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Original Research Article

# DNA methylation in recovery of Maize (*Zea Mays* L.) from maize streak

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***Abstract***

DNA methylation influences regulation of gene expression during cell development and tissue differentiation in plants. This study therefore discussed the role of DNA methylation in the recovery of maize from Maize Streak Diseases (MSD). Bisulfite treatment and DNA sequencing methods were carried out on the 2nd, 4th 6th and 8th white to yellowish streaking leaves of five infected maize varieties (DMR-ESR-Y, TZEBR, and ART/98/SW6) to assess the disease severity using digital phenotyping. The bisulfite treatment utilizes various sensitivities of cytosine and 5-methylcytosine (5-MeC) to deamination by bisulfite under acidic conditions in which cytosine is converted to uracil, whereas 5-MeC remains unreactive. This study showed that DMR-ESR-Y variety significantly ($p<0.05$) tolerated best for MSD, while TZEBR and ART/98/SW6 varieties slightly tolerated MSD but were not significantly different. The analysis of the images from digital phenotyping revealed that DMR-ESR-Y variety had the highest level of resistance, followed by TZEBR while ART/98/SW6 showed mild resistance and DMR-LSR-Y had the least. The leaf length had strong positive correlation with leaf width ($r=0.94$), number of leaves($r=0.64$), plant height ($r=0.96$) and stem height($r=0.96$). The methylation patterns in bisulfite sequences across the same leaf positions were significantly different ($p<0.05$), thus suggesting that CG methylation could contribute to transcriptional gene silencing plant recovery mechanism in maize.

**Keywords**: *Digital phenotyping, DNA methylation, maize streak, bisulfite, transcription*.

## INTRODUCTION

Maize (*Zea mays* L.) is a major grain crop grown in a variety of agro-, zones across Africa, with 25 million hectares under cultivation in Sub-Saharan Africa (Galani et al., 2022; Olakojo et al., 2007). It is one of the industrial raw materials and a major food source processed into various dishes in many developing countries of Asia, Latin America, and Africa and it is regarded as a hunger breaker after a long dry period (Grote et al., 2021; Olawuyi et al., 2015). The International Food Policy Research Institute forecasted a 500 million ton annual maize demand in Sub-Saharan Africa in 2020 (Grote et al., 2021; Rosegrant et al., 2008). However, the large yield gap in maize

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is attributable to both abiotic and biotic constraints (Olowe et al., 2020). Biotic factors such as maize streak virus reduce maize yields in sub-Sahara Africa (Olawuyi et al., 2012).

The maize streak virus (MSV) causes yield losses ranging from 0% to over 100% (Asare-Bediako et al., 2017). Disease severity the amount of diseased plant tissue (infected area) compared to the total amount of susceptible tissue available. When standard area diagrams are utilized as evaluation tools, the accuracy, precision, and reliability of intra- and inter-rater estimates of plant disease severity are greatly enhanced (Moreira et al., 2019). Many computer programs are now commercially accessible proprietary software, being the most extensively used semi-automatic computer tool for measuring disease intensity (Pethybridge and Nelson, 2015). Users can now create macros for processing specific tasks in image analysis using open-source software such as ImageJ or proprietary platforms such as SigmaScan Pro and Adobe Photoshop (Pethybridge and Nelson, 2015). The information on DNA methylation on resistance of plants to MaizeStreak Virus which follows a recovery pathway, needs to be documented in affected plants.

DNA methylation is an addition of a methyl group to the C5 position of cytosine to form 5-methylcytosine (5mC) (Kumar et al., 2018). It is predominantly found at Transposable Elements (TEs) and repeats, ensuring the maintenance of TEs silencing. DNA methylation is involved in important developmental processes and stress responses in both plants and animals (Ferrari et al., 2023). According to Meaney and Szyf (2022), the dynamic interplay between methylation and demethylation determines the level and pattern of DNA methylation in a specific organism. The abundance of Transcriptional Elements (TEs), differences in genome size (C value), and other types of repetitive sequences, according to Wang et al. (2019), may explain the wide variation in total 5-mC contents in plants. Under normal conditions, DNA methylation is a primary mechanism that inhibits the activities of transcriptional elements (TEs), but loss of methylation owing to genetic or environmental perturbations may result in their de-repression (Parveen and Dhawan, 2021; Secco et al., 2015). Therefore, the study investigated the role of DNA methylation in recovery of selected Maize Streak diseases varieties using digital phenotyping as provided by the leaf-doctor application.

## MATERIALS AND METHODS

### Experimental site and duration

The screen house experiment was carried out at the nursery farm of the Department of Botany, University of Ibadan, for three months, from September to December 2018 (Latitude 7$^{o}$ 26'N, and 7$^{o}$ 28'S and Longitude 3$^{o}$ 52'W and 3$^{o}$ 52'E) with a mean altitude of 200m above sea level.

### Soil sterilization and experimental seeds

Topsoil collected from nursery was sieved and sterilized using autoclave at 121$^{0}$C, 15 psi for 15 minutes. Ten varieties of maize collected from the Institute of Agricultural Research and Training (IAR&T) were; ART/98/SW1, ART/98/SW6, BR9943-DMR-LSR, BR9928-DMR-SR, DMR-LSR-Y, DMR-ESR-Y, SUWAN-1 SR, LNTP-Y, PROVITA-Y, and TZEBR (Table 1).

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**The descriptive characteristics of the selected maize varieties are shown in Table 1:**

| S/N | Maize Varieties | Descriptions |
|---|---|---|
| 1 | ART/98/SW1 | Quality protein maize (Akinyosoye, 2022), Tolerant to UV radiation (Olawuyi et al., 2016) |
| 2 | ART/98/SW6 | Tolerant to *Striga lutea*, Tolerant to *Aspergillus niger* (Olawuyi et al., 2012; Olawuyi et al., 2014), Tolerant to UV radiation (Olawuyi et al., 2016), Early maturing traits (Olawuyi and Okoli, 2017), Quality Protein Maize [QPM] (Olakojo et al., 2007). |
| 3 | BR9943-DMR-LSR | Streak resistant, Early maturing. |
| 4 | BR9928-DMR-SR | Streak resistant, Early maturing. |
| 5 | DMR-LSR-Y | Streak resistant Early maturing. |
| 6 | DMR-ESR-Y | Streak resistant, Early maturing. |
| 7 | SUWAN-1 SR | Streak resistant. Early maturing. |
| 8 | LNTP-Y | Early maturing, MSV-susceptible. |
| 9 | PROVITA-Y | Early maturing, MSV- susceptible. |
| 10 | TZEBR | MSV-susceptible, Early maturing. |

## Experimental design and treatments

The experiment was carried out using Complete Randomized Design (CRD). It is laid out in three replications consisting of sixty (60) polythene bags containing ten kilograms (kg) of sterilized dirt. The seeds were treated with MSV, while untreated seeds served as control.

