Status and Relative Distribution of Maize Streak Virus in Western Ethiopia

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Abstract

Maize production in Ethiopia is constrained by both biotic and abiotic factors, and diseases of maize caused by viruses are serious threats to maize production. Maize streak (MS) is one of the most severe and widespread diseases that adversely reduces maize production. Hence, this study was conducted to assess the status and distribution of maize streak and to diagnose Maize streak Virus isolates. Surveys were conducted during the 2019 main cropping season in different agro ecological zones of Gambela, Benishangul-Gumez and Oromia regions. Maize and grasses in maize fields with suggestive MS-like symptoms were sampled for laboratory testing. Out of 127 maize fields visited, based on MS-like symptoms, the disease was prevalent in 100 (79%) fields assessed, from which 100 maize and 5 grass samples were collected for laboratory testing. High prevalence, incidence and severity of MS were recorded at low altitude areas; whereas low incidence and severity were recorded at high altitude maize growing areas. The mean incidences of 64%, 59% and 33%; and severities of 4.3, 4 and 3 (on 1-5 scoring scale) were, respectively, recorded in Gambela, Benshngul-gumeze and Oromia regions. Results of laboratory testing employing PCR showed that out of 100 symptomatic maize and 5 grass samples analyzed, MSV was recovered from 95 (90%) maize and none of the grass samples. Based on the results of the present study, MS is an economic disease of maize at low and mid altitude growing areas. Hence, disease management options involving development and deployment of resistant vanities along with cultural practices should be sought.

Keywords: Intensity, Maize, Maize Streak Virus, Mastervirus, Polymerase Chain Reaction

Introduction

Maize is one of the three leading global cereals that feed the world (Muiru *et al.*, 2010; Shiferaw *et al.*, 2011). Maize, together with rice and wheat, dominate human diets (Ignaciuk, 2014) and provide at least 30% of the food calories of more than 4.5 billion population. The crop provides high yields per unit of land, making it a key crop in ensuring food availability and security for the consumers (Mboya *et al.*, 2011). Maize is a major staple food in Ethiopia and ranks first in production and second in area coverage next to tef (*Eragrostis tef*) (CSA, 2020). A total of 10.5 million tons have been produced across 2.5 million hectares of land in the 2020 cropping season (CSA, 2020). Despite its importance, however, the national average yield (3.4 tons/ha) of maize in Ethiopia is below the world's average (8 tons/ha) (FAOSTAT, 2017).

Maize production in Ethiopia is constrained by both biotic and abiotic factors, and diseases of maize caused by viruses are serious threats to maize production. More than 50 virus species can infect maize (Redinbaugh and Zambrano, 2014) and among these, maize streak virus (MSV), genus Mastrevirus in the family *Geminiviridae* is one of the top ten economically important plant viruses (Rybicki, 2015). Virus species in the genus Mastrevirus have monopartite genomes of approximately 2700 nt encapsidated in geminate particles (King *et al*., 2011). Maize streak (MS) is one of the most severe and widespread diseases that adversely reduces maize production thereby posing a threat to food security (Dhau *et al*., 2018). The most extensively sampled and studied mastrevirus species is *maize streak virus*: the causal agent of maize streak disease (MSD), and the most significant viral disease of maize (Zea mays) in Africa (Oppong *et al*., 2015; Pande *et al*., 2017). Maize streak causes yield losses that range from trace to almost 100% if infection occurs in the first three weeks of planting maize (Alegbejo *et al*., 2002, Danson *et al*., 2006).

The disease is distributed throughout the African continent and surrounding islands (Monjane *et al*., 2011). Globally, MSD is regarded as the third most serious disease of maize after turcicum leaf blight (TLB) and gray leaf spot (GLS) (Pratt & Gordon, 2006). In Africa, however, MSD is a serious problem than both TLB and GLS (Martin & Shepherd, 2009). Epidemics resulting in economic losses of up to 100% have been reported in at least 20 African countries including Ethiopia, Nigeria, Ghana, Sudan, Cameroon, Zimbabwe, Tanzania, Togo, Benin, Burkina Faso, Sao Tome and Uganda (Lagat *et al*., 2008; Owor, 2008). MSD has been identified as a major constraint to maize production in Ethiopia (Alemu *et al*., 1997; Guadie *et al*., 2018).

