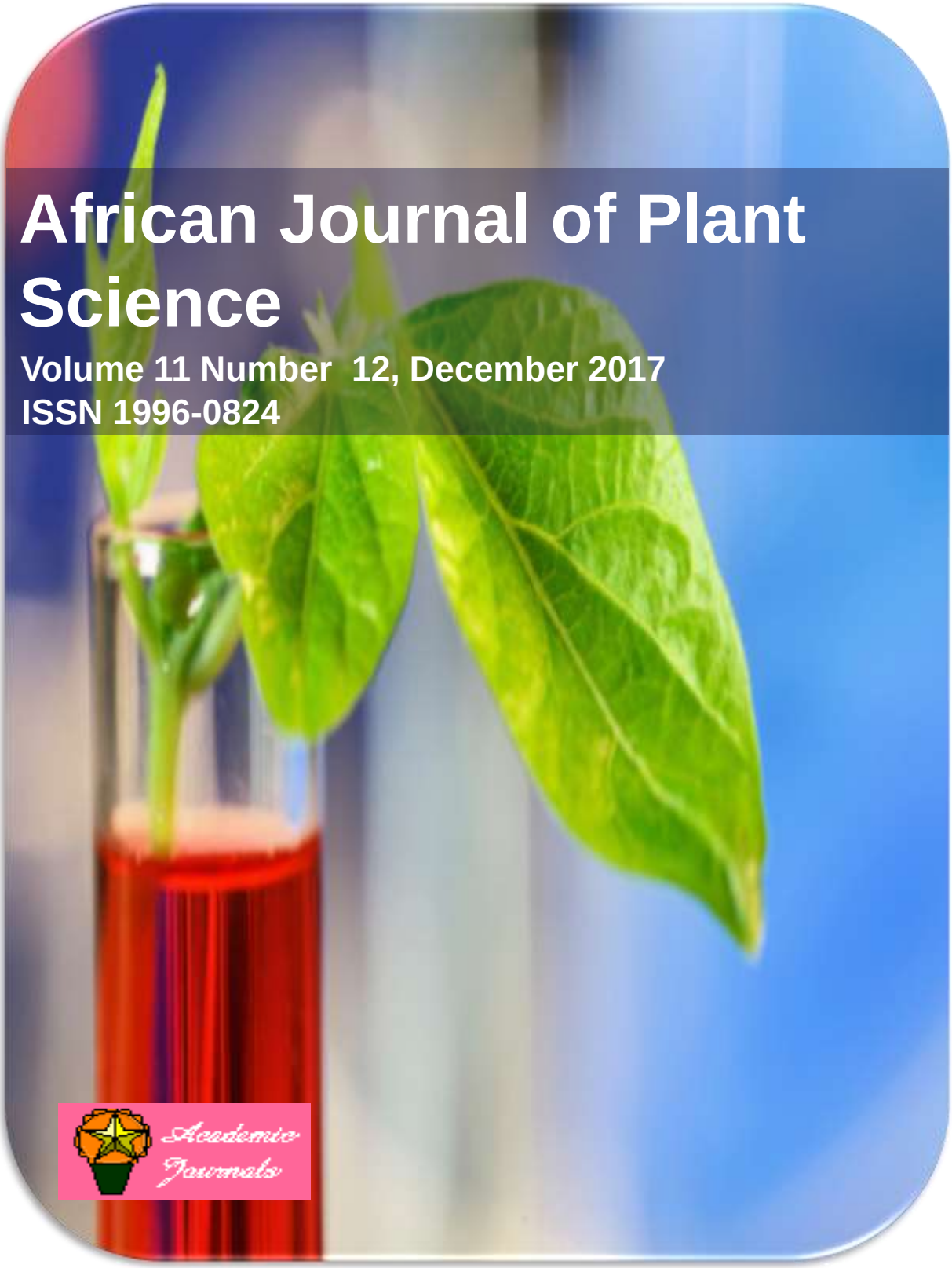

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African Journal of Plant Science

*Full Length Research Paper*

# Potential of cassava starch from TME 419 as suitable gelling agent in micropropagation of cassava (*Manihot esculenta* Crantz)

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**Cassava starch from nine varieties, namely, NR 8082, TMS 97/2205, TMS 97/0162, TMS 92/0057, TMS 98/0505, TMS 92/0326, TMS 30572, TMS 82/0058, and TME 419 were evaluated for their suitability as gelling substitute to conventional gelling agents (gellan gum and agar) in medium using cassava shoot tips and nodal segments as explants. Explants were seeded singly into a 15 ml cassava multiplication medium gelled either in 0.2% gellan gum, 0.7% agar or 7% starch from the nine cassava varieties. Cultures were maintained at 28 ± 2°C, 16 h photoperiod and 30 to 40 $\mu Em^{-2} s^{-1}$ flux intensity supplied by white fluorescent tubes on shelves for four weeks. Percentage survival of explants irrespective of type ranged from 61.5 to 100 with NR 8082 and TMS 97/2205 cassava starch-gelled medium recording the highest score while the mean number of nodes produced per explant ranged between 3.6 ± 1.43 and 5.33 ± 0.87 for shoot tips and 2.73 ± 0.96 and 4.79 ± 0.97 for nodal segments. The nodal segments from TME 419 starch-gelled medium had the highest mean number of nodes though not significantly different (p>0.05) from those from gellan gum and agar media. TME 419 was the most consistent in influencing regeneration of cassava plantlets.**

**Key words:** TME 419, cassava starch, explants, gelling agent, micropropagation.

## INTRODUCTION

Micropropagation technology is more expensive than the conventional methods of plant propagation and requires several types of skills. It is a capital-intensive industry and in some cases the unit cost per plant becomes unaffordable. The major reasons are cost of production and know-how. During the early years of the technology, there were difficulties in selling tissue culture products because the conventional planting material was much cheaper. Now this problem has been addressed by inventing reliable and cost effective tissue culture methods without compromising on quality. This requires a constant monitoring of the input costs of chemicals, media, energy, labour and capital. For example, the cost of medium preparation (chemicals, energy and labour)

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**Table 1.** Functional property of the different gelling agents.

| Gelling agent | Properties | | | | | | |
|---|---|---|---|---|---|---|---|
| | Bulk density (g/ml) | Gelation capacity (% w/v) | Gelatinazation temperature (°C) | Solubility | Swelling capacity (g/ml) | Water absorption capacity | Multiplication rate |
| NR 8082 | 0.69 | 0.50 | 69 | 6.71 | 2.19 | 1.85 | 3.90 |
| TMS 97/2205 | 0.60 | 0.50 | 75 | 6.26 | 1.95 | 2.95 | 4.00 |
| TMS 97/0162 | 0.64 | 0.50 | 68 | 8.75 | 1.80 | 2.16 | 4.00 |
| TMS 92/0057 | 0.66 | 0.5 | 70 | 6.75 | 1.82 | 2.17 | 3.50 |
| TMS 98/0505 | 0.65 | 0.5 | 64 | 5.80 | 1.75 | 2.48 | 3.71 |
| TMS 92/0326 | 0.63 | 1.0 | 62 | 5.72 | 2.12 | 2.80 | 3.60 |
| TMS 30572 | 0.66 | 0.5 | 65 | 8.34 | 1.86 | 2.45 | 4.12 |
| TME 419 | 0.65 | 1.0 | 75 | 4.70 | 1.86 | 2.95 | 4.44 |
| TMS 82/0058 | 0.67 | 1.0 | 69 | 8.71 | 1.92 | 2.40 | 3.78 |
| Gellan gum | 0.69 | 0.5 | 31 | 3.53 | 8.10 | 10.0 | 5.33 |
| Agar | 0.67 | 0.5 | 45 | 3.82 | 7.52 | 7.56 | 5.00 |

can account for 30 to 35% of the micropropagated plant production (Prakash, 1993). Media chemicals cost less than 15% of micro-plant production. In some cases the cost may be as low as 5%. Of the medium components, the gelling agents such as agar contribute 70% of the costs (Prakash, 1993). Other ingredients in the media: water, salts, and sugar have minimal influence on production cost and are reasonably cheap.