## Seed planting and inoculation method

The maize varieties were inoculated artificially by conleafhoppers into insect-proof cages containing 5-day old maize seedlings, while the control is the non-inoculated plants. A forty-eight (48) hour acquisition access period was given to specie of *Cicadulina mbila* species were given on infected maize plants. The inoculated leafhoppers were obtained from the field maintained in insect-proof cages in an insect-proof mesh-screen house at the International Institute of Tropical Agriculture. The insects were frequently disturbed, to ensure uniformity of viral infection. The inoculated plants were transferred and transplanted in the screen house. Agronomic practices such as watering, thinning, were duly carried out on the inoculated plants and control throughout the trial till maturity.

## Diseases assessment and digital phenotyping

Data collected from disease incidence and severity were recorded from symptoms appearance at weekly interval for 8 weeks based on visual evaluation of disease symptoms on individual plants. The symptom rating (1–5 scale) was based on the chlorotic/streaked areas on the leaf surface, the standard procedure used in MSV screening as reported by Barrow (1992), where :

1= no or very few streak symptoms.
2 = light streak symptoms on most leaves.
3 = moderate or mild streak symptoms on most leaves.
4 = abundance symptoms on all leaves (60 -75%) and 5 = severe streak on all leaves (75% and above).

Samsung camera with 21MP was used to take pictures of the infected leaves on black background. Pictures were downloaded, well labeled and uploaded to the leaf doctor application (Pethybridge and Nelson, 2015). The influence of the camera on the total diseased areas were then determined.

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**DNA extraction**

DNA extracted from 70mg of selected diseased leaf samples were carried out using with Dellaporta DNA extraction protocol (Dellaporta et al., 1983). Each leaf sample was grinded in 30 µl of extraction buffer in a sterilized Eppendorf tube and 3µl of 10% Sodium Dodecyl Sulphate (SDS) was then added, vortexed and incubated in a water bath at $65^0$C for 10mins. At room temperature, 10µl of 5M potassium acetate was then added, vortexed and centrifuged at 10000g for 10 minutes.

Supernatants were collected in a new Eppendorf tube, and 20µl of Cold Iso-propanol was added, gently mixed, and stored at -20$^o$C for 60 minutes. The DNA was sedimented by centrifugation at 13000g for 10 minutes, after which the supernatant was carefully decanted and ensured that the pellet was not disturbed. After centrifuging at 10000g for 10 minutes, the DNA pellet was washed with 100 µl of 70% ethanol. Ethanol was decanted from the tube, and the DNA was air-dried at room temperature until no trace of ethanol was visible. To protect and suspend the DNA, the pellets were re-suspended in 15µl of Tris EDTA buffer. Extracted DNA was bisulfite treated using the EZ DNA Methylation TM Kit according to the manufacturer's instructions.

**Primers, thermal cycling PCR and gel electrophoresis**

Primers were selected using the online website of http://eu.idtdna.com/site. Primer sequence was sent to Inquab West Africa for synthesis. Thermal cycling PCR was carried out on the treated DNA to amplify the methylated DNA. The PCR mix consisted of the following (6.25 µl): DNA 2.0, Green buffer 2.50, $MgCl_2$ 0.75 dNTPs 0.25, Methyl-MSV-F primer (5‘-GTTTTTTTTAGTAGGGTTGAAGAGG-3‘) 0.25, Methyl-MSV-R primer (5‘-AAACAATAAATATAAAACCATTACC -3‘) 0.25, Taq polymerase 0.06. 6.0µl of distilled water was added to make the volume 12.5µl. The cycling parameters consisted of a pre-PCR denaturing temperature of 94$^o$C for 1 minute, 35 cycles of 94$^o$C for 1 min, 50$^o$C for 1min 50 seconds and 70$^o$C for 1hr 45secs per cycle and a final termination at 72$^o$C for 7 minutes. The integrity of the amplified gene was checked with a 1.5% Agarose gel electrophoresis stained with Ethidium bromide, viewed and capture in a gel Doc $^{TM}$ EZ imager from BIO-RAD.

**Statistical analysis**

The response of maize cultivars to MSV and healthy (control) were analyzed using Genstat computer programme 2007 for analysis of variance, while means were separated by Duncan Multiple Test Range at 5% probability level. The response of maize cultivars to MSV and healthy (control), and data obtained from the leaf symptomatic areas were analyzed using Genstat computer programme 2007 for analysis of variance while means were separated by Duncan Multiple Test Range at 5% probability level.

**RESULTS**

The mean square interaction of varieties and weeks in Table 2 was highly significant ($P<0.01$) on growth and yield-related characters (leaf length, leaf width, plant height, and stem height) of healthy maize varieties, while higher significant effects were recorded on weeks for shoot biomass at $p<0.01$. The effects of varieties, and weeks are significantly ($p< 0.01$) higher for leaf length, leaf width, number of leaves, stem height, plant height, and diseased shoot and root biomass (Table 3).

The result in Table 4 shows that the effect of Varieties, Treatments and Weeks were highly significant ($p<0.01$) on the growth characters, shoot biomass and disease severity of maize except

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for root biomass. The varieties x weeks produced highly significant effect on shoot biomass and disease severity, while the second order of interaction (Varieties x Treatment x Weeks) had significant effect on leaf length and disease severity of maize (Table 4).

The variety, PROVITA-Y is significantly ($P<0.05$) high for leaf length 4.08cm and plant height 79.00cm, while LNTP-Y and DMR-ESR-Y were higher for stem height (33.18cm) and number of leaves (11.58cm) respectively (Table 5). The SUWAN-1 SR had higher significant effects on leaf width (51.50cm) shoot (0.48g) and root biomass (0.12g).

The mean values of BR9928-DMR-SR and DMR-LSR-Y in Table 5 are not significantly ($P>0.05$) different for leaf length, number of leaves, shoot and root biomass, while leaf length, number of leaves, root and shoot biomass are statistically similar for BB9928-DMR-SR and DMR-LSR-Y. Again, leaf width, plant height, and shoot biomass had similar statistical effects for ART/98/SW6 and BR9943-DMR-LSR, while DMR-LSR-Y and DMR-ESR-Y had the same statistical effect on leaf width, shoot and root biomass.

The varietal effect of BR9943-DMR-LSR on growth and yield-related characters of diseased maize in Table 6 is significantly higher on number of leaves but not significantly different for DMR-ESR-Y, LNTP-Y, TZEBR, PROVITA-Y and ART/98/SW1 varieties while BR9928-DMR-SR is significantly higher on leaf width and disease severity. Also, DMR-ESR-Y is significantly higher for plant height (42.78cm) and shoot biomass (0.46g), while stem height (21.56cm) and leaf length (34.86cm) were higher in TZEBR. The mean of leaf length in ART/98/SW1, ART/98/SW6, BR9943-DMR-LSR, BR9928-DMR-SR, DMR-LSR-Y and SUWAN-1 SR are not significantly ($P>0.05$) different from one another, while leaf width in BR9943-DMR-LSR, DMR-LSR-Y, DMR-ESR-Y LNTP-Y are not statistically different from one another (Table 6).