Except for a few scattered information generated in localized areas (Alemu *et al*., 1997; Guadie *et al*., 2018). Major maize growing agro ecologies was not properly addressed and sufficient information generated regarding the distribution and status of MSD in Ethiopia. This paper therefore reports results of a comprehensive assessments made on the status and distribution of MSD in western regions, which represent the major maize growing areas in Ethiopia.

Materials and Methods

Assessment and Geographical Distribution of Maize Streak Disease

During the 2019 main cropping season, an intensive and extensive surveys for Maize streak (MS) were carried out in major maize growing areas of Oromia (east Welega, west Welega, Jimma, Buno Bedele zones), Benishangule-Gumuz (Asosa zone) and Gambella (Agnuwak and Nuer zones) regions. In these regions maize, in most cases, is cultivated in monoculture as the major crop by small holder farmers (CSA 2020). The surveys were made from July to October 2019 main cropping season.

Most of the surveyed maize fields were at a vegetative growth stage ranging in size from knee height to early tasseling. The surveys were made along road-sides with approximately five to ten kilometer intervals wherever the crop was available. Sampling was done using simple random sampling technique, and assessment was made diagonally along field at five spots. At each spot representative symptomatic sample with characteristic MS symptoms were collected, bulked and desiccated in calcium chloride or silica gel for later laboratory testing. Suspected symptomatic alternative grass hosts of harboring MSV in each field were also collected and preserved as described above for laboratory analysis.

Data related to crop variables such as growth stage, variety, date of sowing, previous crops, crop performance, insect vectors and location, etc were recorded. Altitude and geographical position of survey locations were recorded using geographical positioning system (GPS) at each field. Information on agro-ecological zones was obtained from the Ministry of Agriculture (MOA, 2005).

Table 1. Major agro-ecological zones of surveyed areas for maize streak in Ethiopia

Agro-ecological zone	**Altitude (m.a.s.l)**	**Regions***
Moist lowlands	<1000	Gambela
Moist lower &dry mid-altitudes	1000-1700	W. Oromia & BSG
Moist and semi-moist mid-altitudes	1701-2000	W. Oromia & BSG
Moist upper mid-altitudes	2001-2400	W. Oromia

*W, west; BSG, Benishangule-Gumuz
Adapted from (MOA, 2005)

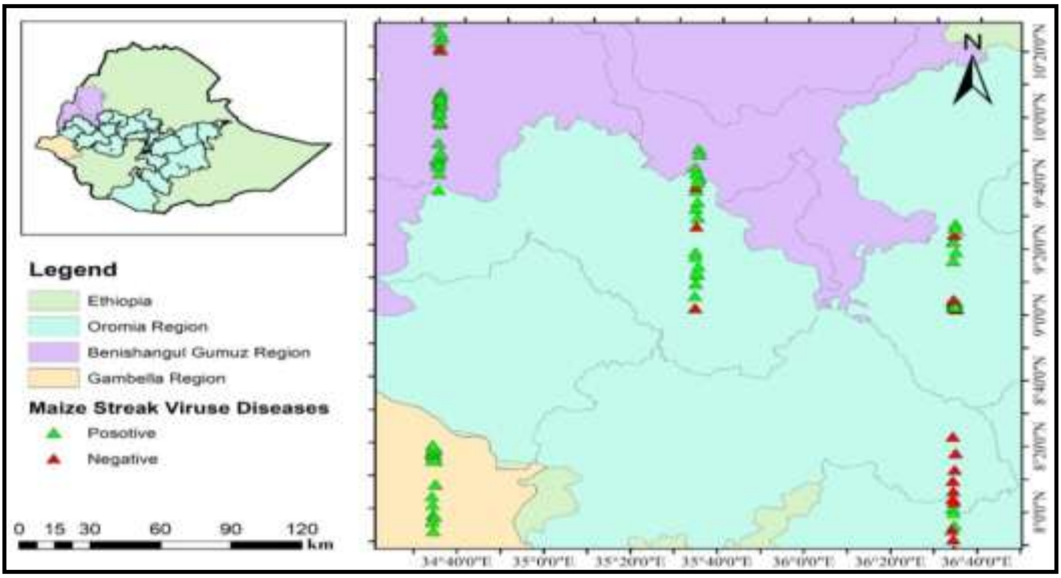

Figure 1.Spatial distribution of MSV in surveyed regions of Ethiopia, 2019 main cropping season

Assessment of MS Prevalence, Incidence and Severity in the Field

Disease prevalence was assessed by determining the number of fields where a particular disease was recorded in relation to the number of fields sampled from surveyed region.

