM IAA to MS media for *L. alba* micropropagation significantly decreased shoot induction, number of shoots multiplied per explant and number of nodes per shoot, as compared to MS media without plant growth hormones (Tavares et al., 2004). Generally, significant rate of shoot multiplications were found in the addition of higher concentrations of BAP alone. Similar results were found for *Lippia junelliana* (Juliani et al., 1999), *L. alba* (Gupta et al., 2001) and *L. filifolia* (Peixoto et al., 2006).

Root induction

Anticipation of CV showed that various strength of IBA alone had highly considerable effect on root percentage, number of roots per shoot and length of roots (Table 4). The root induction results in Table 4 reveals that addition of 1.0 mg/L IBA on ½ strength MS medium resulted to 100% rooting, highest number of roots per shoot (14.4) and a root length of 1.8 cm (Figure 4). Earlier studies on lemon verbena (*L. citriodora*) indicated that root induction on MS media supplemented with 0.5 mg/L IBA resulted in high rate of root induction and root numbers per shoot (Oladzad et al., 2012).

The effect of IBA on rooting of many plants has been

Figure 3. Initiated lemon verbena plants after two weeks of culture.

Table 3. The effect of different concentrations and combinations of BAP and KN on shoot multiplication.

Number of treatments	BAP (mg/L)	Kin (mg/L)	Shoot number/explant
1	0	0	2.5^{g}
2	0.5	0	4.38^{d}
3	1	0	6.29^{b}
4	2	0	9.23^{a}
5	3	0	7.42^{a}
6	4	0	5.64^{c}
7	2	0.5	3.58^{e}
8	2	1	3.27^{e}
9	2	1.5	2.48^{f}
10	2	2	2.42^{f}
CV			8.99

*Means with the same letter in the same column are non-significant at 5% significance level. *CV, Coefficient of variation.

Table 4. Effect of IBA on root induction of micro shoots of Lemmon verbena after 30 days of culture.

Number of treatments	IBA (mg/L)	Shoots with roots (%)	Number of Roots/shoots	Root length (cm)
1	0	96.71	4.76^{d}	3.10^{a}
2	0.5	90	11.95^{b}	2.53^{b}
3	1	100	14.4^{a}	1.82^{c}
4	2	93.3	9.6^{c}	1.19^{d}
5	3	93.3	9.27^{c}	0.64^{e}
CV				

*Means with the same letter in the same column are non-significant at 5% significance level. *CV: Coefficient of variation.

Figure 4. Multiplied lemon verbena plants after 4 weeks of culture.

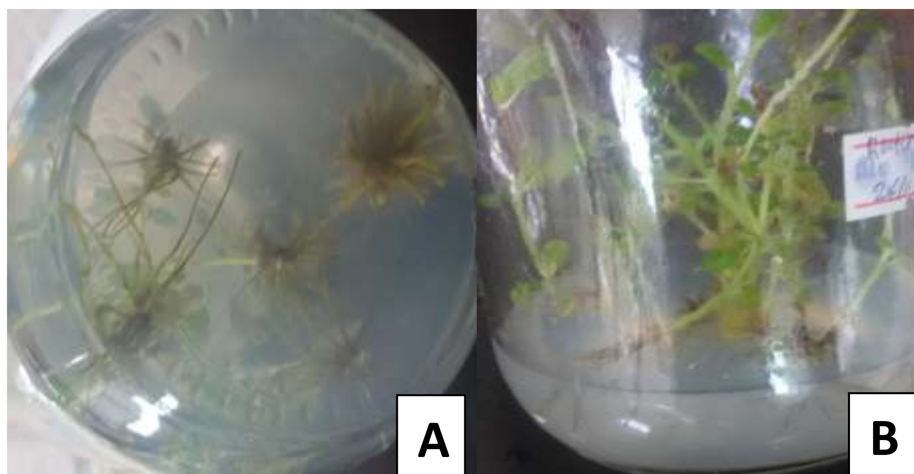

Figure 5. Root induction after 4 weeks from planting on rooting media supplemented (A) with 1.0 mg/L IBA and (B) without IBA.

reported and showed its effectiveness in comparison with NAA (Benelli et al., 2001; Tanimoto, 2005; Ansar et al., 2009). This could be due to slow movement and delayed degradation of IBA as compared to IAA and NAA. Various concentrations of IBA may also induce rooting by increased internal freely available IBA or may synergistically modify the action of endogenous synthesis of IAA (Krieken et al., 1993).

In *A. polystachya, in vitro* root induction of multiplied shoots was found without the addition of plant growth regulators (Sansberro and Mroginski, 1995). Our research output merely indicated that addition of low concentration of IBA to well-developed shoots increased the root induction process (Figure 5A and B). This low IBA concentration also does not stimulate callus formation at shoot base, which is an advantage, since it could acts as a physical barrier to nutrient and water movement (Thorpe et al., 1991; De Klerk, 2002).

Acclimatization

Well-developed plants with good roots were taken from the culture jars and washed with warm water to blow over agar adhering to roots and residue of nutrient media to decrease further adulteration. It was then moved onto seedling tray filled with a collection of heat sterilized river sand, top forest soil and animal manure in a 1:2:1 (v/v/v)

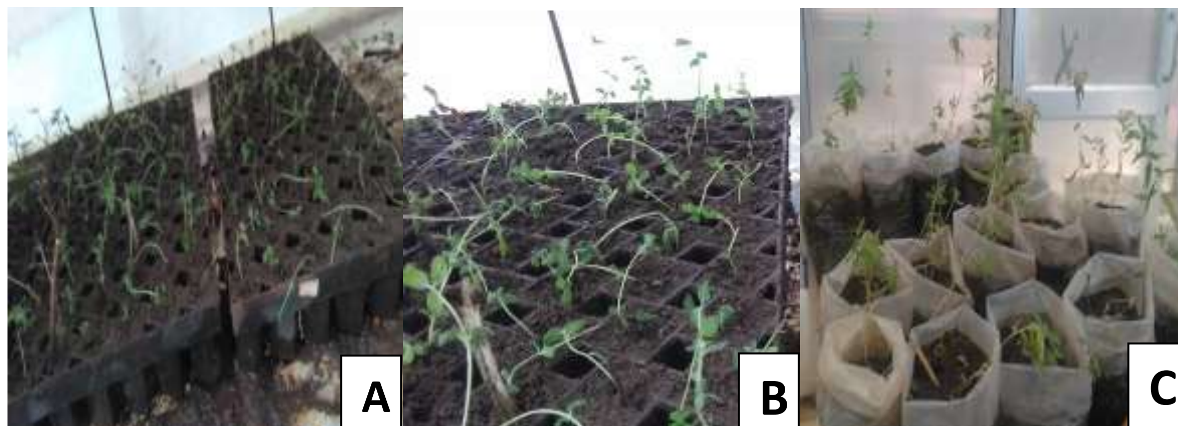

Figure 6. Combination of river sand, top forest soil and animal manure resulting in more than 70% survival and acclimatization in greenhouse (A) during acclimatization (B) after week of acclimatization, and (C) after month of acclimatization.

ratio and placed in fully automated glasshouse for further growth. After two weeks, survival rate greater than 70% was recorded (Figure 6). In acclimatization of apple at the same glasshouse, 65.70% survival rate was reported which is in line with previous finding (Demsachew, 2011).

Deepa et al. (2011) also indicated that vigorous growth and 70% survival rate after well rooted plants were planted to seedling trays filled with a mixture of sterilized ocean sand, soil and vermiculate in a 2:1:1 (v/v/v) ratio. According to Oladzad et al. (2012), high rate of acclimatization were achieved on soils composing a mixture of vermiculite, perlite and soil.

Conclusion

This research describes an efficient procedure for *in vitro* micropropagation and a successful hardening of lemon verbena. The protocol presented here for direct shoot initiation from nodal explants and consequent plant mass propagation will increase the ditch propagation of this crucial medicinal plant. This protocol will also have an impact on cryopreservation and genetic studies aimed at improving the essential oil composition of its extracts.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Evaluation of Tanzanian maize germplasms for identification of resistant genotypes against maize lethal necrosis

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Experiments were conducted in season 2014B at Naivasha maize lethal necrosis screening facility to evaluate Tanzanian maize germplasms for resistance to maize lethal necrosis (MLN). One hundred and fifty-two maize landraces and 33 inbreed lines were artificially inoculated with maize chlorotic mottle virus and sugarcane mosaic virus isolates in two trials arranged in a completely randomized design (CRD) and two replications. Inocula for both virus isolates were prepared, combined and applied to the trials by a 12 L backpack mist blower 4 and 5 weeks after planting. Disease incidence was assessed based on a 1 to 5 MLN rating scale 14, 28, 42 and 72 days post inoculation (dpi) for landraces and 7, 14, 21 and 52 dpi for inbred lines. Significant phenotypic variations (P<0.05) were observed on landraces for symptoms and disease severity scores. Landrace TZA-2793 had the lowest mean score of 3.5 followed by the other four landraces: TZA-3585, TZA-3543, TZA-4505 and TZA-2292, which attained a mean score of 3.75. No significant variations (P>0.05*)* were detected on inbreed lines as all materials were susceptible to MLN with scores ranging from 4.5 to 5 except for resistant check CML494 (mean score of 3.75). In this study, five maize landraces were identified as tolerant candidates against MLN. The identified landraces should be subjected to further MLN testing to explore their potential in breeding for MLN resistance.