The result of combined performance of growth and yield-related characters of healthy and diseased maize in Table 7 shows that the leaf length (40.32cm) and stem height (27.36cm) in LNTP-Y is significantly higher, while PROVITA-Y is significantly higher, but different for leaf width (3.48cm). The number of leaves (9.53), shoot biomass (0.47g) and plant height (57.61cm), in DMR-ESR-Y are significantly higher. The LNTP-Y variety is significantly higher for stem height (27.36cm), SUWAN-1 SR is significantly higher and different for root biomass (0.09g), while BR9928-DMR-SR is significantly higher for disease severity.

The root biomass for all the varieties is not significantly different from one another, while the mean values for disease severity of BR9928-DMR-SR, DMR-LSR-Y, DMR-ESR-Y SUWAN-1SR and PROVITA-Y are statistically different (Table 7).

The result in Table 8 shows that the second leaf positions in ART/98/SW6, DMR-LSR-Y, DMR-ESR-Y, LNTP-Y and TZEBR had the least disease rating score and highest methylated cytosine of (1.00, 37.00%), (1.00, 33.00%), (1.00, 19.00%), (1.00, 16.00%) and (1.00, 15.00%) respectively. Again, the $4^{th}$, $6^{th}$ and $8^{th}$ leaf positions in ART/98/SW6, DMR-LSR-Y, DMR-ESR-Y, LNTP-Y andTZEBR had disease rating scores of 2, 3, and 4 respectively, while (31.00, 29.00, 12.00); (27.00, 21.00, 18.00 ); (15.00, 13.00,6.00); (15.00, 9.00, 6.00) and (12.00, 8.00, 5.00) for methylated cytosine were observed in ART/98/SW6, DMR-LSR-Y, DMR-ESR-Y, LNTP-Y and TZEBR respectively.

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The result of Principal Component Analysis of growth and yield-related character in table 9 is delineated into six (6) principal component axes Prin 1, Prin 2, Prin 3, Prin 4, Prin 5 and Prin 6. Prin 1 accounted for the highest variation with a proportion of 43.20% and eigen value of 3.46. In Prin 1, the leaf length (0.05), leaf width, (0.47), plant height (0.47) and stem height (0.46) are closely related, while the root biomass (0.65) and shoot biomass (0.65) are related in Prin 2. Also, the number of leaves (-0.17), plant height (-0.16), stem height (-0.17) are negatively associated, while leaf length (0.03), plant height (0.03), and stem height (0.02) are positively related in Prin 3. The shoot biomass (0.15) and root biomass (0.17) are related in Prin 4 while leaf width (-0.05) plant height (-0.06) and shoot biomass (-0.08) are negatively related in Prin 5. In Prin 6, the stem height (0.23) is related to disease severity of 0.20.

The result in table 10 shows that the leaf length is strongly positive and correlated with leaf width (r=0.94), no of leaves (r=0.64), plant height (r=0.96), stem height(r=0.96) and weeks (r=0.82). Also, leaf width is positive and strongly associated with number of leaves (r=0.65), plant height (r=0.91), stem height (r=0.93) and weeks (0.83) while number of leaves is positively related to plant height (r=0.61) and stem height (r=0.63). More so, plant height had strong positive association with stem height (r=0.93), while shoot biomass had strong positive association with root biomass at r=0.85. The bisulfite treatment is negatively associated with disease severity at r=0.81

The result showing relationship among healthy maize varieties in figure 1 shows that variety TZEBR branched out from the major cluster. It is also deduced that DMR-LSR-Y and LNTP-Y closely related to each other than SUWAN-1 SR and PROVITA-Y likewise DMR-ESR-Y and ART/98/SW1 are related to each other than to BR928-DMR-SR, while ART/98/SW6 and BR99943-DMR-LSR are closely associated with each other.

In figure 2, DMR-ESR-Y branched out from the two clusters. Cluster 1 which is the major cluster comprised of 7 varieties while cluster 2 has 2 varieties. Also, varieties LNTP and TZEBR in cluster 2 were closely related. SUWAN-1-SR and PROVITA-Y are closely related in cluster 1, while ART/98/SW6 and BR9928-DMR-SR are more related to BR9943-DMR-LSR while DMR-LSR-Y and ART/98/SW1 are closely associated to each other. The result in figure 3 shows that the diseased variety ART/98/SW6 recorded mean area of 64.95cm$^2$. The DMR-LSR-Y, DMR-ESR-Y, LNTP-Y and TZEBR produced leaf area of 78.19cm$^2$, 26.06cm$^2$, 75.70 cm$^2$, and 49.60cm$^2$ respectively. DMR-LSR-Y had the highest diseased area at the eighth leaf with a score of 3 while DMR-ESR-Y had a score of 3 at the second leaf. The highest level of resistance was shownby DMR-ESR-Y followed by TZEBR while ART/98/SW6 showed moderate resistance and DMR-LSR-Y had the least.

The digital phenotype photographs in figure 4 show the leaf positions and recovery of DMR-ESR-Yvariety from MSV infection. The digital phenotype photographs of leaf positions show no recovery of MSV in LNTP-Y variety (Figure 5). There were severe infections without recovery in all the leaf positions shown in the digital phenotype photographs of ART/98/SW6 variety (Figure 6) showed the digital phenotype photographs of leaf positions of TZEBR variety and the extent ofrecovery while variety DMR-LSR-Y showed a mild recovery from MSV infection (Figure 7).

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**Table 2: Mean square interactions of varieties, and weeks on the growth and yield-related characters of healthy maize varieties**

| Source of Variation | Df | Leaf Length | Leaf Width | Number of Leaves | Plant Height | Stem Height | Shoot Biomass | Root Biomass |
|---|---|---|---|---|---|---|---|---|
| Varieties | 9 | 943.13** | 7.72** | 63.62** | 1746.03** | 645.53** | 0.14ns | 0.03ns |
| Weeks | 11 | 21637.61** | 74.39** | 450.47** | 48673.35** | 7439.44** | 61.17** | 1.85 |
| Replicates | 2 | 177.45ns | 7.54ns | 0.76ns | 691.31ns | 72.50ns | 0.05ns | 0.02ns |
| Model | 22 | 1122.76 | 41.04 | 251.32 | 25113.80 | 3990.39 | 30.65 | 0.94 |
| Error | 337 | 98.8336 | 0.49 | 32.02 | 258.68 | 33.22 | 0.17 | 0.03 |
| Corrected total | 359 | | | | | | | |