Low cost alternatives are needed to reduce production cost of tissue-cultured plants. Plant starches have been shown to be good gelling alternatives in plant tissue culture medium to conventional gelling agents such as agar, gellan gum and gelrite (Pierik, 1989; Nagamori and Kobayashi, 2001; NRDC, 2002). The substitution of conventional gelling agent with cassava starch is a welcomed development towards low cost micropropagation. This study confirms the gelling potential of starch from TME 419 cassava in medium over other starches from different cassava varieties in the micropropagation of cassava.

## MATERIALS AND METHODS

### Source of explants

Shoot-tip explants and nodal segments were excised from vigorously growing *in vitro Manihot esculenta* cultivar Egedudu (OY 001) obtained from the gene bank housed at the Biotechnology Unit (Plant Tissue Culture Laboratory) of National Root Crops Research Institute (NRCRI) Umudike, Abia State, Nigeria.

### Starch preparation

Starch was obtained from nine cassava varieties, namely: NR 8082, TMS 97/2205, TMS 97/0162, TMS 92/0057, TMS 98/0505, TMS 92/0326, TMS 30572, TMS 82/0058, and TME 419 according to Mbanaso (2008) and Nkere and Mbanaso (2009).

### Culture medium

The culture medium was Murashige and Skoog (1962) basal medium with 3% sucrose. Medium was solidified with gellan gum, agar or starch at 0.22, 0.7 and 7%, respectively. The pH was adjusted to 5.8. Gellan gum and agar were dissolved by heating while the starches were incorporated as described (Mbanaso, 2008; Nkere and Mbanaso, 2009). The dried cassava starch powder was first made into thick slurry with a part of the medium to be gelled. The remaining part was heated to 78 ± 2°C and the corresponding cold slurry stirred vigorously into it. A 15 ml aliquot each of the different media was then dispensed into culture tubes and autoclaved at 121°C for 15 min.

### Explants culture/Parameters assessed

A total of 275 cultures representing 11 treatments of 25 tubes each (15 shoot tips and 10 nodal segments) were used. Explants were seeded singly into culture tubes containing the prepared medium. Cultures were maintained at 28°C ± 2, 16 h photoperiod and 30 to 40 $\mu Em^{-2}\ s^{-1}$ flux intensity supplied by white fluorescent tubes on culture shelves for four weeks. The number of shoot tips and nodal segments were assessed after two and four weeks in culture. The experiment was repeated twice.

### Statistical analysis

Data were analysed using analysis of variance (ANOVA) and multiple comparison–least significant difference (LSD) of the GenStat (DE3) ver. 7.2.

## RESULTS AND DISCUSSION

The functional properties of the different gelling agents are shown in Table 1. Like the conventional gelling agents, starch from TME 419 cassava variety exhibits low solubility at lower temperatures. However, as with the former, solubility increased as temperature increased. This apparently favoured diffusion and availability of

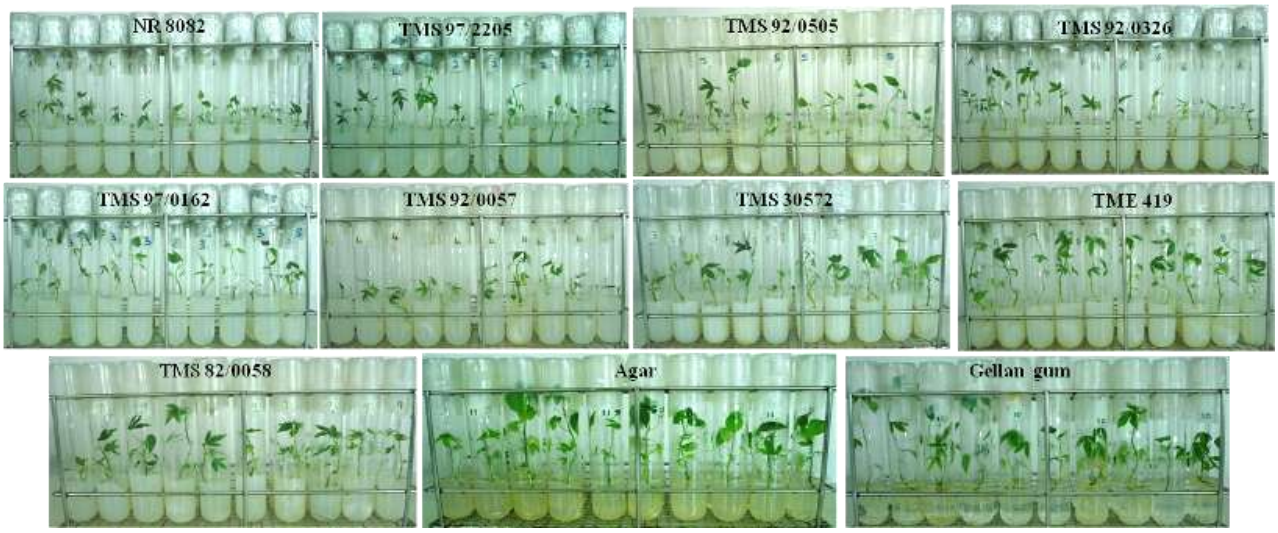


**Figure 1.** Picture of plantlets after 4 weeks in culture in the different gelling agents.

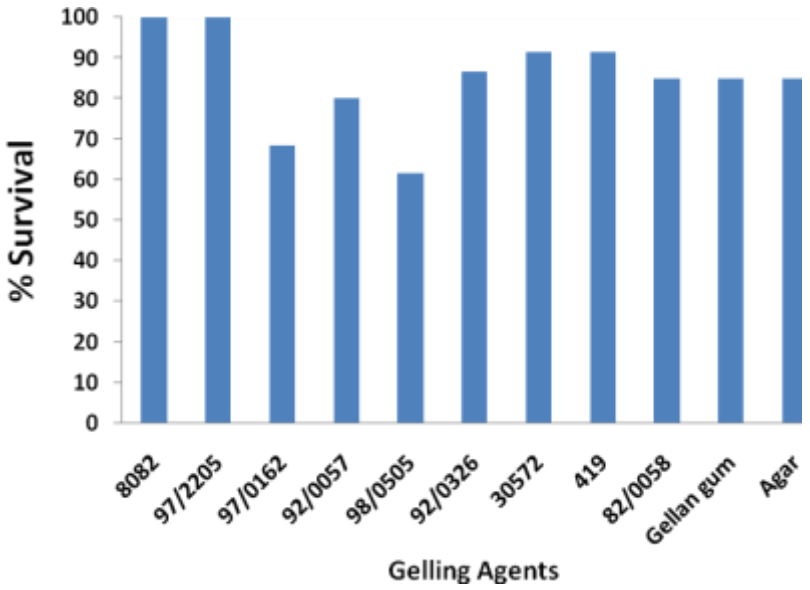


**Figure 2.** Survival of explants in differently gelled medium after 4 weeks in culture.

medium constituents to the plantlets. In addition, TME 419 cassava starch had a relatively higher water absorption capacity compared to other starches from the different cassava varieties (Table 1).