Key words: *Zea mays,* maize chlorotic mottle virus, sugarcane mosaic virus, maize lethal necrosis, maize landraces.

INTRODUCTION

Maize (*Zea mays* L.) is among the world's major cereal crop widely grown for food, feed and income generation for millions of people around the world (Wang et al., 2011; Legesse et al., 2006). In sub-Saharan Africa and

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Latin America, maize stands as the number one staple food for over 1.2 billion people and more importantly for 30 to 50% of low-income household in Eastern and Southern Africa. Most of Africa's rural economies, at least 85%, rely on maize for human consumption as compared to the developed world where most maize grain is used for animal feed, biomass feedstock and for manufacturing industries (FAO, 2012).

Despite the distribution of maize and its importance as staple food in sub-Saharan Africa, the average yield of maize per hectare in Africa is reported to be the lowest, resulting in food shortages (Magenya et al., 2008). Maize yields in most of the African countries, particularly in SSA, are estimated to be lower than 1600 kg ha^{-1} (FAOSTAT, 2012). The low maize productivity is associated with biotic and abiotic factors that impede maize production for market and human consumption. The abiotic constraints include increased drought due to climate change, declining soil fertility, high acidity in soils, soil erosion, high temperatures, lack of early maturing germplasm and lack of improved germplasm for the tropical highlands. The biotic factors are primarily linked to tropical insects, diseases and weeds (Denic et al., 2001; Pingali, 2001).

In Tanzania, maize is a major cereal crop consumed with estimated annual per capita consumption of 113 kg (Hugo et al., 2002). Tanzania maize cultivation is beset by major biotic and abiotic factors such as drought, viral infections, fungal diseases and factors that impede soil fertility, which are common in other tropical and subtropical regions (Bisanda et al., 1998). Plant viruses have been reported to be amongst the most devastating biotic factors that infect maize leading to severely reduced crop quality, and in some cases, complete yield loss (Redinbaugh et al., 2004). Maize chlorotic mottle virus is known to exist in East Africa and this plant virus is considered very devastative to maize crop when it induces maize lethal necrosis (MLN) disease in a combined infection with any of the viruses in the Potyviridae group such as sugarcane mosaic virus (SCMV), wheat streak mosaic virus (WSMV) and maize dwarf mosaic virus (MDMV) (Niblett and Claflin, 1978).

The MLN was originally identified in Peru in 1974 and later in Kansas, USA (1976), Hawaii (1990) and China (2009) (Niblett and Claflin, 1978; Bockelman et al., 1982; Li et al., 2011; Nelson et al., 2011). MLN has become a major disease in maize growing areas of East Africa (Wangai et al., 2012), standing out as the greatest threat to African food security crop (maize) as it can cause serious yield losses of up to 100%, depending on the stage of growth of maize plant when it is attacked. In East Africa, MLN was first identified in Kenya in 2011 and quickly spread to Tanzania in the consecutive year where it was prevalent in Mwanza around Lake Victoria area, central part of Tanzania in Singida and Dodoma regions, and in northern regions of Kilimanjaro, Arusha and Manyara (CIMMYT, 2013). Other countries in Eastern Africa where MLN has been reported include Uganda, Democratic Republic of the Congo, South Sudan, Rwanda and Ethiopia (Adams et al., 2012, 2014).

Symptoms of MLN vary in severity depending on plant age at the time of infection and environmental conditions (Scheets, 2004). A range of specific MLN symptoms that have been reported include severe mottling on the leaves usually starting from the base of young leaves in the whorl and extending upwards toward the leaf tips; stunting and premature aging of the plants, dying of the leaf margins that progresses to the mid rib, necrosis of young leaves in the whorl and eventually plant death (CIMMYT, 2013). Other symptoms stated by Nelson et al. (2011) for infested maize in Hawaii were short ears, which were malformed and partially filled often with prematurely aged husks and shortened male inflorescences (tassels). Plants also become stunted because of shortened internodes (CIMMYT, 2004). Findings show that maize plants are susceptible to MLN at all growth stages and most of these symptoms are obviously restricted to the leaves, stem and ears (Adams et al., 2012).

Virus pathogens implicated in MLN are vector-transmitted (Jiang et al., 1990; Nault et al., 1978) which makes its control more challenging. In most cases, chemical control methods including integrated pest and disease management (IPDM) strategies are commonly adopted for control of insect vectors (Lagat et al., 2008); however, these strategies have not been successful in addressing the incidences of viral diseases in crops (Azizi et al., 2008; Bisanda et al., 1998). Insecticide applications can only kill insect vector found in a maize field within a given time, which is uneconomical to smallholder farmers, especially when it is difficult to afford prices of agrochemicals (Lagat et al., 2008). Under such circumstances, the economical and effective strategy for control of MLN would be breeding for maize host resistance for viruses involved in the disease complex (Kuntze et al., 1995; Redinbaugh et al., 2004).

Effective screening of Tanzanian's maize populations is vital in identifying genetic resistance for MLN. Currently, there is no published report showing resistance to MLN in Tanzanian maize core germplasms. The aim of this study was therefore, to screen maize landraces and inbred lines from Tanzania with MCMV and SCMV isolates under artificial inoculation conditions for the purpose of identifying MLN resistant maize genotypes in Tanzanian maize germplasms that could be used in breeding for MLN resistance.

MATERIALS AND METHODS

Plant materials

The plant materials comprised of 152 maize landraces (Table 1) and 33 maize inbred lines (Table 2). Four commercial East African maize hybrids known for their susceptibility to MLN (Duma 43, Pan 67, H614 and Pioneer) were used as check to screen maize landraces, whereas

Table 1. Representative samples of 50 Tanzanian maize landraces collected from different agro-ecological zones of Tanzania and geographical locations where the collection was done as indicated in NPGRC catalogue of cereal seeds accessions under *ex situ* conservation in Tanzania.

Plant ID			Place of collection				
Entry	NPGRC no.	Local name	District	Village	Latitude	Longitude	Alt (m)
1	TZA-4350	Nakijigo	Ngara	Kashinga	-2.7019 S	30.7058 E	1357
2	TZA-3837	Malombe achinya kala	Newala	Mkongi	-10.5161 S	39.2242 E	660
3	TZA-3543	Soya	Morogoro	Tulo	-6.8836 S	37.6500 E	1298
4	TZA-1758	Mbatagwa (White)	Mbeya Rural	Maganzu	90.0000 S	3323.0000 E	1680
5	TZA-2793	Mkonyoli	Kilombero	Ruaha	-8.8833 S	36.7186 E	487
6	TZA-4164	Ikigoli	Biharamulo	Luganzo	-3.1011 S	31.1292 E	1140
7	TZA-2910	Unknown	Tunduru Rural	Mbatamila	-10.9808 S	36.9694 E	566
8	TZA-4058	Gembe	Sengerema	Busekeseke	-2.5917 S	32.3217 E	1200
9	TZA-2816	Unknown	Pangani	Boza	-5.4028 S	38.9856 E	187
10	TZA-2685	Mampemba (Zigua)	Turiani	Lusanga	-6.1139 S	37.6661 E	395
11	TZA-181	Amangagu	Vwawa	Igamba	901.0000 S	3255.0000 E	1600
12	TZA-67	Unknown	Namanyere	Muimwa	748.0000 S	3107.0000 E	1800
13	TZA-3971	Buhemba	Musoma	Bungwema	-1.9503 S	33.5425 E	1080
14	TZA-3741	Gundugundu	Tandahimba	Mkwiti Juu	-10.4289 S	39.3639 E	490
15	TZA-1728	Ya kienyeji	Njombe	Uwemba	922.0000 S	3448.0000 E	2050
16	TZA-4574	Nchanana	Magu	Mwamabanza	-2.6939 S	37.4183 E	1125
17	TZA-4068	Mnana	Sengerema	Nyakariro	-2.4697 S	32.4056 E	1110
18	TZA-2843	Unknown	Muheza	Potwe-Mpirani	-5.2150 S	38.6189 E	425
19	TZA-111	Makonde/Amala	Sumbawanga	Liapona	820.0000 S	3143.0000 E	1700
20	TZA-1711	Mbegu ya Kihehe	Mufindi	Nzivi	832.0000 S	3535.0000 E	1780
21	TZA-3181	Uruwinga	Kigoma	Kumhasha	-3.6419 S	30.8367 E	1275
22	TZA-3614	Malombe	Mtwara	Nkutimango	-10.4975 S	39.8492 E	200
23	TZA-1754	Unknown	Mbeya Rural	Usoha	859.0000 S	3338.0000 E	2250
24	TZA-1725	Ya Kienyeji	Njombe	Mji Mwema	922.0000 S	3448.0000 E	1900
25	TZA-4197	Gembe	Nyamagana	Lwanima	-2.6072 S	32.9772 E	1220
26	TZA-3167	Urubinga	Kigoma	Nyakasanda	-3.1617 S	30.4689 E	1200
27	TZA-1753	Ya Kienyeji	Mbeya Rural	Kimondo	900.0000 S	3342.0000 E	2360
28	TZA-5621	Bogaqul	Hanang	Jordom	-4.9800 S	35.9414 E	2000
29	TZA-3982	Amaringwa	Musoma	Bungwema	-1.9489 S	33.8764 E	1080
30	TZA-4067	Gembe	Sengerema	Kazungute	-2.5561 S	32.4211 E	1200
31	TZA-3860	Mnumbi	Nachingwea	Likongowele	-10.0531 S	38.6436 E	150
32	TZA-3054	Katumbili	Mufindi	Igomaa	-8.5747 S	34.9447 E	1510
33	TZA-5619	Bogaqul	Hanang	Jordom	-4.9800 S	35.9414 E	2000
34	TZA-4206	Mapo	Ilemela	Sangabuye	-2.3869 S	33.0439 E	1090
35	TZA-4043	Malingwa	Ukerewe	Igallu	-2.0656 S	32.8761 E	1100
36	TZA-1752	Filombe freyu	Makete	Misiwa	911.0000 S	3354.0000 E	2500
37	TZA-78	Maisa	Sumbawanga	Mtimbwa	801.0000 S	3132.0 E	1700
38	TZA-3585	Katumbili	Mtwara	Mtwara	-10.3686 S	39.7100 E	20
39	TZA-3713	Mmakonde	Tandahimba	Tandahimba	-10.9258 S	39.1775 E	20
40	TZA-3567	Ngomeni	Morogoro	Matombo	-7.0100 S	37.6514 E	1391
41	TZA-4020	Malingwa	Ukerewe	Muluseni	-2.1175 S	33.1519 E	1080
42	TZA-2949	Lusewa	Mbinga	Likwela-Nyoni	-11.1019 S	34.9039 E	585
43	TZA-1755	Ya Kienyeji	Mbeya Rural	Galijembe	858.0000 S	3336.0000 E	2100
44	TZA-3585	Katumbili	Mtwara	Mtwara	-10.3686 S	39.7100 E	20
45	TZA-3171	Isega-Iwinga	Kigoma	Muhange	-3.1617 S	30.8622 E	1428
46	TZA-1723	Kibena	Njombe	Itunduma	859.0000 S	3449.0000 E	1780
47	TZA-4203	Gembe	Nyamagana	Kichele	-2.6111 S	32.3167 E	1190
48	TZA-1717	Mbegu ya Kienyeji	Mufindi	Mninga	853.0000 S	3512.0000 E	1900
49	TZA-1713	Mbegu ya Kienyeji	Mufindi	Ibati	833.0000 S	3505.0000 E	1840
50	TZA-5173	Mahindi ya Maramba	Mkinga	Horohoro	-4.6556 S	39.1033 E	120