Note: * P<0.05 significant, ** P<0.01 highly significant, ns is not significant and df is the degree of freedom

**Table 3: Mean Square Interactions of Varieties, and Weeks on the growth and yield-related characters of diseased maize varieties**

| Source of Variation | Df | Leaf Length | Leaf Width | Number of Leaves | Plant Height | Disease Severity | Stem Height | Shoot Biomass | Root Biomass |
|---|---|---|---|---|---|---|---|---|---|
| **Varieties** | 9 | 239.93** | 2.13** | 6.82** | 2.95** | 11.96** | 76.61** | 0.17** | 0.00ns |
| **Weeks** | 11 | 6008.81** | 40.23** | 298.83** | 8489.52** | 77.95** | 4534.54** | 40.00** | 0.65** |
| **Replicates** | 2 | 39.34ns | 0.39ns | 11.20ns | 17.21ns | 2.23ns | 42.34ns | 0.01ns | 0.00ns |
| **Model** | 22 | 3106.13 | 21.02 | 153.22 | 4367.20 | 44.07 | 2302.46 | 20.07 | 0.34 |
| **Error** | 337 | 12.80 | 0.08 | 1.50 | 10.68 | 0.27 | 4.29 | 0.07 | 0.00 |
| **Corrected total** | 359 | | | | | | | | |

Note: * P<0.05 significant, ** P<0.01 highly significant, ns is not significant and df is the degree of freedom.

**Table 4: Mean Square Interactions of Varieties, Treatment and Weeks on the growth-related characters of Combined Healthy and Diseased maizevarieties.**

| Source of variation | Df | Leaf Length | Leaf Width | No of Leaves | Plant Height | Stem Height | Shoot Biomass | Root Biomass | Disease Severity |
|---|---|---|---|---|---|---|---|---|---|
| Varieties | 9 | 253.69** | 4.29** | 36.96** | 723.91** | 294.20** | 0.18ns | 0.02ns | 5. |
| Treatments | 1 | 54723.23** | 127.50** | 475.31** | 170786.00** | 15642.69** | 1.12** | 0.15 ns | 198 |
| Weeks | 11 | 25173.67** | 111.93** | 728.64** | 48658.67** | 11699.77** | 100.05** | 2.35 ns | 38 |
| Varieties*Treatment | 9 | 929.37** | 5.55** | 33.47** | 1317.59** | 427.94** | 0.13ns | 0.01ns | 5. |
| Varieties*weeks | 99 | 27.78ns | 0.13ns | 15.77ns | 42.24ns | 6.57ns | 0.18** | 0.02ns | 0. |
| Treatment*weeks | 11 | 2472.75** | 2.69** | 20.65ns | 8504.20** | 274.21** | 1.12** | 0.15** | 38 |
| Varieties*Treatments *weeks | 99 | 44.69** | 0.11ns | 18.21ns | 58.96ns | 13.76ns | 0.13ns | 0.01ns | 0. |
| Model | 239 | 1575.97 | 6.28 | 53.20 | 3464.31 | 652.17 | 4.80 | 0.13 | 1 |
| Error | 480 | 64.33 | 0.39 | 16.57 | 171.19 | 22.62 | 0.11 | 0.12 | 0 |
| Corrected total | 719 | | | | | | | | |

Note: * P<0.05 significant, ** P<0.01 highly significant, ns is not significant and df is the degree of freedom.

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**Table 5: Performance of growth and yield-related characters of healthy maize varieties**

| Varieties | Leaf Length (cm) | Leaf Width (cm) | Number of Leaves | Plant Height (cm) | Stem Height (cm) | Shoot Biomass (g) | Root Biomass (g) |
|---|---|---|---|---|---|---|---|
| ART/98/SW1 | 3.40cde | 44.92b | 8.50bc | 72.97ab | 27.11cd | 0.41a | 0.06a |
| ART/98/SW6 | $3.11^{e}$ | $46.36^{ab}$ | $9.30^{abc}$ | $69.61^{bc}$ | $30.92^{ab}$ | $0.44^{a}$ | $0.11^{a}$ |
| BR9943-DMR-LSR | $3.53^{bcd}$ | $47.89^{ab}$ | $9.25^{ab}$ | $70.31^{bc}$ | $28.67^{bcd}$ | $0.40^{a}$ | $0.07^{a}$ |
| BR9928-DMR-SR | $3.33^{ed}$ | $46.06^{b}$ | $8.25^{bc}$ | $63.69^{c}$ | $28.28^{bcd}$ | $0.35^{a}$ | $0.06^{a}$ |
| DMR-LSR-Y | $3.31^{ed}$ | $50.19^{ab}$ | $8.50^{bc}$ | $68.75^{bc}$ | $32.53^{a}$ | $0.43^{a}$ | $0.09^{a}$ |
| DMR-ESR-Y | $3.73^{abc}$ | $47.03^{ab}$ | $11.58^{a}$ | $72.44^{ab}$ | $25.83^{d}$ | $0.47^{a}$ | $0.07^{a}$ |
| SUWAN-1 SR | $3.79^{ab}$ | $51.50^{a}$ | $8.94^{abc}$ | $72.72^{ab}$ | $31.17^{ab}$ | $0.48^{a}$ | $0.12^{a}$ |
| LNTP-Y | $3.99^{a}$ | $49.28^{ab}$ | $8.67^{abc}$ | $70.86^{abc}$ | $33.81^{a}$ | $0.46^{a}$ | $0.06^{a}$ |
| PROVITA-Y | $4.08^{a}$ | $48.94^{ab}$ | $8.61^{abc}$ | $79.00^{a}$ | $29.28^{bc}$ | $0.32^{a}$ | $0.03^{a}$ |
| TZEBR | $2.50^{f}$ | $33.14^{c}$ | $6.08^{c}$ | $52.81^{d}$ | $18.78^{e}$ | $0.37^{a}$ | $0.04^{a}$ |

Mean with the different letters in the same column are significant at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