The growth and proliferation of explants in the differently gelled medium are as shown in Figure 1. The overall percentage survival irrespective of the explant type ranged from 61.5 to 100 (Figure 2). Worthy of note is zero mortality among the explants cultures in medium gelled in NR 8082 and TMS 97/2205 unlike the conventional gelling agent (Figure 2).

The mean number of nodes produced by the plantlets regenerated from the explants cultured in the differently gelled medium is shown in Table 2. After two weeks in culture, plantlets from shoot tips generally produced more nodes than those from nodal segments. At the fourth week in culture by which time the plantlets were ready for subculture, mean number of nodes from shoot tips had exceeded 5 in both conventional gelling agents although this did not differ significantly ($p>0.05$) from the mean number produced by plantlets gelled in starch from TME 419 only. For nodal segments more nodes were produced in plantlets from the later but did not differ significantly ($p>0.05$) from gellan gum, agar, TMS 82/0058, TMS 98/0505 and TMS 30572. Starch from TME 419 was most consistent in influencing regeneration

**Table 2.** Number of nodes after 2 and 4 weeks in culture.

| Gelling agent | 2 weeks in culture | | 4 weeks in culture | |
|---|---|---|---|---|
| | Shoot tip | Nodal segment | Shoot tip | Nodal segment |
| NR 8082 | 2.80 ± 0.42 | 2.33 ± 0.96 | 3.90 ± 1.2 | 3.40 ± 0.99 |
| TMS 97/2205 | 2.50 ± 0.53 | 2.07 ± 0.26 | 4.00 ± 0.94 | 2.73 ± 0.96 |
| TMS 97/0162 | 2.83 ± 0.41 | 2.46 ± 0.91 | 4.00 ± 0.71 | 3.46 ± 1.13 |
| TMS 92/0057 | 2.75 ± 0.46 | 2.14 ± 0.66 | 3.50 ± 0.76 | 3.17 ± 1.47 |
| TMS 98/0505 | 2.57 ± 0.79 | 2.25 ± 0.71 | 3.71 ± 1.50 | 4.25 ± 0.71 |
| TMS 92/0326 | 2.50 ± 0.71 | 2.31 ± 0.75 | 3.60 ± 1.43 | 3.00 ± 1.05 |
| TMS 30572 | 3.00 ± 0.71 | 2.47 ± 0.51 | 4.12 ± 1.58 | 4.21 ± 1.05 |
| TME 419 | 3.10 ± 0.74 | 2.93 ± 0.48 | 4.44 ± 1.88 | 4.79 ± 0.97 |
| TMS 82/0058 | 3.00 ± 0.47 | 2.75 ± 0.75 | 3.78 ± 1.30 | 4.33 ± 1.07 |
| Gellan gum | 3.33 ±0.50 | 2.93 ± 0.92 | 5.33 ± 0.87 | 4.67 ± 2.07 |
| Agar | 4.00 ± 0.58 | 2.73 ± 0.80 | 5.00 ± 1.63 | 4.42 ± 1.59 |
| $LSD_{(0.05)}$ | 0.52 | 0.51 | 1.12 | 0.82 |

of cassava plantlets, generating more nodes if subcultured. This result confirms an earlier evaluation reports on the better performance of TME 419 cassava starch as gelling agent in medium for ginger micropropagation (Nkere et al., 2009).

Several agar alternatives (wheat flour corn starch, laundry starch, potato powder, rice powder and semolina) have been shown to be good substitutes for the micropropagation of various plants (Prakash, 1993). Corn-starch (CS) along with low concentration of Gelrite (0.5 g 'Gelrite' + 50.0 g CS/l) has been used for the propagation of fruit trees, such as apple, pear and raspberry, banana, sugarcane, ginger and turmeric with better shoot proliferation than in agar (Zimmerman, 1995). She found that, corn starch was relatively less expensive ($1.8 $kg^{-1}$) compared with $200 $kg^{-1}$ of agar. "Isubgol" (a colloidal mucilaginous husk derived from the seeds of *Plantago ovate*), at 3% in MS medium has been used for the propagation of chrysanthemum (Babbar and Jain, 1998; Bhattacharya et al., 1994). The cost of 'Isubgol' is about $4 $kg^{-1}$. It has also been shown that addition of 8.0% tapioca starch to the MS medium severed as a good substitute for 'Bacto-agar' for potato shoot-culture (Getrudis and Wattimena, 1994).

The relatively low performance of explants (Shoot tip and nodal segment) in NR 8082 and TMS 97/2205 starch gelled medium as against the high survival rate of the explants is not unusual as it has been reported that some gelling agents contain inhibitory substances that hinder morphogenesis and reduce the growth rate of cultures (Powell and Uhrig, 1987). This once again brings to the fore that the adoption of a starch as a gelling agent would depend on proper screening and evaluation.

## Conclusion

The result from this study has shown that cassava starch from the genotype TME 419, could serve as a good gelling agent alternative to agar or gellan gum for *in vitro* multiplication of cassava. This is a welcomed development in cost reduction especially in resource poor laboratories where the price of conventional gelling agents is significant in micropropagation.

## CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# Various macro and micro-morphological features of two species of *Cakile*

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**The morphology and anatomy of *Cakile maritima* subsp. *aegyptiaca* and *Cakile arabica* are organized in this work to invent the different characters between the two studied species. The species were collected fresh from Mersa-Matruh in Egypt and Dammam city in Eastern region of Saudi Arabia. The main characteristic to distinguish between the different *Cakile* species is the morphology of the fruit, *C. maritima* was characterized by the occurrence of 2 opposite lateral horns in its fruit, and the absence of this in the other species. In this paper many characteristics other than fruit morphology are noticed to differentiate between them as habit of stem, type of leave, texture of sepal, seed shape, stem outline, types of cortex, pericycle tissue and number of vascular bundles. These characteristics can be used as tools for identification between the different species belonging to the same genera.**

**Key words:** Vegetative part, flowering part, anatomy, *Cakile maritima*, *Cakile arabica.*

## INTRODUCTION

The *Cakile* is one genus in the family Brassicaceae, its species are annual succulent halophyte plants, Clausing et al., (2000). Species of *Cakile* are widely distributed in sandy coasts throughout the world as sandy beach of North Atlantic Ocean, the Baltic, Mediterranean, North and White seas, the Caribbean and Gulf of Mexico and the Great lakes, and is established in Australia, Japan and on the Pecific Coast of North America, one species, *Cakile arabica* Vel. et Bornm is found in deserts of Middle as (Iraq, Kuwait and Saudi Arabia). The number of species of the genus *Cakile* is undefined. Pobedimova (1963) recorded 15 species on the basis of the morphology only. While Rodman (1974) verified seven species: (*Cakile arabica, Cakile arctica, Cakile constricta, Cakile edentula, Cakile geniculata, Cakile lanceolat, and Cakile maritima*) based on morphological and chemical analysis. Recently Warwick and Sauder (2005) recognized 6 species on the basis of morphological and molecular evidence. In Egypt the genus is represented by one species and one subspecies (*Cakile maritima* Scop. subsp. *aegyptiaca* (Willd.), according to Tackholm (1974) and Boulos (1999). In Saudi Arabia, one species (*Cakile arabica* Vel. et Bornm*)* is recorded according to Mandaville, (1990) and Chaudhary, (1999).