Table 2. Tanzanian maize inbred lines obtained from Selian Agricultural Research Institute in Arusha, Tanzania.

Entry	Name	Pedigree
1	TZ-24	KAT 12/2-92-1-1-2
2	TZ-25	KAT 12-1-4-2
3	TZ-23	KAT 12-4-2-2
4	TZ-33	KIL 4-78-2-3
5	TZ-32	KIL 4-78-4-3
6	TZ-01	KS 03-OB15-1
7	TZ-08	KS 03-OB15-111
8	TZ-09	KS 03-OB15-118
9	TZ-10	KS 03-OB15-120
10	TZ-11	KS 03-OB15-125
11	TZ-12	KS 03-OB15-126
12	TZ-13	KS 03-OB15-153
13	TZ-14	KS 03-OB15-156
14	TZ-15	KS 03-OB15-188
15	TZ-16	KS 03-OB15-198
16	TZ-02	KS 03-OB15-3
17	TZ-03	KS 03-OB15-45
18	TZ-04	KS 03-OB15-53
19	TZ-05	KS 03-OB15-83
20	TZ-06	KS 03-OB15-85
21	TZ-07	KS 03-OB15-92
22	TZ-31	L511-15-1-3-1-1
23	TZ-26	MV 1-89-2
24	TZ-27	MV 3-34-2-8
25	TZ-28	MV 38-1-2-1-2
26	TZ-29	MV 501-6-86-3-1-1
27	TZ-30	P43-1-1-1-BBB
28	TZ-21	TMV 1-5-28-3-1
29	TZ-22	TMV 2-65-2-1-2-2
30	TZ-17	TUX 5-50-1-1-2-2
31	TZ-18	TUX 5-50-1-2-6-1
32	TZ-19	TUX 5-50-1-3-1-1
33	TZ-20	TUX 5-50-1-5-2-1

the International Maize and Wheat Improvement Center (CIMMYT) lines, CML494 and CML 395, were used as resistant and susceptible checks, respectively, to compare MLN response of maize inbreed lines. Maize landraces were requested from the National Plant Genetic Resources Center (NPGRC) located at the Tropical Pesticide Research Institute (TPRI) in Arusha, Tanzania. These materials were collected by the NPGRC from farmers in different agro-ecological and geographical locations in Tanzania (Figure 1). Maize inbred lines of Tanzania origin were requested from Selian Agricultural Research Institute (SARI) also located in Arusha, Tanzania.

Production of inoculum

The isolates of the virus combination known to cause maize lethal necrosis were collected from MLN hotspots in Kenya, confirmed for presence of MCMV or SCMV by enzyme-linked immunosorbent assay (ELISA). The two isolates were propagated on a susceptible hybrid H614 and maintained in two separate screen houses at Naivasha MLN screening facility. The screen houses were sprayed at weekly intervals with broad-spectrum insecticides to stringently minimize the chances of vector survival that could lead to contamination.

Inoculum preparation, MLN artificial inoculation and phenotyping

Young leaves with typical chlorotic symptoms of MCMV infected maize and that with mosaic symptoms of SCMV infected maize were separately collected in labelled plastic bags from each screen house and transferred to the laboratory for inoculum preparation.

Symptomatic leaves for each virus isolate were collected separately, weighed and cut into 1 to 2 cm long pieces using scissors and blended in a heavy-duty blender by adding a ratio of 1 g of leaf materials to 20 ml of 10 mM potassium-phosphate buffer (pH 7.0). The resulting homogenized mixture was sieved through cheesecloth. The inoculum extracts were mixed by adding one part of MCMV and four parts of SCMV (1:4) in one container to obtain optimized virus combination known to cause MLN in East Africa (Gowda et al., 2015). Carborundum was added in each combination at a rate of 1 g/L of extracts. Motorized backpack mist blower (SOLO 423, 12 L capacity) was used for the inoculum application in the trials 4 and 5 weeks after planting (plants were at four to five leaf stages).

Inoculated materials were planted in two trials; one involving maize landraces and the other inbreed line using a completely randomized design (CRD) and two trial replications. Each genotype was comprised of at least 13 plants in single rows 3 m long and spaced 0.25 m within and 0.75 m apart in season 2014B at Naivasha MLN Screening Facility located at Naivasha (latitude 0°43′S, longitude 36°26′E, 1896 m ASL) in Kenya. Disease severity was recorded 14 days after the second inoculation for maize landraces and seven days for maize inbreed lines. Rating was based on MLN severity scoring scale (1 to 5) (Kumar, 2009); where 1 = No MLN symptom, 2 = fine chlorotic streaks on lower leaves, 3 = chlorotic mottling throughout plant, 4 = excessive chlorotic mottling and dead heart and 5 = complete plant necrosis. Plants were evaluated and four scores were recorded for data analysis. The fourth disease scores were recorded 30 days after the third one.

Data analysis

Data were subjected to analysis of variance (ANOVA) using GenStat Release 16.1 and testing mean separation using LSD test at 5%. The source of variations in the analysis included replications and genotype effects. Therefore, the model used in the analysis was:

$$Y_{ik} = \mu + P_i + G_k + E_{ik}$$

Where, μ is mean; P_i is *ith* replication; G_k is *kth* genotype and E_{ik} is the error term. Disease severity scores were used to assess the effect of MLN inoculation on the genotypes involved in this study. Histograms were plotted for each scoring date to show MLN symptoms progression and the frequency of genotypes response to the disease.

RESULTS

Analysis of variance (ANOVA)

Significant phenotypic variations *($P<0.05$)* were observed on landraces for symptoms and disease severity scores (Figure 2). Landrace TZA-2793 had the lowest mean score of 3.5 followed by the other four landraces: TZA-3585, TZA-3543, TZA-4505 and TZA-2292, which attained a mean score of 3.75 (Supplementary material Table 1). There were no significant differences observed among the inbred lines. All inbred lines attained the mean score values between 4.5 and 5.0 except for the resistant