**Table 6: Performance of growth and yield-related characters of diseased maize varieties**

| Variety | Leaf Length (cm) | Leaf Width (cm) | Number of Leaves | Plant Height (cm) | Disease Severity | Stem Height (cm) | Shoot Biomass (g) | Root Biomass (g) |
|---|---|---|---|---|---|---|---|---|
| ART/98/SW1 | $26.86^{c}$ | $2.29^{c}$ | $7.22^{a}$ | $37.11^{d}$ | $3.67^{bc}$ | $17.86^{e}$ | $0.21^{c}$ | $0.03^{a}$ |
| ART/98/SW6 | $27.92^{c}$ | $2.35^{c}$ | $6.56^{bc}$ | $38.58^{cd}$ | $3.64^{bc}$ | $18.25^{cd}$ | $0.37^{ab}$ | $0.04^{a}$ |
| BR9943-DMR-LSR | $28.31^{c}$ | $2.70^{b}$ | $7.53^{a}$ | $38.42^{cd}$ | $3.56^{bc}$ | $18.89^{bcd}$ | $0.28^{bc}$ | $0.04^{a}$ |
| BR9928-DMR-SR | $28.03^{c}$ | $2.88^{a}$ | $6.42^{c}$ | $38.86^{c}$ | $4.14^{a}$ | $17.86^{ed}$ | $0.30^{bc}$ | $0.05^{a}$ |
| DMR-LSR-Y | $27.47^{c}$ | $2.57^{b}$ | $6.58^{bc}$ | $36.94^{d}$ | $3.72^{b}$ | $19.61^{b}$ | $0.33^{bc}$ | $0.05^{a}$ |
| DMR-ESR-Y | $21.33^{b}$ | $2.66^{b}$ | $7.47^{a}$ | $42.78^{a}$ | $2.17^{f}$ | $21.03^{a}$ | $0.46^{a}$ | $0.05^{a}$ |
| SUWAN-1 SR | $27.47^{c}$ | $2.36^{c}$ | $7.11^{ab}$ | $34.19_{e}$ | $3.19^{d}$ | $19.61^{b}$ | $0.32^{bc}$ | $0.05^{a}$ |
| LNTP-Y | $31.36^{b}$ | $2.68^{b}$ | $7.47^{a}$ | $42.36^{ab}$ | $2.92^{e}$ | $20.92^{a}$ | $0.38^{ab}$ | $0.04^{a}$ |
| PROVITA-Y | $27.92^{c}$ | $2.87^{a}$ | $7.31^{a}$ | $35.03^{e}$ | $3.44^{c}$ | $18.61^{bcd}$ | $0.32^{bc}$ | $0.04^{a}$ |
| TZEBR | $34.86^{a}$ | $2.99^{a}$ | $7.50^{a}$ | $40.86^{ab}$ | $2.72^{e}$ | $21.56^{a}$ | $0.38^{ab}$ | $0.03^{a}$ |

Mean with the different letters in the same column are significant at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

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**Table 7: Genotypic performance on growth and yield-related characters of combined healthy and diseased maize varieties.**

| Variety | Leaf Length (cm) | Leaf Width (cm) | Number of Leaves | Plant Height (cm) | Stem Height (cm) | Shoot Biomass (g) | Root Biomass (g) | Disease Severity |
|---|---|---|---|---|---|---|---|---|
| **ART/98/SW1** | 35.89$^{cd}$ | 2.85$^{e}$ | 7.86$^{bc}$ | 55.04$^{ab}$ | 22.14$^{e}$ | 0.31$^{b}$ | 0.04$^{a}$ | 1.83$^{b}$ |
| **ART/98/SW6** | 36.99$^{bc}$ | 2.73$^{e}$ | 7.79$^{bc}$ | 54.10$^{ab}$ | 24.58$^{bcd}$ | 0.40$^{ab}$ | 0.08$^{a}$ | 1.82$^{bc}$ |
| **BR9943-DMR-LSR** | 38.10$^{abc}$ | 3.12$^{cd}$ | 8.39$^{b}$ | 54.36$^{ab}$ | 23.78$^{cde}$ | 0.34$^{b}$ | 0.05$^{a}$ | 1.78$^{bc}$ |
| **BR9928-DMR-SR** | 37.04$^{bc}$ | 3.10$^{cd}$ | 7.33$^{bc}$ | 51.28$^{ab}$ | 23.07$^{de}$ | 0.32$^{b}$ | 0.06$^{a}$ | 2.07$^{a}$ |
| **DMR-LSR-Y** | 38.69$^{abc}$ | 2.94$^{de}$ | 7.54$^{bc}$ | 52.85$^{ab}$ | 26.07$^{ab}$ | 0.38$^{ab}$ | 0.07$^{a}$ | 1.86$^{b}$ |
| **DMR-ESR-Y** | 39.18$^{ab}$ | 3.20$^{bc}$ | 9.53$^{a}$ | 57.61$^{a}$ | 23.43$^{de}$ | 0.47$^{a}$ | 0.06$^{a}$ | 1.08$^{f}$ |
| **SUWAN-1 SR** | 39.49$^{ab}$ | 3.07$^{cd}$ | 8.03$^{bc}$ | 53.46$^{ab}$ | 25.21$^{bc}$ | 0.40$^{ab}$ | 0.09$^{a}$ | 1.60$^{d}$ |
| **LNTP-Y** | 40.32$^{a}$ | 3.33$^{ab}$ | 8.07$^{bc}$ | 56.61$^{ab}$ | 27.36$^{a}$ | 0.42$^{a}$ | 0.05$^{a}$ | 1.46$^{e}$ |
| **PROVITA-Y** | 38.43$^{abc}$ | 3.48$^{a}$ | 7.96$^{bc}$ | 57.01$^{a}$ | 23.94$^{cd}$ | 0.42$^{cd}$ | 0.04$^{a}$ | 1.72$^{c}$ |
| **TZEBR** | 34.00$^{d}$ | 2.75$^{e}$ | 6.79$^{c}$ | 46.83$^{c}$ | 20.17$^{f}$ | 0.32$^{b}$ | 0.04$^{a}$ | 1.36$^{e}$ |

Mean with the same letters in the same column are not significant at $p \leq 0.05$ according to Duncan Multiple Range Test (DMRT).

**Table 8: Disease rating scores and methylated cytosine on leaf positions of maize**

| Maize varieties | Leaf position | Disease rating scores | Methylated cytosine (%) |
|---|---|---|---|
| **ART/98/SW6** | 2.00 | 1.00 | 37.00 |
| | 4.00 | 2.00 | 31.00 |
| | 6.00 | 3.00 | 29.00 |
| | 8.00 | 4.00 | 12.00 |
| **DMR-LSR-Y** | 2.00 | 1.00 | 33.00 |
| | 4.00 | 2.00 | 27.00 |
| | 6.00 | 3.00 | 21.00 |
| | 8.00 | 4.00 | 18.00 |
| **DMR-ESR-Y** | 2.00 | 1.00 | 19.00 |
| | 4.00 | 2.00 | 15.00 |
| | 6.00 | 3.00 | 13.00 |
| | 8.00 | 4.00 | 6.00 |
| **LNTP-Y** | 2.00 | 1.00 | 16.00 |
| | 400 | 2.00 | 15.00 |
| | 6.00 | 3.00 | 9.00 |
| | 8.00 | 4.00 | 6.00 |
| **TZEBR** | 2.00 | 1.00 | 15.00 |
| | 4.00 | 2.00 | 12.00 |
| | 6.00 | 3.00 | 8.00 |
| | 8.00 | 4.00 | 5.00 |