The incidence of MS was determined by visually observing and recording the number of maize plants showing the disease symptoms and the percentage incidence was calculated as follows:

$$\text{Incidence}(\%) = \frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}} \text{x}100$$

Disease severity was assessed as the area of plant tissue affected by disease, expressed as a percentage of the total area at regular intervals using a 1–5 scale (Table 2) (Blankson *et al*., 2018).

Table 2. Visual rating scale for maize streak disease severity

Rating scale	Description	Expression in terms of Severity
1.	No symptoms	No infection
2.	Very few streaks on leaves, light streaking on old leaves gradually decreasing on young leaves	Mild infection
2.	Moderate streaking on old and young leaves Slight stunting	Moderate infection
4.	Severe streaking on about 60-75% of leaf area, Plants stunted	severe infection
5.	Severe streaking on more than 75% leaf area, severely stunted or dead	Very severe infection plants

Molecular Characterization of Maize Streak Virus

DNA extraction and Polymerase Chain Reaction

Young leaf samples collected from maize plants showing MS symptoms from different maize growing regions of Ethiopia were used for DNA extraction. Total DNA was extracted using modified CTAB extraction method as described by Doyle and Doyle (1991). The extracted genomic DNA was amplified by Polymerase Chain Reaction (PCR) using degenerate Primer pair of forward 5’-CCA AA (GT) (AGT) TC AGC TCC TCC G-3' and Primer reverse 5'-TTG G(CGA)C CG(AC) (ACG)GA TGT A(CG)AG-3'. The primers were expected to amplify a 1300 base pair (bp) fragment. PCR amplification of MSV was performed in 25 µl reaction volume containing 11µl Buffer, 10 µl SDH_2O, 1µl each primer, 1µl Taq polymerase and 1µl genomic DNA. PCR was run on standard thermal cycler (PCR sprit thermal cycler) at Ambo Agricultural Research Centre (AmARC), virology laboratory with reaction condition of 94 °C for 1min, followed by 30 cycles of 93 °C for 45sec, 54 °C for 30sec and 72 °C for 90sec, and a final extension step of 72°C for 3 min.

Electrophoresis and UV visualization

PCR products were assessed by electrophoresis in 0.8% agarose gel in TAE (Tris-Acetate-EDETA) buffer, stained with ethidium bromide, and viewed under ultraviolet light trans-illuminator. For reference and comparison of PCR products, 100 bp DNA size markers were used along with the samples.

Data analysis

Descriptive statistics was performed for analysis of data related to prevalence, incidence and severity.

Results and Discussion

Prevalence, Incidence and Severity of MS across Locations and Varieties

A total of 127 maize fields were visited, and 105 maize and 5 grass samples with MS-like symptoms were collected. Most frequently encountered MS-like symptoms supposed to have associated with MSV infection are: Broken to almost chlorotic strips centered on the tertiary leaf veins and uniformly distributed across the leaf surface, pale yellow streaks, mosaic and chlorosis on leaf lamellae, broken or longitudinal chlorotic streaks along leaf veins, wilting and drying of leaf margins, necrosis and mottling on the entire plant (Fig 2). The characteristic symptoms described in this study have similarly been reported in detail by various researchers (Shepherd *et al*., 2010; Mesfin *et al*., 1995). It is interesting to note that visual assessment of MS based on field symptoms, in most cases, corresponded with laboratory test result substantiating that characteristic symptoms are in most cases diagnostic and useful for field assessment.