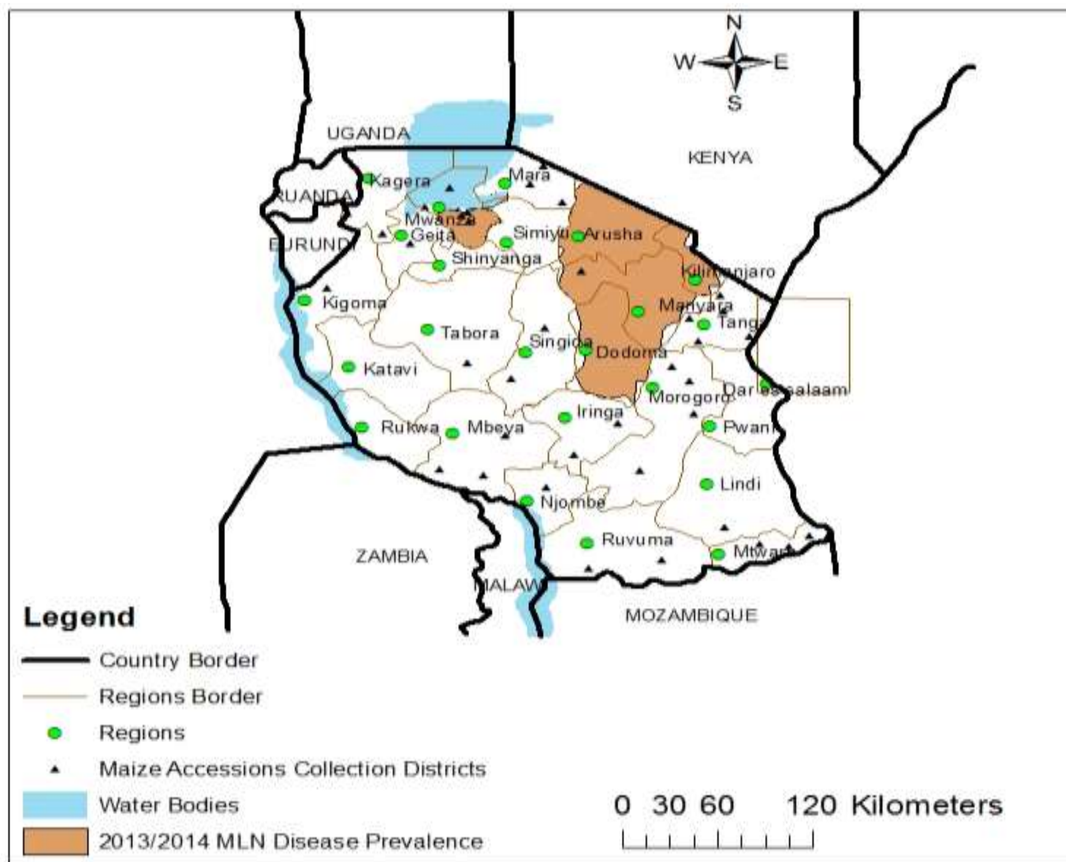

Figure 1. Map showing MLN disease prevalence in Tanzania (2013/2014) and districts where maize landraces in this study were collected.

check line CML494 which differed from inbred lines tested materials with a mean score of 3.75 (Supplementary material Table 2).

Maize lethal necrosis symptoms

Chlorotic mottle symptoms were observed between 9 and 14 days post inoculation (dpi). All maize genotypes in the experiments exhibited a range of MLN symptoms including mild to acute leaf chlorosis, higher density of chlorotic spots and stunting of plants. At the advanced stages of the disease, older leaves became severely chlorotic and necrotic tissues developed from leaf margins to the mid-ribs resulting in complete death of most plant materials in all the trials.

There were noticeable variations in the development of symptoms between the landraces and the inbred lines. Most of the inbreed lines were stable at the first evaluation but deteriorated quickly in subsequent scoring dates. In contrast, landraces also developed similar symptoms with most of the entries; only few of the landraces showed distinctive variation in symptoms development including within entry variations. The varied landraces within the same entry had plants with mild chlorotic spots (Figure 2) but most did not undergo complete plant necrosis and appeared to have a certain degree of tolerance to MLN.

Maize lethal necrosis disease severity

Reaction of maize landraces

The results showed that, all materials screened had mean scores ranging from 3.5 to 5.0 (Figure 3 and Table 3) in reference to rating scale of 1 to 5 (Kumar, 2009). Landrace TZA-2793 had a mean score of 3.5 at the last MLN score rating which was the lowest among all the landraces. Other maize landraces, which include TZA-3567, TZA-3585, TZA-3543 and TZA-4505 were found to have mean scores of 3.75. The remaining 147 landraces were susceptible to MLN with severity scores ranging from 4 to 5. Similarly, the control hybrid cultivar, Pan 67 also known to be susceptible to MLN had a score of 3.75. Other hybrids such as Duma 43, H614 and Pioneer had scores of 4, 4 and 4.5, respectively, indicating susceptibility to MLN.

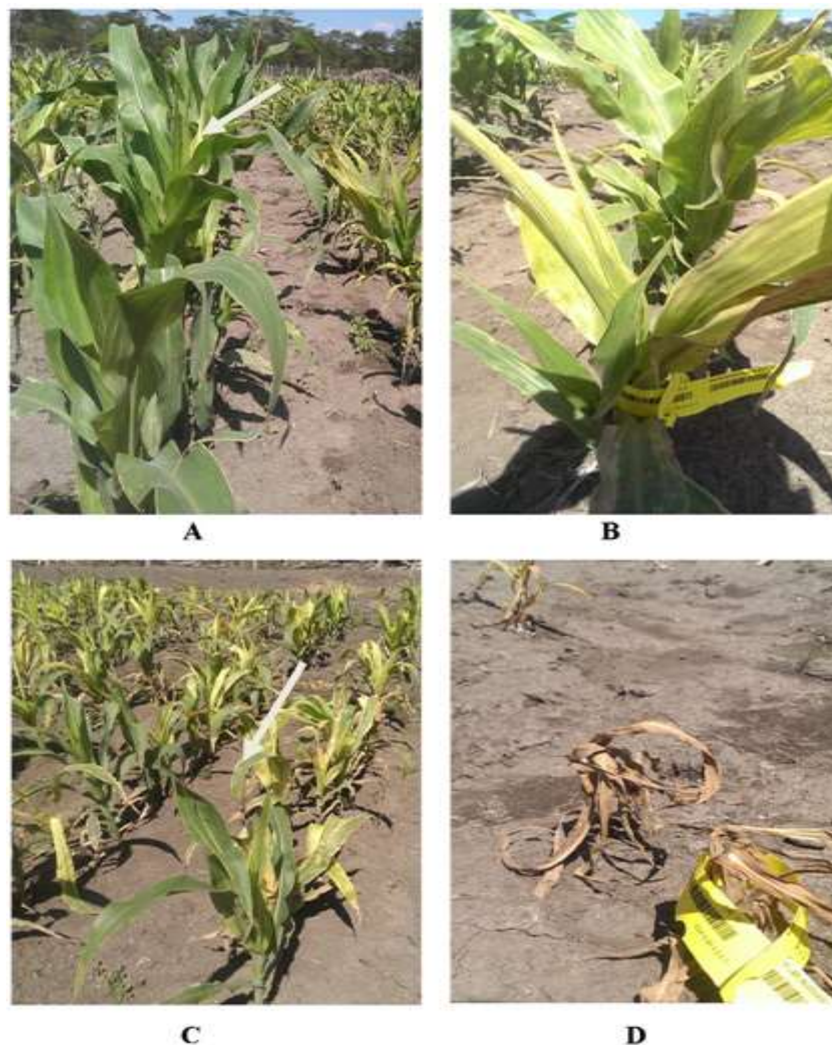

Figure 2. Maize lethal necrosis disease symptoms on Tanzanian maize landraces at Naivasha MLN screening facility. (A) Mild leaf chlorosis; (B) higher density of chlorotic spots; (C) necrotic tissues developed from leaf margins to the mid-ribs; (D) complete plant death.

Reaction of the Tanzanian maize inbred lines

Trials involving maize inbred lines had a resistant check line CML494, which had a mean disease severity score of 3.75. The susceptible control line CML395 proved to be highly susceptible to MLN with a final severity score of 5. All 33 Tanzanian inbred lines were highly susceptible to MLN disease with severity scores ranging from 4.5 to 5 (Figure 4).

DISCUSSION

Maize lethal necrosis disease (MLN) is caused by a co-infection of *maize chlorotic mottle virus* (MCMV) and any of the potyvirus infecting cereals such as *sugarcane mosaic virus* (SCMV). The former is transmitted by maize thrips (*Frankliniella williamsi*) and the latter by maize aphids (*Ropalosiphum maidis*) (Wangai et al., 2012). However, reports suggest that MCMV alone is a threat to maize production and may cause significant yield losses of up to 15% under natural disease pressure and up to 59% in experimental plots in the absence of the counterpart potyviruses (Castillo, 1976). Different strategies have been suggested for the control of MLN including cultural practices, use of insecticides and breeding for host resistance, which is considered the more viable approach to manage MLN (Nelson et al., 2011). Phenotypic diversities are essential prerequisites for cultivar identification and production; thus, to identify potential sources of natural resistance to MCMV, a collection of Tanzanian maize germplasm, including