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**Table 9: Principal component axis of growth, disease severity and yield-related characters of maize**

| Characters | Prin 1 | Prin 2 | Prin 3 | Prin 4 | Prin 5 | Prin 6 |
|---|---|---|---|---|---|---|
| Leaf Length | 0.50 | 0.10 | 0.03 | -0.12 | -0.12 | 0.19 |
| Leaf Width | 0.47 | -0.07 | 0.22 | -0.09 | -0.05 | -0.84 |
| No of Leaves | 0.25 | -0.17 | -0.05 | 0.95 | 0.02 | 0.04 |
| Plant Height | 0.47 | -0.16 | 0.03 | -0.11 | -0.06 | 0.37 |
| Stem Height | 0.46 | -0.17 | 0.02 | -0.12 | 0.79 | 0.23 |
| Shoot Biomass | -0.13 | 0.65 | 0.15 | 0.15 | -0.08 | -0.18 |
| Root Biomass | -0.10 | 0.65 | 0.20 | 0.17 | 0.05 | -0.01 |
| Disease Severity | -0.09 | -0.09 | -0.25 | 0.94 | 0.02 | 0.20 |
| Eigenvalues | 3.46 | 1.93 | 0.95 | 0.81 | 0.31 | 0.26 |
| Proportion (%) | 43.20 | 24.18 | 11.87 | 10.04 | 3.80 | 3.20 |

**Table 10: Correlation coefficients among the growth and yield-related characters of maize.**

| | Leaf Length (cm) | Leaf Width (cm) | No of Leaves | Plant Height (cm) | Stem Height (cm) | Shoot Biomass (g) | Root Biomass (g) | Disease Severity | Treatment | Varieties | Weeks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Leaf Width | | | | | | | | | | | |
| No of Leaves | 0.94** | | | | | | | | | | |
| No of Leaves | 0.64** | 0.65** | | | | | | | | | |
| Plant Height | 0.96** | 0.91** | 0.61** | | | | | | | | |
| Stem Height | 0.96** | 0.93** | 0.63** | 0.93** | | | | | | | |
| Shoot Biomass | 0.35 | 0.29 | 0.12 | 0.36 | 0.41 | | | | | | |
| Root Biomass | 0.32 | 0.25 | 0.10 | 0.33 | 0.37 | 0.85** | | | | | |
| Severity | -0.21 | -0.09 | -0.04 | -0.28 | -0.15 | -0.02 | -0.04 | | | | |
| Treatment | 0.37 | 0.28 | 0.15 | 0.43 | 0.31 | 0.03 | 0.06 | -0.81** | | | |
| Varieties | 0.01 | 0.06 | -0.02 | -0.02 | -0.00 | 0.01 | -0.02 | -0.07 | 0.00 | | |
| Weeks | 0.82** | 0.83** | 0.58* | 0.76** | 0.88** | 0.46 | 0.39 | 0.16 | 0.00 | 0.00 | |
| Replicates | 0.01 | 0.04 | 0.00 | 0.01 | 0.03 | 0.03 | 0.01 | -0.02 | 0.00 | 0.00 | 0.00 |

Note: * P<0.05 significant, ** P<0.01 highly significant, *** P<0.001 highly significant

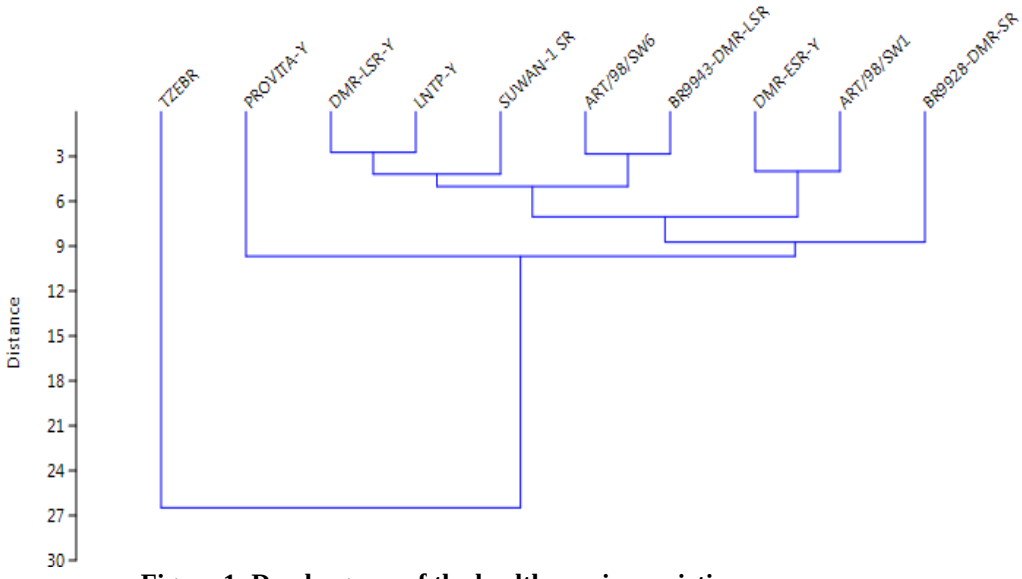


**Figure 1: Dendrogram of the healthy maize varieties**

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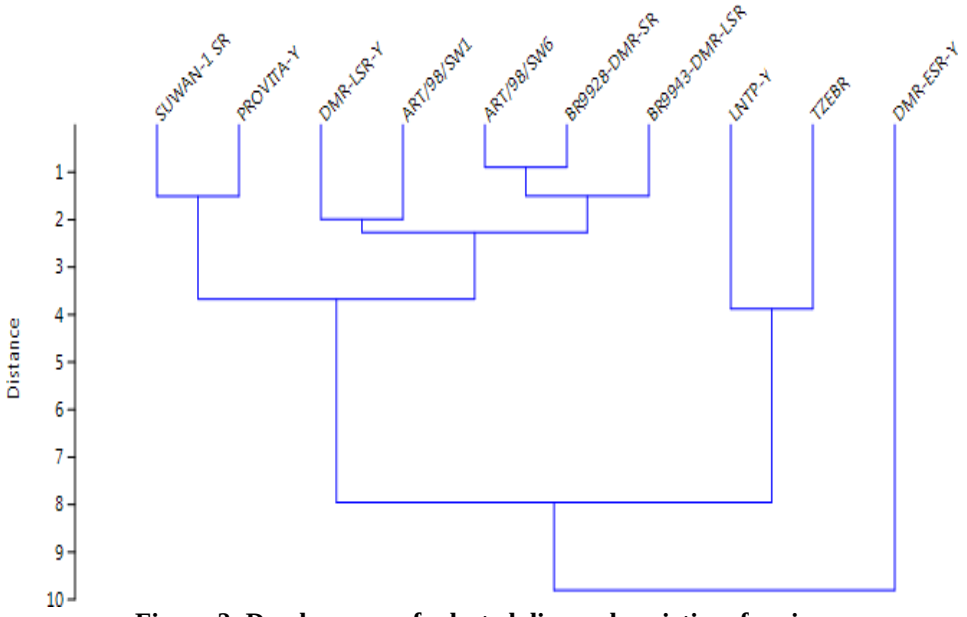