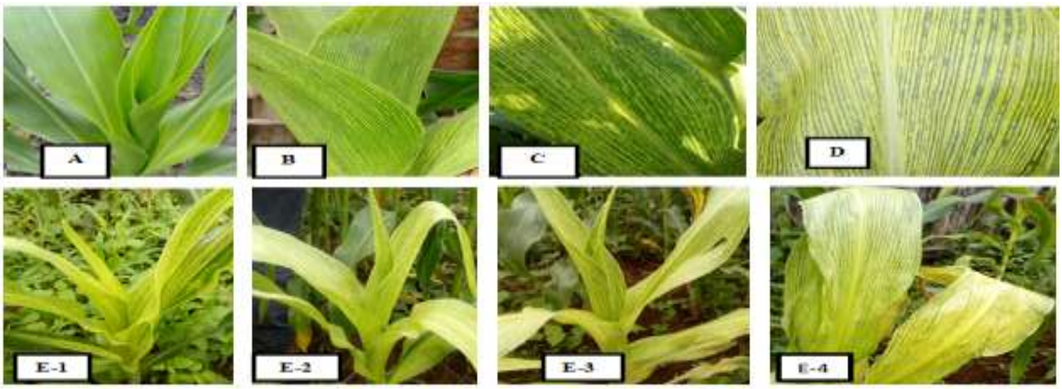

Figure 2. Shows different MS symptoms associated with plant growth stage at the time of infection: (A) no symptoms (no infection), (B) light streaking on old leaves (mild infection); (C) moderate streaking on old and young leaves, slight stunting (severe infection); (D) severe streaking on about 60-75% of leaf area, plants stunted (severe infection); (E1-E4) Severe streaking on more than 75% leaf area, plants severely stunted or dead (very severe infection).

Out of 127 maize fields visited, based on MS-like symptoms, the disease was prevalent in 100 (79%) fields assessed. Results of this investigation depicted that the disease prevalence, incidence and severity were highest in Gambella region followed by Benishangule-Gumuz and Oromiya regions, in this order (Table 3). This finding is in agreement with what reported by Mesfin *et al*. (1991) who indicated the exceptionally high MS outbreak in Gambella region during the 1986 crop season. In this work, other regions in the west other than Gambella are also succumbed to the disease. Mesfin *et al*. (1991) reported that MSV was prevalent at lower altitudes, this being attributed to higher population density of *Cicadulina spp* especially *C. mbila* found at lower altitude. This indicated that the altitude range was one of the deriving factors affecting MS distribution and prevalence by being favorable for the insect vectors build up and activity. Earlier, similar findings in Ethiopia reported the distribution and importance of maize streak disease in some parts of the country (Guadie *et al*., 2018; Alemu *et al*., 1997).

The mean prevalence of MS was, respectively, 94 and 93% in Gambela and Benishangul Gumez regions, while the mean disease prevalence was 65% in Oromia region (Table 3). As indicated in Table 3, the mean prevalence was assessed in Agnuwak zone from three districts (Abobo, Abol and Gambela) and the disease had 100% prevalence in Abobo and Gambela while 75% in Abol distract and the average mean prevalence was 92% in Agnuwak zone. The prevalence of MS in Nuer zone was assessed from two districts (Itang and Lare) and had 100% prevalence in both districts and the average mean prevalence of MS in Nuer zone was 100%. Of four zones, namely Jimma, Buno Bedele, east and west Wolega Zones assessed in Oromiya, the mean prevalence of the MS was respectively 64, 0, 62 and 85%. Except Buno Bedele where the disease was not detected, MS was prevalent in three Oromia zones surveyed, the highest prevalence being recorded in west Wellega. The prevalence of MS was also assessed from one zone (Asossa) of Benishangul-Gumez region where the mean prevalence recorded was 93%.

Likewise, MS incidence was also higher in Gambella, followed by Benishangule-Gumuz and Oromia, where the mean incidences of up to 64% in Gambela, 59% in Benishangul Gumez and 33% in Oromia regions were recorded (Table 3). Of the surveyed areas in Gambella region, higher incidences of MS were recorded in Agnuwak zone, Gambela district and Itang and Lare districts of Nuer zone. In Oromiya, low incidence of MS was recorded in Buno Bedele and Jimma zones and relatively higher incidence recorded in east and west Wolega zones (Table 3). Recent report showed that MS incidence of up to 58% was also found in Ethiopian maize germplasm (Guadie *et al*., 2018).