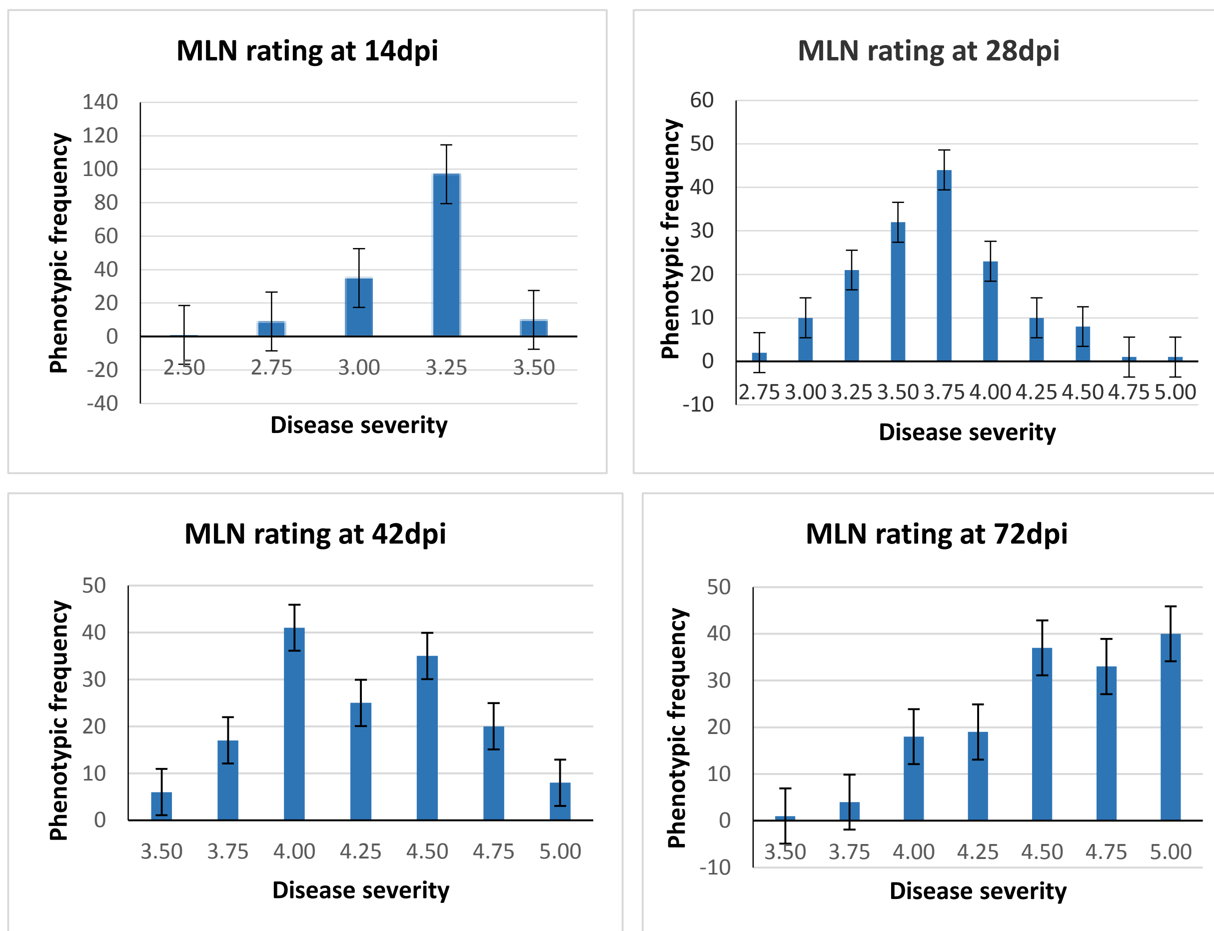

Figure 3. MLN disease responses and score distribution for Tanzanian maize landraces evaluated for MLN disease resistance at Naivasha maize lethal necrosis screening facility (14, 28, 42 and 72 dpi).

Table 3. Responses of selected Tanzanian maize landraces and control hybrid Pan 67 evaluated against MLN disease under artificial inoculation conditions.

Landrace	Kernel color	MLN severity score rating dates				Response to MLN
		MLN1 (14 dpi)	MLN2 (28 dpi)	MLN3 (42 dpi)	MLN4 (72 dpi)	
TZA_2793	Yellow	3.00	3.25	3.75	3.50	Tolerant
TZA_3567	White	3.00	3.00	3.50	3.75	Tolerant
TZA_3585	White	3.00	3.50	3.50	3.75	Tolerant
TZA_3543	White	2.75	3.00	3.75	3.75	Tolerant
TZA_4503	White	2.75	3.00	3.50	3.75	Tolerant
Pan 67	White	2.50	3.25	3.75	3.75	Tolerant

MLN, Maize lethal necrosis; MLN1, first rating date; MLN2, second rating date; MLN3, third rating date; MLN4, fourth rating date; dpi, days post inoculation.

maize landraces from different agro ecological zones (Figure 1) and maize breeding lines of Tanzania origin were evaluated for resistance against maize lethal necrosis disease (MLN).

In this study, we employed two artificial inoculation tests for maize landraces and maize inbred lines due to genetic variability of the maize landraces and that of maize inbred lines which were used as test materials. The two virus isolates, maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV) used to

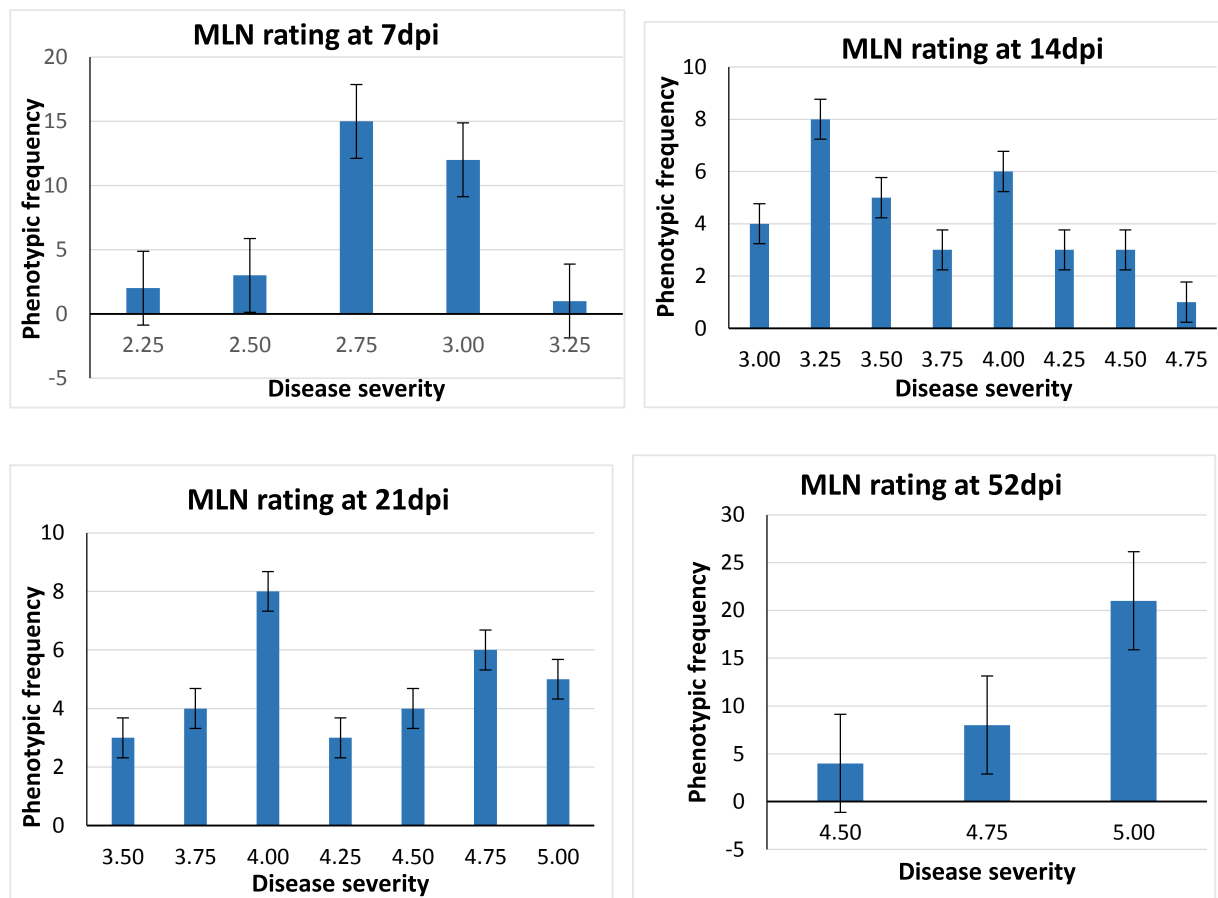

Figure 4. MLN disease responses and score distribution for Tanzanian maize inbreed lines evaluated for MLN resistance at Naivasha maize lethal necrosis screening facility (7, 14, 21 and 52 dpi).

facilitate phenotypic selection, led to development of typical MLN symptoms similar to those previously reported in double inoculated maize plants (Drake et al., 2007; Scheets, 1998).

Many of the materials utilized for MLN screening in this study were found susceptible to MLN. However, five Tanzanian maize landraces with the potential to tolerate MLN were identified (Table 3). Landraces TZA-2793, TZA-3567, TZA-3585, TZA-3543 and TZA-4505 displayed mild MLN symptoms under artificial inoculation conditions and were considered as tolerant. As these materials were of different genetic background, they displayed significant variations in their reaction to MLN and symptoms, which were noticed even within the same entry landrace lines where some individuals showed varied symptoms. These results are in agreement with those of Raji et al. (2009) who identified within line variations in African cassava landraces and suggested it is a result of geographical or regional variations where the germplasms were collected. This is a good indicator that, if the identified landraces are purified, the revealed lines may be very useful for use in future work involving MLN breeding for disease resistance. Landrace TZA-2793 was of particular interest as at the final scoring date, new growth of healthy leaves was observed which enabled this genotype to reduce the symptoms of MLN; however, the experiment was terminated before the end of the crop cycle. This provides possible opportunities of continued investigations on different screening environments and at all crop growth stages to explore the potentiality of using this landrace in MLN maize breeding programs. In the same trial involving maize landraces, the hybrid Pan67 also displayed a score rating of 3.75 which is also considered as tolerant. This hybrid could have displayed this performance because of its hybrid vigor (Sanghera et al., 2011).