The *Cakile maritima* and its subspecies are common species of this genus and it is widely distributed throughout the world Barbour, (1972). It is a naturally salt-tolerant plant that shows potential for economical (oilseed), nutrient food and chemotherapeutic utilization (Ksouri et al. 2007).

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Studies on this genus are limited and most of them are on the adaptation of these plant to its environment as, Wright (1927), Ball (1964), Davy et al. (2006), Daniela et al. (2010) and Jianu et al. (2014). Morphological and anatomical studies of the studied species are very scarce Al-Taisan and Gabr, (2017). The main objective for this paper is to prove the presence of any differences between the two studied species.

## MATERIALS AND METHODS

Two species of *Cakile* were collected; *Cakile maritima* Scop. subsp. *aegyptiaca* (Willd.), from the coastal part of Mersa-Matruh in Egypt and *Cakile arabica* Vel. et Bornm, from Al- Rawda area - Dammam city in the Eastern region of Saudi Arabi. The species were collected by the author in March (2015). The species were identified according to the plant key of Tackholm (1974), Mandaville (1990), Boulos (1999) and Chaudhary (1999).

Foliar and floral details were examined with the aid of binocular stereo microscope under incident light and photographs. For anatomical investigation, each specimen was fixed according to Nassar and El-Sahhar, (1998) in F.A.A. (formalin - glacial acetic acid - 70% alcohol) with the ratio of 5: 5: 90 by volume. The stems and leaves (petiole and blade) were hand sectioned; the stems were taken from second internodes. The sections were stained according to Dilcher, (1974) in safranin (1% solution in 50% ethanol) and light green (1% solution in 96% ethanol) then photographed.

## RESULTS

### Morphology

#### *Cakile maritima* Scop. subsp. *aegyptiaca* (Willd.)

The *Cakile maritima* Scop. subsp. *aegyptiaca* is an annual, succulent herb that can grow up to 60 cm. long and glabrous. The stem is decumbent, terete, solid and branched. Internodes are 2-4 cm x 0.2 - 0.4 cm. The leaves are up to 6 cm. long while the lower leaves are oblong-ovate in outline and petiolate. The petiole is glabrous and up to 2.5 cm. long. Blade is 2- 3.5 cm x 1 - 1.5 cm, simple with entire to sinuate-dentate margin and acute apex. The upper leaves are simple and petiolate. The petiole is up to 1.5 cm. long. Blade is 1.5- 2.5 cm. × 0.5 - 1 cm, oblong ovate, dentate with acute apex (Table 1, Plate 1 and 2).

Inflorescence types are raceme. Flower length is 3 - 7 mm. long and pedicellate. Pedicels are glabrous, 2- 4 mm. long and thick. Sepals are glabrous, green- yellow, 2.5 - 4 mm × 1- 1.5 mm and ovate oblong in outline with narrow membranous margin. Petals color is violet-lilac, 4 -6.5 mm × 1- 2.5 mm clawed with obovate limb and obtuse apex. Stamens length is 3-5 mm. long, with glabrous filament and ovate-triangle anthers. Ovary is smooth with inconspicuous style and flattened stigma (Plate 1).

Siliqua is 1.5 - 2 cm × 0.4 - 0.6 cm, ribbed, glabrous, horned and indehiscent with 2- segmented. The upper segment is longer than the lower with pyramidal shape and one seeded. The lower segment is short, cylindrical with two prominent lateral projections basally and one seeded. Beak length is 2-3 mm long and seedless. Seeds are D-shaped, 2.5-3.5 mm x 1 long, 2 mm wide, brown with sub-terminal hillum and has smooth surface.

#### *Cakile arabica* Vel. et Bornm

The *Cakile arabica* species is an annual, glabrous and succulent herb arising from tap root. The stem is erect, terete, solid and ascending in branch. Internodes are 2 – 4.5 cm × 0.1 - 0.3 cm. Leaves are alternate and pinnate. Lower leaves are oblong-ovate in outline and petiolate. Petiole is glabrous and up to 7.5 cm. long. Blade is 9.5 to 15 cm × 4.5 to 10 cm and pinnately divided into 4 to 7 narrowly linear lobes. The upper leaves are pinnate and petiolate. The petiole is up to 1.7 cm. long, the Blade 4 to 6 cm × 3 to 5.5 cm and ovate with 1-3 lateral lobes.

Inflorescence types are raceme. Flower length is 3 to 7 mm. long and pedicellate. Pedicels are glabrous and thick with 1.5 to 3.5 mm long. Sepals are hairy, green violet, 4 to 5.5 mm × 1 to 1.5 mm and ovate oblong in outline with narrow membranous margin. Petals are violet, 5 to 6.5 mm × 1.5 to 2 mm clawed, limb obovate with obtuse apex. Stamens length is 4.5 to 5.5 mm long with glabrous filament and long ovate anthers. Ovary is smooth with inconspicuous style and flattened stigma.

Siliqua is 1.6 to 2 cm × 0.2 to 0.3 cm, ribbed, glabrous and indehiscent with 2- segment. The upper segment is longer than the lower with pyramidal shaped and is one seeded. The lower segment is short, cylindrical and one seeded. Beak is long and seedless. Seeds are oblong, 3 to 3.5 mm × 0.5 to 1 mm brown with sub-terminal hillum and smooth surfaced (Plate 2).

### Anatomy

#### *Cakile maritima* Scop. subsp. *aegyptiaca* (Willd.)

##### *Stem anatomy*

The outline in cross section is pentagonal. Epidermal cells are radially elongated cells covered with thick andwarty cutin. Cortex is wide and consists of 5 - 6 layers of scalerenchyma followed by 1 to 2 layers of polygonal parenchyma. Starch sheath is will defined. Pericycle consists of patches of fibers alternate with parenchymatous cells. Vascular cylinder is composed of 9 to 10 bundles, each with will defined patches of phloem and wide xylem vessels (Plate 3). The medullary rays are wide; 6 to 9 series of thin walled parenchyma cells. Pith is wide, solid and homogenous, consists of round thin cell wall parenchymatous cells. Schizogenous canals are recorded in cortex and pith (Table 2, Plate 3 and 4).

**Table 1.** The main different morphological characters among the two studied species.

| Character \ Species | *C. maritime* subsp. *aegyptiaca* | *C. arabica* |
|---|---|---|
| Duration | Annual Herb | Annual Herb |
| Nature | Succulent | Succulent |
| Stem habit | Decumbent | Erect |
| Internode length (cm.) | 2-4 | 2- 4.5 |
| Internode width (cm.) | 0.2-0.4 | 0.1-0.3 |
| Type of Leaf | Simple | Pinnate |
| Petiole length (cm.) | 1.5-2.5 | 1.7-7.5 |
| Blade length (cm.) | 1.5-3.5 | 4-15 |
| Blade width (cm.) | 0.5-1.5 | 3-10 |
| Color of sepal | Green- yellow | Green violet |
| Texture of Sepal | Glabrous | Hairy |
| Color of petal | Violet-lilac | Violet |
| Shape of stamen | Ovate-triangle | Long ovate |
| Fruit | Horned | Not horned |
| Shape of seed | D-shaped | Oblong |


**Plate 1.** Different morphological features of *Cakile maritima* subsp. *aegyptiaca* 1-lower leaves; 2- Upper leaves;3- Flower;4- Sepal; 5- Petal;6- Stamen;7- Fruit;8- Seed.