**Figure 2: Dendrogram of selected diseased varieties of maize.**

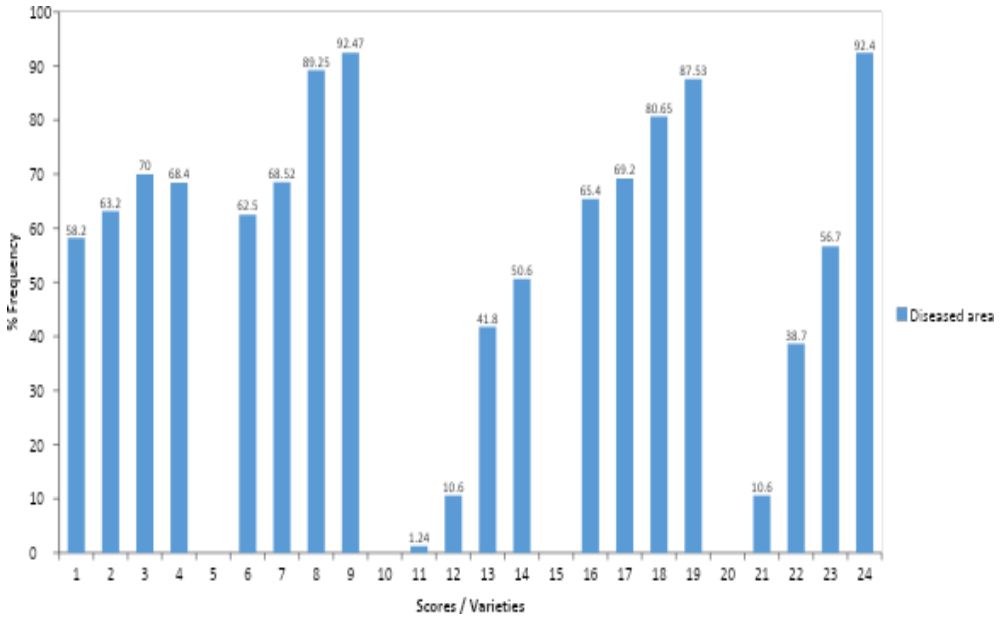


**Figure 3: Frequencies of the disease areas in relation to varieties and scores of the digitally phenotyped leaves of maize**

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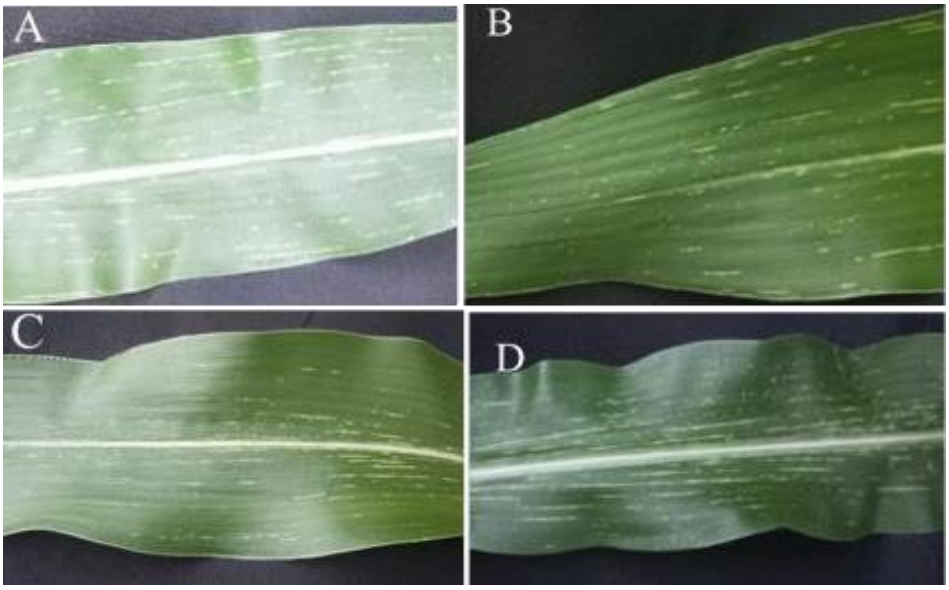


**Figure 4: Digital phenotype photographs of leaf positions showing the recovery of DMR-ESR-Y variety from MSV infection.**

KEYS: A – MSV symptoms in: A- 2 nd leaf, B - 4th leaf, C- 6th leaf, and D - 8th leaf.

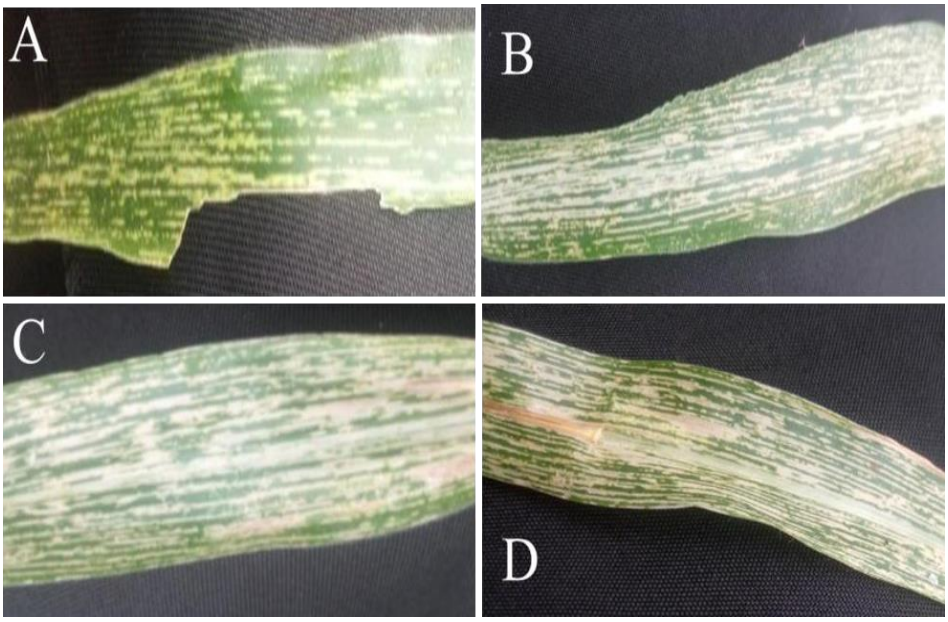


**Figure 5: Digital phenotype photographs of leaf positions of variety ART/98/SW6 showing no recovery from MSV infection.**

KEYS: MSV symptoms in: A - A – MSV symptoms in: A- 2 nd leaf, B - 4th leaf, C- 6th leaf, and D - 8th leaf.