All Tanzanian maize inbred lines were generally more susceptible to the infection of MLN; thus, it is concluded that, the resistance of maize to MCMV cannot be identified in this set of breeding materials and therefore more efforts are needed to screen more maize germplasm available in Tanzania. The CIMMYT line CML494, which was earlier identified as resistant in previous trials by CIMMYT in different screening

environment showed some symptoms in this trial; however, it was rated as tolerant. This probably shows the role of environmental conditions in the incidence of MLN disease. This is in line with the work of Scheets (1998) who evaluated MLN disease synergy using maize line (N28Ht) under different environmental conditions.

Maize landraces have been reported as among major source of genes that may be useful in breeding programs, particularly when breeding for biotic and abiotic stresses (Prassana et al., 2010); the same has been reported for other crops such as cassava (Raji, 2003) and barley (Adawy et al., 2008). It is important perhaps to continue conducting more investigation and utility of maize landraces to seek for more possibilities of exploring complete MLN resistance in Tanzanian landraces because, recently, a significant number of landraces have not been screened for resistance against MLN. CIMMYT and other partners involved in maize breeding programs have made progress aimed at identifying sources of natural resistance against MLN and particularly focusing on MCMV resistance because resistance for the corresponding potyvirus (SCMV) that co-infect with MCMV to induce MLN in East Africa has been identified and mapped on chromosome 3(Scmv2) and 6 (Scmv1) (Xia et al., 1999). Many of the genotypes screened have shown susceptibility to the disease, although some materials have shown promise as good sources of tolerance and/or resistance (Mahuku and Kimunye, 2015).

Management of MLN in East Africa also relies on the use of cultural practices. These approaches have not been reported to significantly address the incidences of MLN in the region. Together with searching for natural source of resistance, it is imperative to conduct studies to understand MLN epidemiology and the interaction existing between host/vector/pathogen in Tanzania and elsewhere in East Africa so as to provide more appropriate MLN management practices to maize farmers. It is also suggested that, the five landraces identified in this study should be purged and subjected to further MLN testing to explore the potential of using these materials in breeding for MLN disease resistance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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SUPPLEMENTARY MATERIAL

Table 1. Means of MLN disease severity scores for Tanzanian maize landraces and control of commercial hybrid cultivars obtained at different MLN evaluation intervals (at 14, 28, 42 and 72 days post inoculation).

Entry	Maize genotype	MLN rating at 14 dpi	MLN rating at 28 dpi	MLN rating at 42 dpi	MLN rating at 72 dpi	Response to MLN
1	TZA_1742	2.25^{a}	3.50^{abcd}	4.00^{abc}	4.50^{bcd}	Susceptible
2	H614	2.50^{ab}	3.25^{abc}	3.50^{a}	4.00^{abc}	Susceptible
3	Pan 67	2.50^{ab}	3.25^{abc}	4.00^{abc}	3.75^{ab}	Tolerant
4	TZA_3914	2.50^{ab}	3.25^{abc}	4.00^{abc}	4.00^{abc}	Susceptible
5	TZA_3926	2.50^{ab}	3.75^{bcde}	4.50^{bcd}	4.50^{bcd}	Susceptible
6	TZA_3951	2.50^{ab}	4.25^{defg}	5.00^{d}	5.00^{d}	Susceptible
7	TZA_3957	2.50^{ab}	3.25^{abc}	4.00^{abc}	4.50^{bcd}	Susceptible
8	TZA_4000	2.50^{ab}	4.00^{cdef}	4.75^{cd}	5.00^{d}	Susceptible
9	TZA_4047	2.50^{ab}	3.75^{bcde}	4.75^{cd}	5.00^{d}	Susceptible
10	TZA_4212	2.50^{ab}	3.75^{bcde}	4.25^{abcd}	4.25^{abcd}	Susceptible
11	TZA_4350	2.50^{ab}	3.5^{abcd}	4.25^{abcd}	4.25^{abcd}	Susceptible
12	TZA_1723	2.75^{bc}	4.00^{cdef}	4.25^{abcd}	5.00^{d}	Susceptible
13	TZA_1724	2.75^{bc}	3.25^{abc}	3.75^{ab}	4.00^{abc}	Susceptible
14	TZA_1741	2.75^{bc}	3.75^{bcde}	4.50^{bcd}	4.75^{cd}	Susceptible
15	TZA_1744	2.75^{bc}	3.25^{abc}	4.00^{abc}	4.00^{abc}	Susceptible
16	TZA_1755	2.75^{bc}	3.50^{abcd}	4.00^{abc}	4.50^{bcd}	Susceptible
17	TZA_1757	2.75^{bc}	3.50^{abcd}	4.00^{abc}	4.50^{bcd}	Susceptible
18	TZA_181	2.75^{bc}	3.75^{bcde}	4.25^{abcd}	4.50^{bcd}	Susceptible
19	TZA_212	2.75^{bc}	3.50^{abcd}	4.00^{abc}	4.50^{bcd}	Susceptible
20	TZA_2816	2.75^{bc}	3.50^{abcd}	4.00^{abc}	4.25^{abcd}	Susceptible
21	TZA_2843	2.75^{bc}	4.25^{defg}	4.75^{cd}	4.75^{cd}	Susceptible
22	TZA_3536	2.75^{bc}	3.25^{abc}	3.50^{a}	4.00^{abc}	Susceptible
23	TZA_3543	2.75^{bc}	3.00^{ab}	3.75^{ab}	3.75^{ab}	Tolerant
24	TZA_3544	2.75^{bc}	3.50^{abcd}	4.50^{bcd}	4.75^{cd}	Susceptible
25	TZA_3885	2.75^{bc}	3.50^{abcd}	4.50^{bcd}	5.00^{d}	Susceptible
26	TZA_3942	2.75^{bc}	3.25^{abc}	4.00^{abc}	4.00^{abc}	Susceptible
27	TZA_3958	2.75^{bc}	3.50^{abcd}	4.00^{abc}	4.00^{abc}	Susceptible
28	TZA_3964	2.75^{bc}	3.50^{abcd}	4.00^{abc}	4.75^{cd}	Susceptible
29	TZA_3971	2.75^{bc}	3.50^{abcd}	3.75^{ab}	4.00^{abc}	Susceptible
30	TZA_4016	2.75^{bc}	3.00^{ab}	4.25^{abcd}	4.50^{bcd}	Susceptible
31	TZA_4043	2.75^{bc}	3.75^{bcde}	4.25^{abcd}	4.50^{bcd}	Susceptible
32	TZA_4052	2.75^{bc}	3.25^{abc}	4.25^{abcd}	4.50^{bcd}	Susceptible
33	TZA_4058	2.75^{bc}	3.50^{abcd}	4.50^{bcd}	4.75^{cd}	Susceptible
34	TZA_4067	2.75^{bc}	4.00^{cdef}	4.50^{bcd}	5.00^{d}	Susceptible
35	TZA_4186	2.75^{bc}	3.50^{abcd}	4.25^{abcd}	4.75^{cd}	Susceptible
36	TZA_4203	2.75^{bc}	3.25^{abc}	3.75^{ab}	4.25^{abcd}	Susceptible
37	TZA_4206	2.75^{bc}	3.50^{abcd}	4.50^{bcd}	5.00^{d}	Susceptible
38	TZA_4273	2.75^{bc}	4.50^{efg}	4.50^{bcd}	5.00^{d}	Susceptible
39	TZA_4505	2.75^{bc}	3.00^{ab}	3.50^{a}	3.75^{ab}	Tolerant
40	TZA_5101	2.75^{bc}	3.00^{ab}	3.75^{ab}	4.25^{abcd}	Susceptible
41	TZA_5200	2.75^{bc}	3.00^{ab}	3.75^{ab}	4.25^{abcd}	Susceptible
42	TZA_5201	2.75^{bc}	3.50^{abcd}	3.75^{ab}	4.25^{abcd}	Susceptible
43	TZA_5619	2.75^{bc}	4.00^{cdef}	4.75^{cd}	5.00^{d}	Susceptible
44	TZA_707	2.75^{bc}	3.75^{bcde}	4.00^{abc}	4.00^{abc}	Susceptible
45	TZA_78	2.75^{bc}	3.25^{abc}	4.00^{abc}	4.25^{abcd}	Susceptible
46	TZA_93	2.75^{bc}	4.00^{cdef}	4.50^{bcd}	5.00^{d}	Susceptible
47	Duma 43	3.00^{cd}	3.50^{abcd}	4.00^{abc}	4.00^{abc}	Susceptible
48	Pioneer	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.50^{bcd}	Susceptible

Table 1. Contd.