## Leaf anatomy

### *Petiole*

The outline in cross section is crescent with two prominent ridges. Epidermis is composed of radially elongated cell mixed with bulliform cells and covered with thick and warty cutin. Ground tissue is consisted of 4- 6 layers of chlorenchyma tissue found abaxially and in ridges followed by round to irregular thin cell wall


**Plate 2.** Different morphological feature of *Cakile arabica*. 1-lower leaves; 2- Upper leaves;3- Flower;4- Sepal; 5- Petal;6- Stamen;7- Fruit;8- Seed.

**Table 2.** The main different anatomical characters among the two studied species**.**

| Characters \ Species | | *C. maritime* subsp. *aegyptiaca* | *C. arabica* |
|---|---|---|---|
| Stem | outline | Pentagonal | Terete |
| | Epidermal cell | Radial | Tangential |
| | Cortex layer | 6-8 | 4-6 |
| | Cortex tissue | sclerenchyma | chlorenchyma |
| | Pericycle | Fibers + Parenchyma | Parenchyma |
| | No. of vascular bundles | 9-10 | 15-17 |
| Petiole | Bulliform cell | Present | Absent |
| | No. of vascular bundles | 11 | 7 |
| Blade | Epidermal cell | Radial | Tangential |
| | Bulliform cell | Present | Absent |
| | Mesophyll | Discontinuous | Continuous |
| | Type of mesophyll | Isobilateral | Centric in rachis – Isobilateral in lobe |
| | Main vascular bundles | one | 11 in rachis- one in lobe |

parenchyma cells. Vascular system is 11 bundles arranged in crescent form, one main and 10 (5, 5) small, unequal size in each side. Each bundle has well-defined patches of phloem, wide xylem vessels and surrounded by bundle sheath of wide parenchyma. The vascular bundles are associated with fibers. Schizogenous canals are present.

**Blade**

The outline in cross section is in duplicate. Epidermal

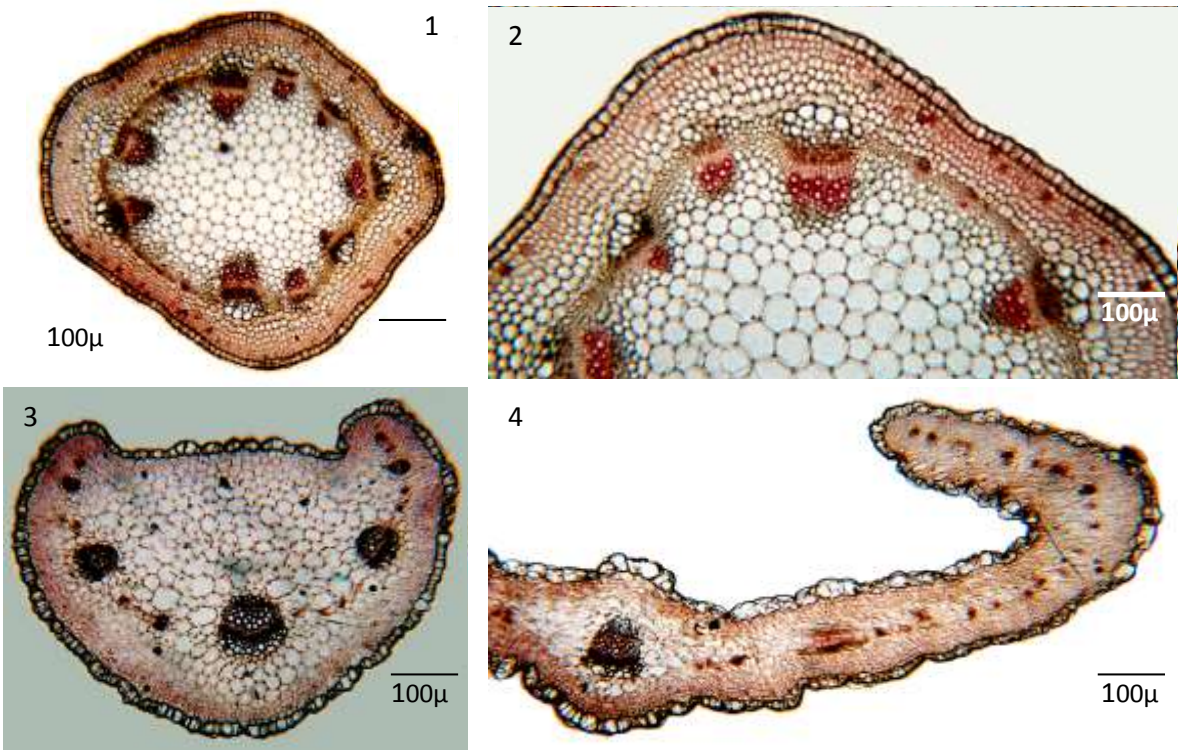


**Plate 3.** Different anatomical features of *Cakile maritima* subsp. *Aegyptiaca*.1-Stem outline; 2- Stem sector; 3- Petiole outline; 4- Blade.