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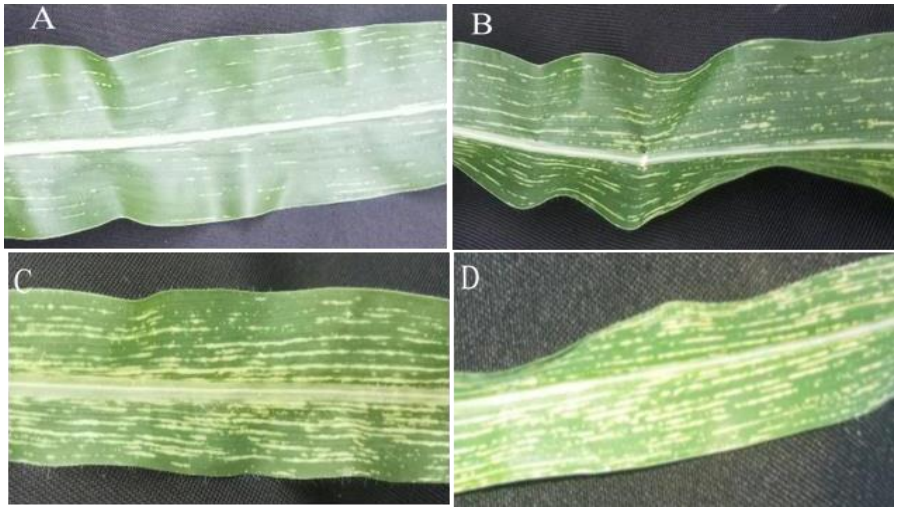


**Figure 6: Digital phenotype photographs of leaf positions of variety ART/98/SW6 showing the extent of MSV infection.**

KEYS: MSV symptoms in: A - A – MSV symptoms in: A- 2 nd leaf, B - 4th leaf, C- 6th leaf, and D - 8th leaf.

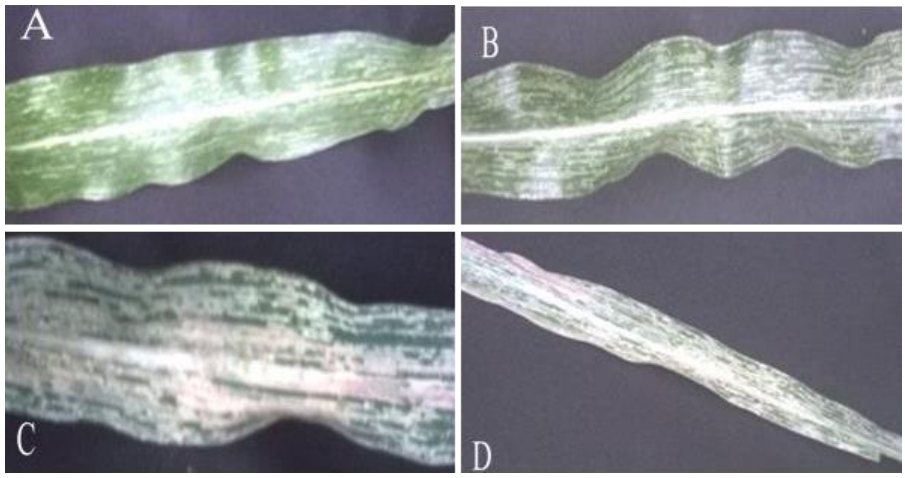


**Figure 7: Digital phenotype photographs of leaf positions of variety DMR-LSR-Y showing mild recovery from MSV infection.**

KEYS: MSV symptoms in: A - A – MSV symptoms in: A- 2 nd leaf, B - 4th leaf, C- 6th leaf, and D - 8th leaf.

## DISCUSSION

Methylated cytosine regions were observed, and the varieties followed the same methylation pattern and frequencies. This indicates the contributions of DNA methylation to the resistance and recovery of maize varieties from disease caused by Maize Streak Virus (MSV). This is inline with the findings of Kitimu et al. (2015) and Rodríguez-Negrete et al. (2013).

The findings from this study showed that variety TZEBR was moderately tolerant to MSV, DMR-ESR-Y showed strong resistance, while ART/98/SW6 and DMR-LSR-Y that were severely affected showed recovery due to higher production of methylated cytosine.The moderately tolerant varieties expressed symptoms of viral infection, and their methylation frequencies are negligibly affected though, not significantly different ($p>0.05$) from the resistant maizevarieties. This in accordance with the reports of Zhu et al. (2021).

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Biotic stresses such as pathogen infection generally increase the overall level of DNA methylation and slows down the metabolism of the plants. This helped the maize cultivar to overcome the temporary challenge of infection. This is in agreement with the reports of Lamalakshmi Devi et al. (2017). From the sequenced DNA, methylated cytosine of the 2nd leaf in all the varieties followed similar pattern, but slightly higher in DMR-ESR-Y. For the 4th leaf across all varieties, methylated cytosine were few. The same was observed in the 6th and 8th leaf positions across the sequenced varieties.

From the dendrogram constructed for diseased varieties, DMR-ESR-Y (resistant variety) branched out from the main clusters (figure 2) indicates resistant characteristics, while TZEBR branched out for healthy maize due to its best performance in growth and yield related characters. The result of correlation showed that leaf length had strong positive correlation with leaf width (r=0.94), no of leaves(r=0.64), plant height (r=0.96) and stem height(r=0.96) respectively. Also, leaf width had strong positive association with number of leaves (r=0.65), plant height(r=0.91) and stem height (r=0.93) respectively. Number of leaves had strong positive correlations with plant height (r=0.61) and stem height (r=0.63). More so, plant height had a strong positive correlation with stem height (r=0.93), while shoot biomass had strong positive association with root biomass at r=0.85. This indicate that the leafy characters contributed to the development of shoot a root biomass of maize. Again, leaf width, number of leaves, plant height and stem height reveal the impact of methylation in the assessment of growth and yield characters, this implies that leaf traits should be considered in future breeding of maize.

It was also found from the table that the higher the diseased area, the lower the number of methylated cytosine. This shows the contributions of cytosine methylation to the recovery of the leaves frommaize streak virus. DNA methylation could impact gene expression due to the presence of methylated cytosine which is a regulatory element of genes that can act as transcription binding factor and hence affect gene regulation. As a result, a biomarker might be created by accurately estimating changes in DNA methylation at specific loci between normal and diseased states.

## CONCLUSION

The DMR-ESR-Y variant was shown to have the best tolerance to maize streak virus in this study. The digital phenotyping supported the rating scores of 1 to 5 levels of Maize Streak Disease resistance.

More efficient protocols can be used for determining the extent of the contribution of DNA methylation in maize recovery from maize streak virus as these will give better results for genome-wide sequencing.

## Conflict of interest

The authors declare no conflict of interests

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