49	TZA_111	3.00^{cd}	4.50^{efg}	4.75^{cd}	4.75^{cd}	Susceptible
50	TZA_163	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.75^{cd}	Susceptible
51	TZA_1711	3.00^{cd}	4.50^{efg}	4.50^{bcd}	5.00^{d}	Susceptible
52	TZA_1713	3.00^{cd}	3.75^{bcde}	4.50^{bcd}	4.75^{cd}	Susceptible
53	TZA_1718	3.00^{cd}	4.25^{defg}	4.50^{bcd}	4.75^{cd}	Susceptible
54	TZA_1725	3.00^{cd}	3.50^{abcd}	4.00^{abc}	4.25^{abcd}	Susceptible
55	TZA_1727	3.00^{cd}	3.75^{bcde}	4.25^{abcd}	4.50^{bcd}	Susceptible
56	TZA_1728	3.00^{cd}	3.25^{abc}	4.25^{abcd}	4.75^{cd}	Susceptible
57	TZA_1731	3.00^{cd}	3.50^{abcd}	4.25^{abcd}	5.00^{d}	Susceptible
58	TZA_1732	3.00^{cd}	4.00^{cdef}	4.25^{abcd}	4.50^{bcd}	Susceptible
59	TZA_1739	3.00^{cd}	3.50^{abcd}	4.00^{abc}	4.50^{bcd}	Susceptible
60	TZA_1745	3.00^{cd}	3.50^{abcd}	4.50^{bcd}	4.25^{abcd}	Susceptible
61	TZA_1752	3.00^{cd}	4.00^{cdef}	4.50^{bcd}	5.00^{d}	Susceptible
62	TZA_1753	3.00^{cd}	3.50^{abcd}	4.50^{bcd}	4.50^{bcd}	Susceptible
63	TZA_1754	3.00^{cd}	3.75^{bcde}	4.25^{abcd}	4.25^{abcd}	Susceptible
64	TZA_1758	3.00^{cd}	3.50^{abcd}	4.50^{bcd}	5.00^{d}	Susceptible
65	TZA_2259	3.00^{cd}	4.25^{defg}	4.75^{cd}	5.00^{d}	Susceptible
66	TZA_2263	3.00^{cd}	3.75^{bcde}	4.25^{abcd}	4.50^{bcd}	Susceptible
67	TZA_2264	3.00^{cd}	3.75^{bcde}	4.75^{cd}	5.00^{d}	Susceptible
68	TZA_2267	3.00^{cd}	3.75^{bcde}	4.50^{bcd}	4.75^{cd}	Susceptible
69	TZA_2271	3.00^{cd}	4.00^{cdef}	4.75^{cd}	5.00^{d}	Susceptible
70	TZA_2292	3.00^{cd}	3.50^{abcd}	3.75^{ab}	4.00^{abc}	Susceptible
71	TZA_2330	3.00^{cd}	3.00^{ab}	3.75^{ab}	4.00^{abc}	Susceptible
72	TZA_2333	3.00^{cd}	3.25^{abc}	4.00^{abc}	4.50^{bcd}	Susceptible
73	TZA_2338	3.00^{cd}	4.50^{e}fg	5.00^{d}	5.00^{d}	Susceptible
74	TZA_2369	3.00^{cd}	3.75^{bcde}	4.75^{cd}	5.00^{d}	Susceptible
75	TZA_2719	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.75^{cd}	Susceptible
76	TZA_2721	3.00^{cd}	3.25^{abc}	4.50^{bcd}	5.00^{d}	Susceptible
77	TZA_2731	3.00^{cd}	3.75^{bcde}	4.50^{bcd}	4.50^{bcd}	Susceptible
78	TZA_2793	3.00^{cd}	3.25^{abc}	3.75^{ab}	3.50^{a}	Tolerant
79	TZA_2813	3.00^{cd}	2.75^{a}	3.75^{ab}	4.25^{abcd}	Susceptible
80	TZA_2824	3.00^{cd}	3.00^{ab}	3.75^{ab}	4.25^{abcd}	Susceptible
81	TZA_2829	3.00^{cd}	3.25^{abc}	3.75^{ab}	4.00^{abc}	Susceptible
82	TZA_2840	3.00^{cd}	3.5^{abcd}	4.00^{abc}	4.25^{abcd}	Susceptible
83	TZA_2904	3.00^{cd}	4.00^{cde}f	4.75^{cd}	5.00^{d}	Susceptible
84	TZA_2910	3.00^{cd}	3.75^{bcde}	4.50^{bcd}	4.75^{cd}	Susceptible
85	TZA_2933	3.00^{cd}	3.25^{abc}	4.00^{abc}	4.50^{bcd}	Susceptible
86	TZA_3054	3.00^{cd}	3.50^{abcd}	4.00^{abc}	4.50^{bcd}	Susceptible
87	TZA_3167	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.75^{cd}	Susceptible
88	TZA_3171	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.50^{bcd}	Susceptible
89	TZA_3181	3.00^{cd}	4.25^{de}fg	4.50^{bcd}	4.75^{cd}	Susceptible
90	TZA_3206	3.00^{cd}	3.75^{bcde}	4.25^{abcd}	4.75^{cd}	Susceptible
91	TZA_3310	3.00^{cd}	4.00^{cde}f	4.25^{abcd}	5.00^{d}	Susceptible
92	TZA_3312	3.00^{cd}	5.00g	5.00^{d}	5.00^{d}	Susceptible
93	TZA_3546	3.00^{cd}	4.00^{cde}f	4.50^{bcd}	5.00^{d}	Susceptible
94	TZA_3559	3.00^{cd}	3.75^{bcde}	3.75^{ab}	4.00^{abc}	Susceptible
95	TZA_3567	3.00^{cd}	2.75^{a}	3.50^{a}	3.75^{ab}	Tolerant
96	TZA_3569	3.00^{cd}	3.75^{bcde}	4.75^{cd}	5.00^{d}	Susceptible
97	TZA_3585	3.00^{cd}	3.50^{abcd}	3.50^{a}	3.75^{ab}	Tolerant
98	TZA_3713	3.00^{cd}	3.75b^{cde}	4.50b^{cd}	4.75^{cd}	Susceptible
99	TZA_3741	3.00^{cd}	3.00^{a}b	4.00^{a}b^{c}	4.00^{a}b^{c}	Susceptible
100	TZA_3744	3.00c^{d}	3.75bc^{de}	5.00^{d}	5.00^{d}	Susceptible

Table 1. Contd.

101	TZA_3795	3.00^{cd}	$4.25^{de}fg$	4.50^{bcd}	5.00^{d}	Susceptible
102	TZA_3827	3.00^{cd}	3.75^{bcde}	4.50^{bcd}	4.50^{bcd}	Susceptible
103	TZA_3854	3.00^{cd}	3.75^{bcde}	4.25^{abcd}	4.75^{cd}	Susceptible
104	TZA_3855	3.00^{cd}	3.75^{bcde}	4.50^{bcd}	5.00^{d}	Susceptible
105	TZA_3860	3.00^{cd}	3.50^{abcd}	3.75^{ab}	4.25^{abcd}	Susceptible
106	TZA_3974	3.00^{cd}	3.25^{abc}	3.75^{ab}	4.00^{abc}	Susceptible
107	TZA_3982	3.00^{cd}	$4.00^{cde}f$	4.25^{abcd}	4.75^{cd}	Susceptible
108	TZA_4010	3.00^{cd}	$4.25^{de}fg$	4.50^{bcd}	4.50^{bcd}	Susceptible
109	TZA_4020	300^{cd}	3.50^{abcd}	4.00^{abc}	4.75^{cd}	Susceptible
110	TZA_4035	300^{cd}	$4.50^{e}fg$	5.00^{d}	5.00^{d}	Susceptible
111	TZA_4063	3.00^{cd}	$4.50^{e}fg$	5.00^{d}	5.00^{d}	Susceptible
112	TZA_4064	3.00^{cd}	$4.25^{de}fg$	4.25^{abcd}	4.50^{bcd}	Susceptible
113	TZA_4068	3.00^{cd}	3.75^{bcde}	4.25^{abcd}	4.50^{bcd}	Susceptible
114	TZA_4078	3.00^{cd}	$4.25^{de}fg$	4.75^{cd}	5.00^{d}	Susceptible
115	TZA_4092	3.00^{cd}	$4.00^{cde}f$	4.25^{abcd}	4.50^{bcd}	Susceptible
116	TZA_4130	3.00^{cd}	$4.00^{cde}f$	4.50^{bcd}	4.75^{cd}	Susceptible
117	TZA_4163	3.00^{cd}	3.75^{bcde}	4.50^{bcd}	5.00^{d}	Susceptible
118	TZA_4164	3.00^{cd}	3.00^{ab}	4.00^{abc}	4.50^{bcd}	Susceptible
119	TZA_4165	3.00^{cd}	3.50^{abcd}	5.00^{d}	5.00^{d}	Susceptible
120	TZA_4167	3.00^{cd}	$4.00^{cde}f$	4.50^{bcd}	4.75^{cd}	Susceptible
121	TZA_4181	3.00^{cd}	$4.50^{e}fg$	4.75^{cd}	4.50^{bcd}	Susceptible
122	TZA_4185	3.00^{cd}	3.75^{bcde}	4.75^{cd}	5.00^{d}	Susceptible
123	TZA_4197	3.00^{cd}	3.50^{abcd}	4.25^{abcd}	4.75^{cd}	Susceptible
124	TZA_4205	3.00^{cd}	3.75^{bcde}	4.50^{bcd}	4.75^{cd}	Susceptible
125	TZA_4351	3.00^{cd}	$4.00^{cde}f$	4.00^{abc}	4.75^{cd}	Susceptible
126	TZA_4574	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.50^{bcd}	Susceptible
127	TZA_4667	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.50^{bcd}	Susceptible
128	TZA_5102	3.00^{cd}	$4.00^{cde}f$	4.75^{cd}	5.00^{d}	Susceptible
129	TZA_5105	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.75^{cd}	Susceptible
130	TZA_5129	3.00^{cd}	3.50^{abcd}	4.00^{abc}	4.50^{bcd}	Susceptible
131	TZA_5138	3.00^{cd}	$4.00^{cde}f$	4.50^{bcd}	4.75^{cd}	Susceptible
132	TZA_5162	3.00^{cd}	3.75^{bcde}	4.25^{abcd}	5.00^{d}	Susceptible
133	TZA_5169	3.00^{cd}	3.50^{abcd}	4.00^{abc}	4.75^{cd}	Susceptible
134	TZA_5170	3.00^{cd}	3.25^{abc}	4.00^{abc}	4.25^{abcd}	Susceptible
135	TZA_5171	3.00^{cd}	3.25^{abc}	3.50^{a}	4.00^{abc}	Susceptible
136	TZA_5173	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.25^{abcd}	Susceptible
137	TZA_5618	3.00^{cd}	3.75^{bcde}	3.75^{ab}	4.00^{abc}	Susceptible
138	TZA_5621	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.50^{bcd}	Susceptible
139	TZA_589	3.00^{cd}	$4.00^{cde}f$	4.50^{bcd}	5.00^{d}	Susceptible
140	TZA_599	3.00^{cd}	3.25^{abc}	4.00^{abc}	4.50^{bcd}	Susceptible
141	TZA_604	3.00^{cd}	3.75^{bcde}	4.00^{abc}	4.50^{bcd}	Susceptible
142	TZA_608	3.00^{cd}	3.75^{bcde}	4.25^{abcd}	4.25^{abcd}	Susceptible
143	TZA_615	3.00^{cd}	$4.00^{cde}f$	4.25^{abcd}	4.75^{cd}	Susceptible
144	TZA_62	3.00^{cd}	$4.00^{cde}f$	4.75^{cd}	5.00^{d}	Susceptible
145	TZA_67	3.00^{cd}	3.75^{bcde}	4.75^{cd}	5.00^{d}	Susceptible
146	TZA_687	3.00^{cd}	3.25^{abc}	4.00^{abc}	4.25^{abcd}	Susceptible
147	TZA_1717	3.25^{d}	$4.00^{cde}f$	4.75^{cd}	4.75^{cd}	Susceptible
148	TZA_2685	3.25^{d}	3.50^{abcd}	4.50^{bcd}	4.75^{cd}	Susceptible
149	TZA_2733	3.25^{d}	3.50^{abcd}	4.50^{bcd}	4.50^{bcd}	Susceptible
150	TZA_2949	3.25^{d}	4.75fg	5.00^{d}	5.00^{d}	Susceptible
151	TZA_3548	3.25^{d}	3.00^{ab}	4.00^{abc}	4.50^{bcd}	Susceptible
152	TZA_3605	3.25^{d}	$4.50^{e}fg$	5.00^{d}	5.00^{d}	Susceptible