The severity of MS was significantly higher in Gambela and Benishangul-Gumz compared to Oromia region. The severity score of MS was higher in Agnuwak, Nuer and Asosa zones and relatively low severity was recorded in Jimma, east and west Wolega zones where as no disease recorded in Buno Bedele zone. Mean severity of up to 4.3 (on 1-5 severity scoring scale) was recorded in all surveyed western regions of Ethiopia (Table 3) attributing to higher yield loss. Of the surveyed regions, the highest severities between 4 & 5 (on 1-5 scoring scale) were recorded in Gambella. This is followed by Benishangule-Gumuz and Oromia regions, in this order (Table 3).

The present study also disclosed the existence of variations in the level of disease prevalence and incidence in different altitude ranges assessed, where relatively higher prevalence and incidence were recorded at lower altitudes ranging from 400-1600 and vice versa at higher altitudes ranging from 2000-2400 masl. For instance, the disease incidence was higher in Benishangul-Gumuz and Gambela regions at locations having 400 to 1500 masl (Table 4), which perhaps can be attributed to warmer climates favoring vector populations for virus transmission, which in turn contributing to more severe disease symptoms development (Redinbaugh and Zambrano 2014). The high disease incidence detected in the field is expected to cause a considerable reduction in yield and quality. In highly susceptible and late sown genotypes, MS reported to cause up to 100% yield losses in infected crops (Alegbejo *et al*., 2002; Kyetere *et al*., 1999; Lagat *et al*., 2008).

Table 3. The mean prevalence, incidence and severity of MS across district during the 2019 main cropping season

Region	Zone	District	Prevalence (%)	Incidence (%)	Severity (1-5)
Gambella	Agnuwak	Abobo	100	63	4
		Abol	75	45	4
		Gambella	100	85	5
	Mean		92	64	4
	Nuer	Itang	100	63	4
		Lare	100	65	5
	Mean		100	64	5
Grand Mean			94	64	4
Oromia	Jimma	Gumay	60	26	2
		Seka Chekorsa	67	33	3
		Shebe Sombo	75	42	3
		Kersa	80	29	3
		Mana	40	13	1
	Mean		64	28	2
	Buno Bedele	Didesa	0	0	0
		Gechi	0	0	0
	Mean		0	0	0
	East Welega	Guto Gida	78	44	3
		Diga	100	57	4
		Wayu Tuka	50	18	2
		Sibu Sire	20	14	2
	Mean		62	33	3
	West Welega	Gimbi	80	47	3
		Lalo Asabi	100	60	4
		Boji Dirmeji	67	35	3
		Nejo	100	40	4
		Leta Sibu	50	22	2
		Kiltu Kara	100	60	4
		Mene Sibu	100	58	4
	Mean		85	46	2
Grand mean			65	33	3
Benishangul-Gumuz	Asosa	Banbasi	100	67	5
		Homosha	80	51	3
		Asosa	100	58	4
Grand Mean			93	59	4

Table 4. Disease prevalence (%), Incidence (%) and severity (1-5scale) of Maize Streak across different Altitude ranges in Ethiopia, 2019 main season

S.N	Altitude Range masl	NFA	NFI	Prevalence (%)	Incidence (%)	Severity (1-5 scale)
1	<1000	23	22	96	63	4
2	1000-1700	23	22	96	63	4
3	1701-2000	52	36	69	33	3
4	2001-2400	8	1	13	5	1

NFA- Number of fields assessed
NFI- Number of fields infected

Table 5. Correlation of Altitude verses Maize Streak disease incidence and severity

	Altitude	Incidence	Severity
Altitude	1	-.510**	-.446**
Incidence	-.510**	1	.948**
Severity	-.446**	.948**	1