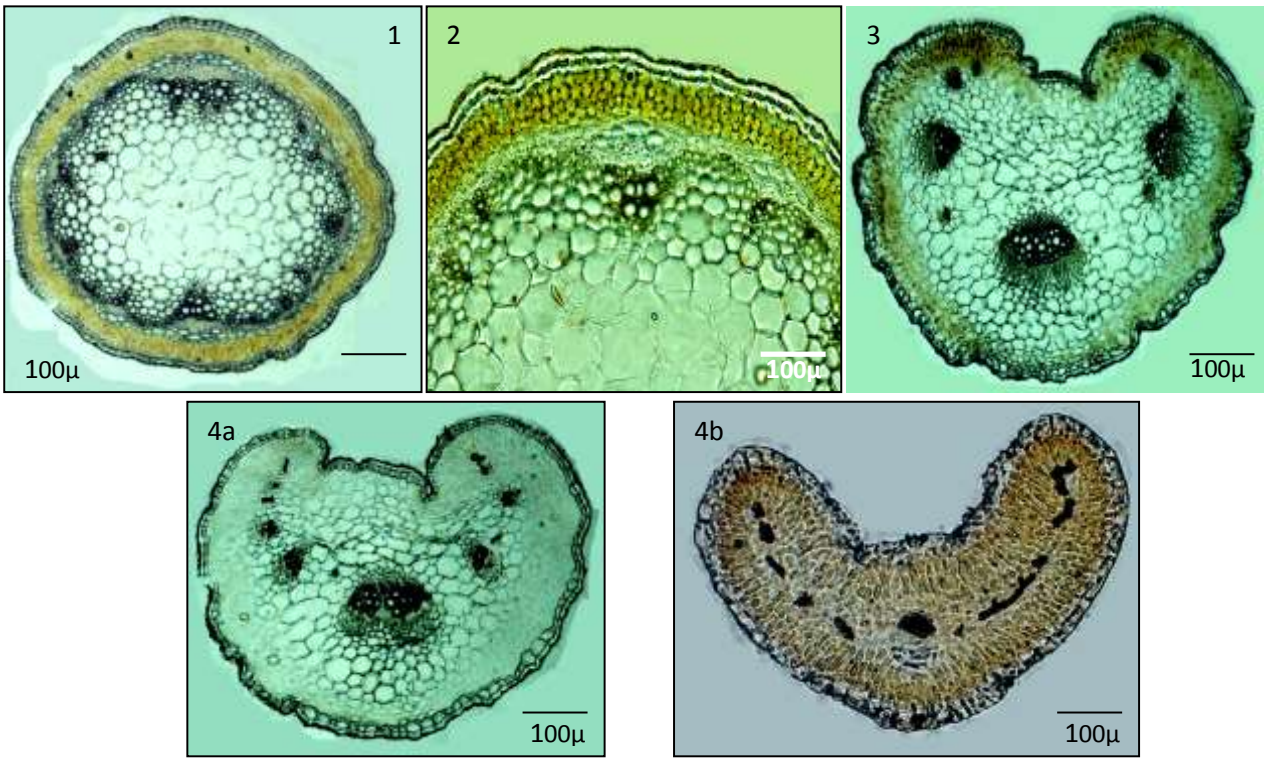


**Plate 4.** Different anatomical feature of *Cakile arabica.*1-Stem outline; 2- Stem sector; 3- Petiole outline; 4a- Rachis of blade; 4b Lobe of blade.

cells are radial mixed with bulliform cells covered with thick and warty cutin. The epidermis is interrupted by anisocytic semi depressed stomata. Mesophyll is isobilateral, composed of 4 to 5 layers of short cubic cells of palisade tissue discontinuous adaxially at midrib region followed by one layer of thin cell wall parenchyma cells.

Vascular system is composed of one large main bundle at midrib region and many small bundles in each side at wing region. Each bundle surrounded by bundle sheath of wide parenchyma and associated with fibers.

### *Cakile arabica* Vel. et Bornm

#### *Stem anatomy*

The outline in cross section is terete. Epidermal cells are tangentially elongated cells shielded by thick and warty cutin. Cortex consists of 3 to 4 layers of chlorenchyma cells followed by 1 to 2 layers of parenchyma. Pericycle consists of parenchymatous cells. Vascular cylinder is eustele, composed of 15 to 17 bundles; each with will defined patches of phloem and will defined xylem vessels. The medullary rays are wide. Pith is wide and homogenous and consists of thin walled round to polygonal parenchymatous cells. Schizogenous canals are recorded in cortex and pith (Plate 4).

#### Leaf anatomy

#### *Petiole*

The outline in cross section is ± crescent with two prominent ridges. Epidermis is composed of radially elongated cells covered with thick and warty cutin. Ground tissue is consisted of 3 to 4 layers of chlorenchyma tissue found abaxially and in ridges followed by round to irregular thin cell wall parenchyma cells. Vascular system consists of 7 bundle, one main and 6 (3, 3) subsidiary in each side. Each bundle with well-defined patches of phloem, wide xylem vessels and surrounded by bundle sheath of wide parenchyma cells. The vascular bundles are associated with fibers (sclerenchyma), the number of row of sclerenchyma ranges from 4 to 5 row. Schizogenous canals are present.

#### Blade- c.1- Rachis

The outline in cross section is ± crescent with two prominent ridges. Epidermis is composed of radially elongated cells mixed with some tangential and covered with thick and warty cutin. Mesophyll is centric, composed of palisade in the form of outer 3-4 layers of loose cells, followed by parenchyma tissue which is composed of 4 - 6 layers of large thin-walled round to polygonal. The vascular system is in the form of 11 collateral bundles, two (united) main vascular bundles and 9 (5,4) subsidiary schizogenous canal are recorded.

#### 2- Lobe

The outline in cross section is in duplicate. Epidermal cells are tangential mixed with some radial cells and covered with thick and warty cutin. The epidermis is interrupted by anisocytic, semi depressed stomata. Mesophyll is isobilateral, composed of 3 to 4 layers of long palisade tissue continuous adaxially at midrib region followed by one layer of thin cell wall parenchyma cells. Vascular system is composed of one large main bundle at midrib region and 4-5 small bundles in each side.

**The key:** The studied characters were used in the construction of an indented key to the assorted species.

(i) Decumbent stem, simple leaves, d-shaped seed, pentagonal stem outline, scalerenchyma tissue present in the cortex and the type of the mesophyll is isobilateral..... *Cakile maritima* subsp. *aegyptiaca*.

(ii) Erect stem, pinnate leaves, oblong shaped seed, teret stem outline, scalerenchyma tissue absent in the cortex and the type of the mesophyll is centric..... *Cakile arabica*

## DISCUSSION

The *Cakile* fruit is a characteristically shaped, fleshy, usually single-seeded, indehiscent, heteroarthrocarpic silique and consists of a proximal capsule that stays attached to the parent, and a deciduous beaked distal capsule that separates easily at the joint when fully ripe (Hall et al. 2006). It has a thick, corky inner tissue that allows it to float on water, allowing it to disperse to great distances, Maun and Payne (1989) and Donohue (1997, 1998a, b).

The main characteristic to distinguish between the different *Cakile* species is the morphology of the fruit, *Cakile maritima* characterized by occurrence of 2 opposite lateral horns in its fruit, and the other species do not have these horns.

This study recorded different morphological and anatomical features between *Cakile maritima* subsp. *aegyptiaca* and *Cakile arabica* beside the different in fruit morphology.

The stem is decumbent and more succulent in *Cakile maritima* subsp. *aegyptiaca*, and erect in *Cakile arabica*. Leaves are simple and small in *Cakile maritima* subsp. *aegyptiaca*, while pinnate and longer in *Cakile arabica*.

Sepals are glabrous and green yellow in *Cakile maritima* subsp. *aegyptiaca*, and hairy and green violet in *Cakile arabica*. Seeds are d-shaped in *Cakile maritima* subsp. *aegyptiaca*, and oblong in *Cakile arabica*. The stem outline pentagonal in *Cakile maritima* subsp. *aegyptiaca*, and terete in *Cakile Arabica*, cortex wide and contain scalerenchyma tissue in *Cakile maritima* subsp. *aegyptiaca*, and consists of chlorenchyma tissue in *Cakile arabica*, the pericycle in *Cakile maritima* subsp. *aegyptiaca* and consists of patches of fibers alternate with parenchyma while consists of parenchyma only in *Cakile arabica*. The stem vascular bundles are little in *Cakile maritima* subsp. *aegyptiaca*, rather than in *Cakile arabica*. Petiole vascular bundles are 11 in *Cakile maritima* subsp. *aegyptiaca*, and 7 in *Cakile arabica*. Bulliform cells are present in the leaves of *Cakile maritima* subsp. *aegyptiaca*, and absent in *Cakile Arabica*. Mesophyll discontinuous in *Cakile maritima* subsp. *aegyptiaca*, while continuous in *Cakile Arabica*, Isobilateral *Cakile maritima* subsp. *aegyptiaca*, while centric and isobilateral in *Cakile Arabica*. The number of vascular bundle in midrib region is one in *Cakile maritima* subsp. *aegyptiaca*, and11 in the rachis of *Cakile arabica*

## Conclusion

The two species, *Cakile maritima* subsp. *aegyptiaca*, and *Cakile arabica* have different morphological characters such as habit of stem, type of leave, texture of flower (sepal) and seed shape. They also have some different anatomy characters such as, stem outline, tissue of cortex and pericycle, types of mesophyll and number of vascular bundles. The present study recommends that future studies should use these characteristics as a tool for identification of the different species belonging to the same genera.

## CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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*Full Length Research Paper*

# 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:

$$Yik = \mu + Pi + Gk + Eik$$

Where, $\mu$ is mean; *Pi* is *ith* replication; *Gk* is *kth* genotype and *Eik* 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

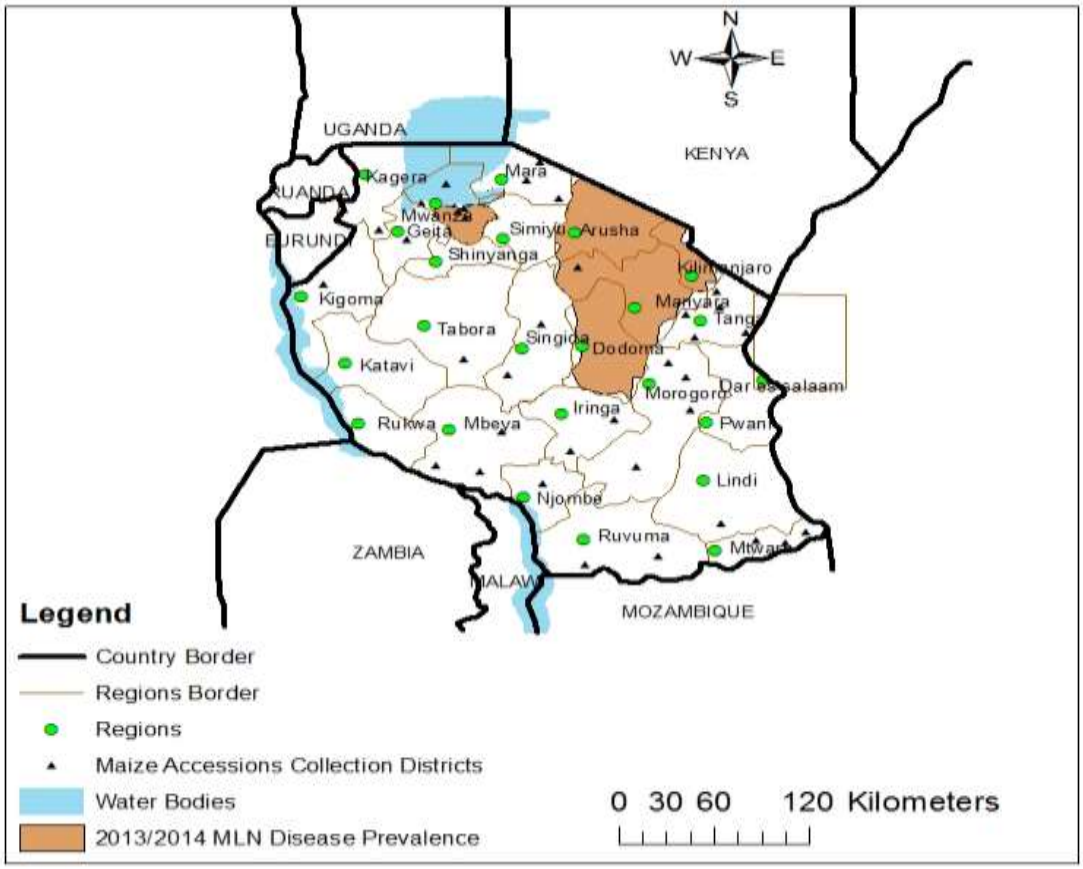


**Figure 1.** Map showing MLN disease prevalence in Tanzania (2013/2014) and districts where maize landraces in this study were collected.

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.

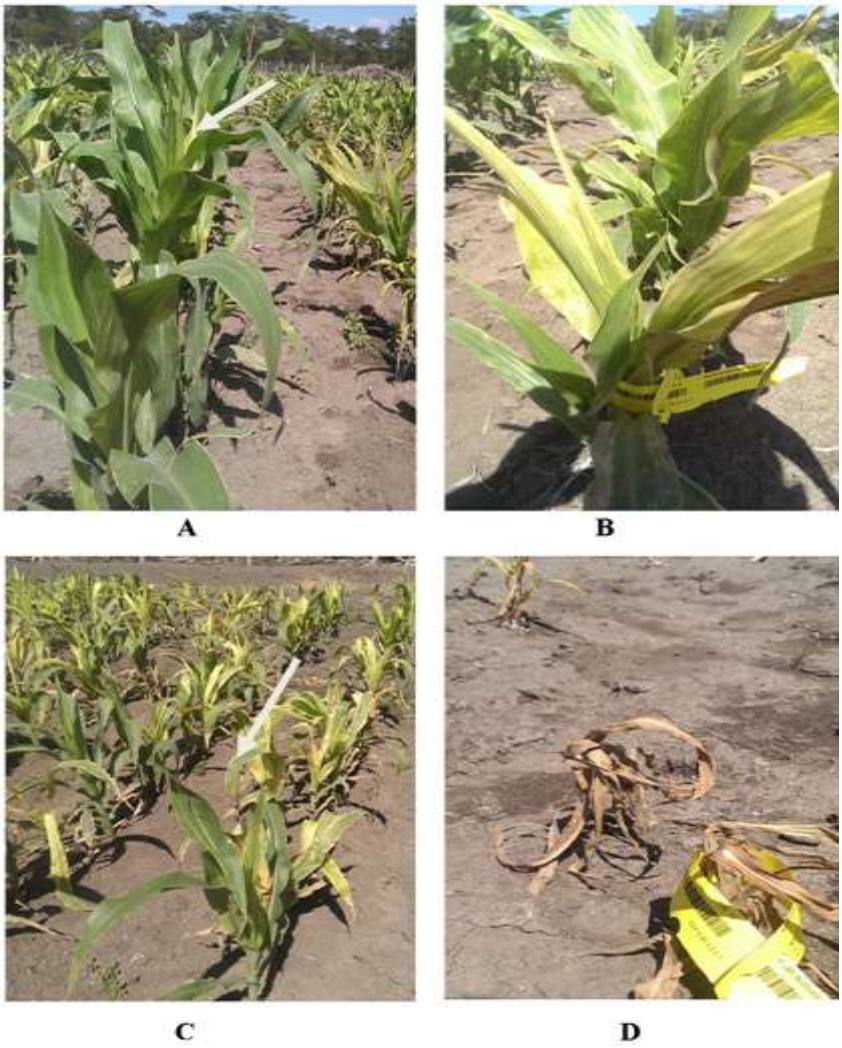


**Figure 2.** Maize lethal necrosis disease symptoms on Tanzanian maize landraces at Naivasha MLN screening facility. (A) Mild leaf chlorosis; (B) higher density of chlorotic spots; (C) necrotic tissues developed from leaf margins to the mid-ribs; (D) complete plant death.