Table 1. Contd.

153	TZA_3614	3.25^{d}	4.25^{defg}	4.75^{cd}	4.75^{cd}	Susceptible
154	TZA_3837	3.25^{d}	3.75^{bcde}	4.00^{abc}	4.50^{bcd}	Susceptible
155	TZA_3961	3.25^{d}	4.00^{cde}f	4.75^{cd}	5.00^{d}	Susceptible
156	TZA_4320	3.25^{d}	3.75^{bcde}	4.00^{abc}	4.00^{abc}	Susceptible
	F value	1.11	1.58	1.89	1.79	
	P value	0.26	0.002	<0.001	<0.001	
	S.E	0.24	0.46	0.39	0.39	
	CV%	8.20	12.40	9.20	8.60	
	L.S.D	0.47	0.90	0.78	0.78	

Figures followed by the same letter(s) in columns are not significantly different (P=0.05). dpi, days post inoculation.

Table 2. Means of MLN disease severity scores for Tanzanian maize inbred lines and control CIMMYT lines obtained at different MLN evaluation intervals (at 7, 14, 28 and 52 days post inoculation).

Entry	Maize genotype	MLN rating at 7 dpi	MLN rating at 14 dpi	MLN rating at 21 dpi	MLN rating at 52 dpi	Response to MLN
1	KAT 12-4-2-2	2.25^{a}	3.25^{ab}	4.00^{abc}	4.75^{b}	Susceptible
2	KIL 4-78-4-3	2.25^{a}	3.00^{a}	3.75^{ab}	4.50^{b}	Susceptible
3	CML494	2.50^{ab}	3.25^{ab}	3.50^{a}	3.75^{a}	Tolerant
4	KS 03-OB15-120	2.50^{ab}	3.25^{ab}	4.25^{abcd}	5.00^{b}	Susceptible
5	P43-1-1-1-BBB	2.50^{ab}	3.00^{a}	4.25^{abcd}	5.00^{b}	Susceptible
6	TUX 5-50-1-1-2-2	2.50^{ab}	3.75^{abcd}	4.75^{cd}	5.00^{b}	Susceptible
7	KAT 12-1-4-2	2.75^{abc}	3.50^{abc}	4.00^{abc}	4.75^{b}	Susceptible
8	KIL 4-78-2-3	2.75^{abc}	3.50^{abc}	4.50^{bcd}	5.00^{b}	Susceptible
9	KS 03-OB15-125	2.75^{abc}	4.50^{de}	5.00^{d}	5.00^{b}	Susceptible
10	KS 03-OB15-188	2.75^{abc}	4.00^{bcde}	3.75^{ab}	4.75^{b}	Susceptible
11	KS 03-OB15-198	2.75^{abc}	4.50^{de}	5.00^{d}	5.00^{b}	Susceptible
12	KS 03-OB15-45	2.75^{abc}	3.25^{ab}	3.50^{a}	4.75^{b}	Susceptible
13	KS 03-OB15-83	2.75^{abc}	4.25^{cde}	4.25^{abcd}	5.00^{b}	Susceptible
14	KS 03-OB15-85	2.75^{abc}	3.25^{ab}	3.75^{ab}	5.00^{b}	Susceptible
15	KS 03-OB15-92	2.75^{abc}	3.75^{abcd}	4.00^{abc}	4.75^{b}	Susceptible
16	MV 1-89-2	2.75^{abc}	3.50^{abc}	4.75^{cd}	5.00^{b}	Susceptible
17	MV 3-34-2-8	2.75^{abc}	3.50^{abc}	4.00^{abc}	5.00^{b}	Susceptible
18	MV 38-1-2-1-2	2.75^{abc}	3.25^{ab}	3.75^{ab}	4.75^{b}	Susceptible
19	TMV 1-5-28-3-1	2.75^{abc}	3.25^{ab}	4.00^{abc}	4.50^{b}	Susceptible
20	TMV 2-65-2-1-2-2	2.75^{abc}	4.00^{bcde}	4.50^{bcd}	5.00^{b}	Susceptible
21	TUX 5-50-1-3-1-1	2.75^{abc}	3.00^{a}	3.50^{a}	4.50^{b}	Susceptible
22	KAT 12/2-92-1-1-2	3.00^{bc}	3.25^{ab}	4.00^{abc}	4.75^{b}	Susceptible
23	KS 03-OB15-1	3.00^{bc}	4.00^{bcde}	5.00^{d}	5.00^{b}	Susceptible
24	KS 03-OB15-111	3.00^{bc}	4.00^{bcde}	5.00^{d}	5.00^{b}	Susceptible
25	KS 03-OB15-118	3.00^{bc}	4.25^{cde}	4.75^{cd}	5.00^{b}	Susceptible
26	KS 03-OB15-126	3.00^{bc}	3.00^{a}	3.50^{a}	4.50^{b}	Susceptible
27	KS 03-OB15-153	3.00^{bc}	4.00^{bcde}	4.50^{bcd}	5.00^{b}	Susceptible
28	KS 03-OB15-156	3.00^{bc}	4.00^{bcde}	4.75^{cd}	5.00^{b}	Susceptible
29	KS 03-OB15-3	3.00^{bc}	4.25^{cde}	4.75^{cd}	5.00^{b}	Susceptible
30	KS 03-OB15-53	3.00^{bc}	3.75^{abcd}	4.50^{bcd}	5.00^{b}	Susceptible
31	L511-15-1-3-1-1	3.00^{bc}	4.50^{de}	4.75^{cd}	5.00^{b}	Susceptible
32	MV 501-6-86-3-1-1	3.00^{bc}	3.50^{abc}	4.00^{abc}	5.00^{b}	Susceptible
33	TUX 5-50-1-5-2-1	3.00^{bc}	3.25^{ab}	4.00^{abc}	4.75^{b}	Susceptible
34	CML395	3.25^{c}	4.25^{cde}	4.75^{cd}	5.00^{b}	Susceptible
35	TUX 5-50-1-2-6-1	3.25^{c}	4.75^{e}	5.00^{d}	5.00^{b}	Susceptible

Table 2. Contd.

F value	1.61	2.81	2.33	1.86
P value	0.085	0.002	0.008	0.038
S.E	0.26	0.43	0.46	0.27
CV%	9.4	11.6	10.8	5.5
L.S.D	0.53	0.87	0.94	0.55

Figures followed by the same letter(s) in columns are not significant different (P=0.05). dpi, days post inoculation.

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