**. Correlation is significant at the 0.01 level (2-tailed).

From the present result, one can conclude that MS disease is distributed in almost all maize growing agro-ecologies with higher prevalence and severity recorded at low and mid altitudes. The high prevalence, incidence and severity of MS at lower and mid altitude maize growing agro-ecologies may partly attributed to the conducive environment for both the virus and vectors transmitting MSV. It is worthwhile to note that high level of correlations was observed between altitude ranges and MS disease distribution, incidence and severity. There were high percentages of disease incidence and severity at lower altitudes and vice versa at higher altitudes. The presence of several grasses in the family poaceae serving as alternate hosts to MSV along with high vector populations and activity at lower altitudes may also immensely contributed for high MS prevalence, incidence and severity. The present results support earlier findings that reported the virus was favored by warmer climate in sub-Saharan Africa (Monjane *et al*., 2011; Oluwafemi *et al*., 2014; Pande *et al*., 2017).

The exceptionally high MS incidence and severity recorded in the surveyed locations in Gambella region might have associated with high vector (s) population and activity. For example, earlier report by Mesfin *et al*. (1991) has shown that efficient MSV vector in the genus Cicadulina such as *C. mabila* is recorded at high intensity in surveyed areas in Gambella, whose activity and population has direct relation to MSV incidence and severity. Furthermore, Mesfin *et al*. (1991) has also reported cereal (Maize, Triticum sp, Avena sp. and sugar cane) and grass (Digitaria, Setaria, Hyparrhenia, Panicum, Pennisetum and Sorghum spp.) are hosts of

Cicadulina spp. vectoring MSV. MSV has also known to have wide host ranges. In addition to maize, cereals such as rice (Oryza sativa L.), wheat (Triticum aestivum L.), oats (Avena sativa L.), barley (Hordeum vulgare L.), rye (Secale cereale L.), finger millet (Eleusine coracana L.), pearl millet (Pennisetum typhoides L.), sorghum (Sorghum bicolor L.) and sugarcane (Saccharum officinarum L.) (Willment *et al*., 2001; Van Antwerpen *et al*., 2011) are infected by MSV.

A wide range of grasses in the genera Axonopus, Brachiaria, Coix, Eleusine, Paspalum, Imperata, Rottboellia, Eragrostis and Setaria (Willment *et al*., 2001; Lett *et al*., 2002; Fajemisin, 2003), most of which are naturally growing in valleys and irrigation schemes, are also attacked by MSV. All cereals and most grasses identified as hosts for MSV and its insect vectors are widely grown in Ethiopia (Bekele *et al*., 2017; Stroud and Parker, 1989). Furthermore, some of the grasses just identified as hosts of *cicadulina* spp. are also known as alternate hosts of MSV (Alemu *et al*., 1997). Hence, having common hosts for both the virus and its vectors simply imply the presence of suitable substrate/medium for their survival and multiplication serving as sources of inoculum and vectors from which the newly growing maize crop can get infected.

Similarly, prevalence, disease incidence and severity of Maize Streak were assessed across maize varieties evaluated. The study revealed that higher average MS incidence and severity were scored on varieties Gambela composite, local and BH540 than other varieties (Fig 3), implying that these varieties appear more susceptible to Maize Streak disease.

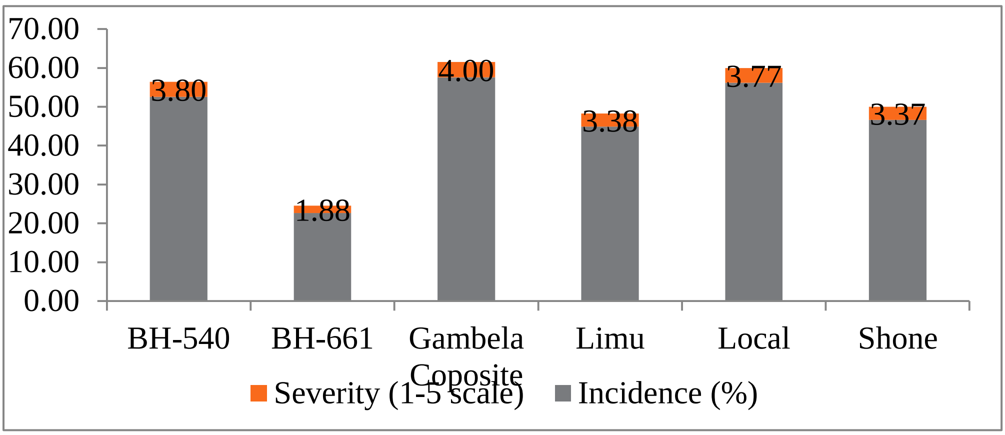

Figure 3. The mean incidence and severity of maize streak disease by varieties sampled across the surveyed locations during the 2019 main cropping season