### *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

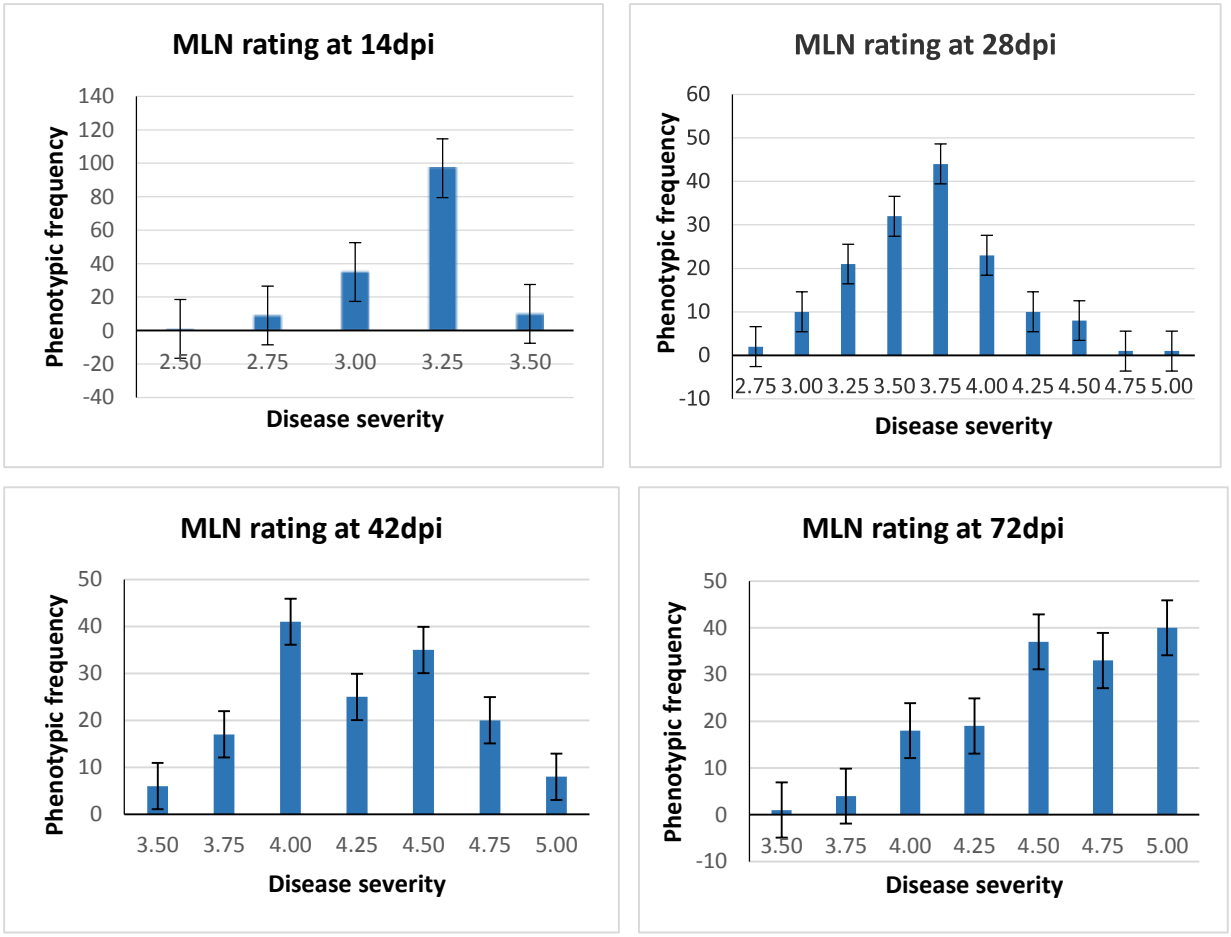


**Figure 3.** MLN disease responses and score distribution for Tanzanian maize landraces evaluated for MLN disease resistance at Naivasha maize lethal necrosis screening facility (14, 28, 42 and 72 dpi).

**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

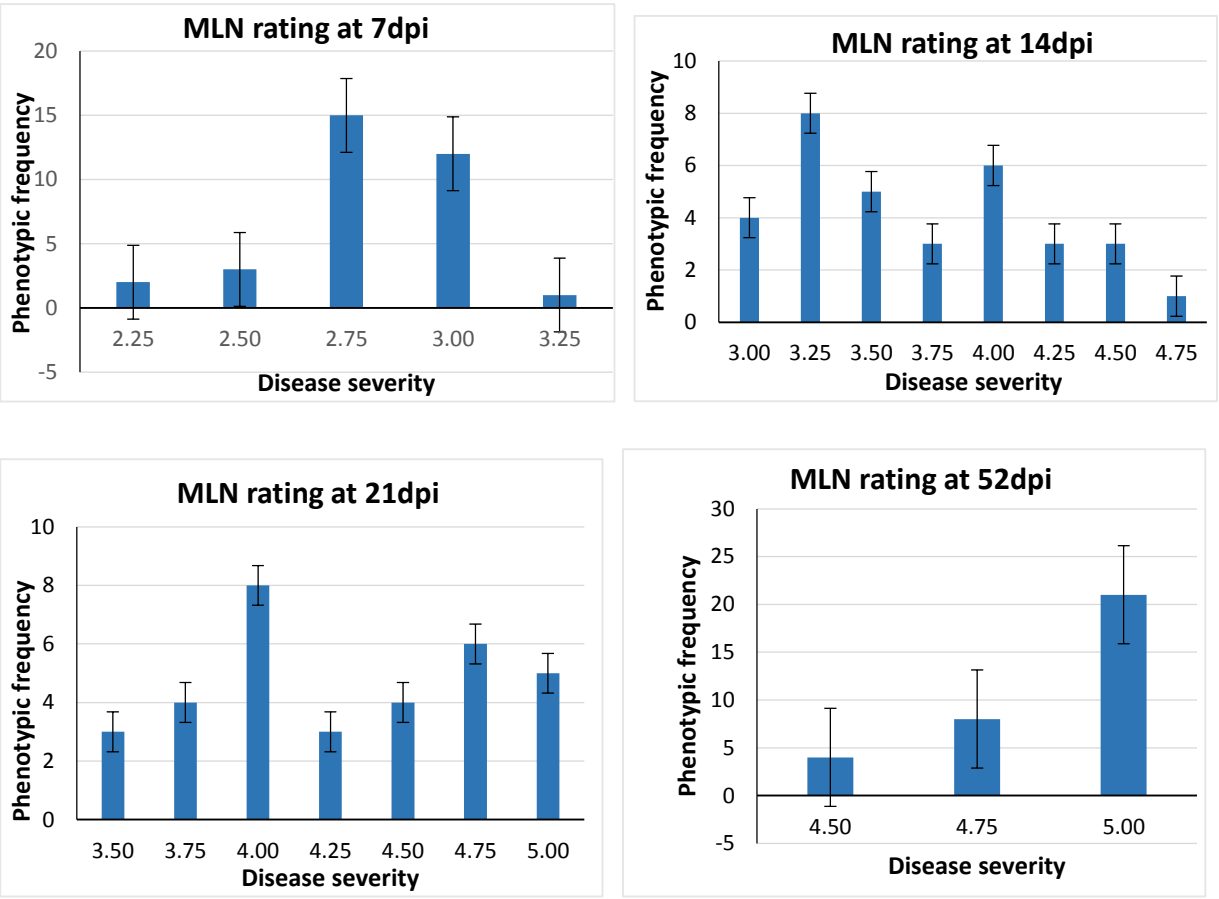


**Figure 4.** MLN disease responses and score distribution for Tanzanian maize inbreed lines evaluated for MLN resistance at Naivasha maize lethal necrosis screening facility (7, 14, 21 and 52 dpi).

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.

## ACKNOWLEDGEMENTS

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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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