Molecular Characterization of Maize Streak Virus

The PCR amplification using the degenerate primer pairs consistently yielded an approximately 1300-bp product for ninety five out of one hundred five samples tested (Figure 4). Absence of amplification for five symptomatic maize and five grass samples may be due to misdiagnosis in the field for other viruses which may develop similar or closely related symptoms with MSV such as Maize lethal necrosis (MLN). This primer was amplified nearly 95% of the samples at expected base products, which was comparative result with the reports of Willment, *et al*. (2001). Results of field assessments and molecular detection revealed that MS-causing viruses were distributed in major maize growing areas of Ethiopia surveyed during this study. Symptomatology and molecular diagnosis of MS showed that the disease was detected in nearly 95% of the fields surveyed, indicating that both diagnostic methods can be used interchangeably if attentively done. The bands of some isolates were weak, indicating low quantities of PCR products, which in turn attributed to low DNA extract.

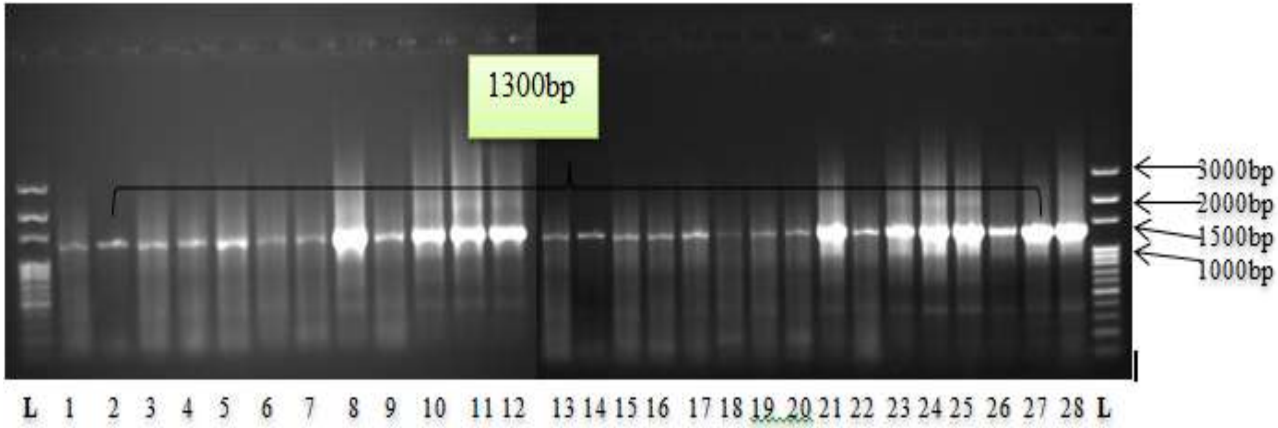

Figure 4. A 1.3-kilo base product was amplified using a MSV replicative form-specific degenerate primer pair, 215–234 and 1770–1792 (Willment et al., 2001). L=The 100 bp DNA Ladder ready-to-use molecular weight marker (Solis BioDyne, Estonia), Size range of 100 – 3,000 bp and 12 numbers of bands. Nos. 1-28 is representative samples collected from surveyed regions.

Conclusion

The study revealed that MS disease was distributed in almost all major maize growing agro-ecologies of western Ethiopia from low to high altitude areas assessed. The results of the present study provided broader information on the distribution, incidence, severity and molecular characterization of MSV in surveyed regions of Ethiopia.

High prevalence, incidence and severity of MS were recorded at low and mid-altitude areas surveyed where as low incidence and severity were recorded at high altitude maize growing areas. The disease recorded in all available local and commercial maize varieties.

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