Nat. Volatiles & Essent. Oils, 2021; 8(5): 9035-9042

NVEO
Natural Volatiles & Essential Oils

Evaluation of Agronomy and Resistance Stability of Transgenic Sugarcane with Mosaic Virus-resistant through Pathogen-derived Resistance Approach

Choirul Ainiyati [1*], Sudiarso [2], Soemarno [2]

[1] Postgraduate Program For Agriculture, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia.

[2] Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia.

**corresponding author e-mail : choirulainiyati@gmail.com*

ABSTRACT

Sugarcane mosaic virus (SCMV) is one of important sugarcane disease that caused mosaic symptom and yield loss due to mosaic disease reaches 20% at disease incidence rates above 50%. Pathogen Derived Resistance (PDR) is a molecular mechanism that disrupts pathogen expression in host plants. Transgenic sugarcane has been obtained using the PDR (Pathogen derived resistance) technique that expresses SCMV-coat-protein established, and has been tested to be resistant to SCMV until the third generation. This study aims to determine the resistance stability of transgenic sugarcane by considering growth and yield at SCMV post inoculation. The result showed that the resistance character of transgenic sugarcane to SCMV virus was inherited to the next generation (the 4th generation). The resistance of transgenic sugarcane was confirmed by molecular analysis using the RT-PCR method with a capsid protein-based gene targeting to explain the symptoms caused by SCMV. The DNA fragment of capsid protein has been successfully amplified in all sugarcane lines, proving that the symptom that appeared on the leave was caused by SCMV. This research showed that line B10.3 and B11.3 had better growth performance compared to the other transgenic line, Cane yield and sucrose content were also high and concluded that this line was resistant to SCMV infection and had genetic stability. On the other hand, the hight incidence percentage in line B11.1 and B10.2 did not affect the growth and production. The possibility of recovery mechanism from SCMV infection occurred in transgenic sugarcane lines B11.1 and B10.2 during the grand growth phase after infection resulted a normal growth performance. The recovery mechanism in transgenic sugarcane using pathogen-derived resistance approaches need to be proved with experimental research.

Keynotes : *Sugarcane mosaic virus* (SCMV), Pathogen Derived Resistance (PDR), resistance stability, growth, yield

Introduction

Sugarcane known as a crop that can accumulate sucrose, biomass producer, ethanol/biofuel, and the biggest sugar producer, in 2020 Indonesian sugar production from sugarcane reached 2,130,720 tons, it has been decreased compared to 2019 which produce 2.22 million tons of sugar (Dirjenbun, 2020). The reduction of sugar production is caused by weather condition, which has been affected sugarcane cultivation. On the other hand, the growth and production of sugarcane are caused by pests and disease attacks. The mosaic disease is categorized as sugarcane disease caused by abiotic stress. This disease is caused by a virus such as *sugarcane streak mosaic virus* (SCSMV) dan *sugarcane mosaic virus* (SCMV), which have been infected sugarcane through mechanic and non-mechanic dissemination. Putra *et al.* (2013) reported that Yield loss due to mosaic disease reaches 20% at disease incidence rates above 50%, especially on susceptible varieties like PS 864. SCMV virus incidence on sugarcane is reported to be around 20-50%, even reaching 78% on a sugarcane plantations in East Java (Darsono *et al.,* 2018).

Some research has been developed to reduce SCMV incidence on sugarcane. The research which has been conducted to eliminate the SCMV virus consists of hot water treatment, meristem culture, and chemotherapy using antiviral ribavirin and acyclovir through somatic embryogenesis (Dewanti *et al.,* 2015). Ribavirin and acyclovir application on sugarcane explants are capable to eliminate the SCMV virus and have a 100% success rate, but when these antiviral applicated in the free-virus sugarcane field, the plant still had a risk to be infected by the SCMV virus (Apriasti *et al.,* 2018). There is a need to conduct research that has the aim to improve sugarcane resistance toward the SCMV virus. Some research reported that the SCMV attack on sugarcane cultivation can be solved using the genetic transformation method. Genetic transformation can be conducted by inserting a gene that can be improved plant endurance toward SCMV attack, such as SCMV-CP. This gene can be stably expressed when transformed into the genome of the Badila cultivar. Genetic transformation methods that can be used to insert these genes are RNA silencing through RNA interference (RNAi) and Pathogen Derived Resistance (PDR) method (Yao *et al.,* 2017). RNA interference (RNAi) is a post-transcriptional molecular mechanism initiated by double-stranded RNA (ds-RNA), which is the 12-24th homology nucleotide sequence of the gene that its expression is suppressed. RNA target will be recognized as broken RNA and will be degraded so it cannot be expressed into protein (Aslam *et al.,* 2018). Pathogen Derived Resistance (PDR) is a molecular mechanism that disrupts pathogen expression in host plants. PDR through coat-protein virus expression is reported to have the capability on inhibited the disassembly process of viral particles that infected the cell, so it can suppress the infection process and the movement of viruses in the host plant (Baulcomlbe, 1996). Currently, transgenic sugarcane has been obtained using the PDR (Pathogen derived resistance) technique that expresses SCMV-coat protein using the plasmid vectors pRION-927 bp (p927) and pRION-702 bp (p702) on *Agrobacterium tumefaciens* so that the sugarcane is resistant to virus attack (Apriasti *et al*., 2018). The sugarcane has been able to be planted in a greenhouse, however, in the field it has never been studied, so it is necessary to carry out continuous research.

Materials and Methods

Research location

This research is located in Agrotechnopark research station, University of Jember, Jember Regency, East Java on the lowland with an altitude of ± 76 m above sea level. The type of soil was regosol.

Method

The research was conducted from the beginning of the rainy season in December 2018 to the end of the dry season in October 2020. This research was conducted twice, The first was non-GMO and GMO mule sugarcane which took place from the early rainy season in December 2018 until the end of the dry season in October 2019 and continued with sugarcane yields from ratooning that has been grown until the end of the dry season on October 2020.

This research was conducted using two types of sugarcane, Non-Transgenic (NT) and Transgenic Pathogen Derived Resistance (PDR). Non-Transgenic (NT sugarcane used bululawang varieties and transgenic sugarcane used transgenic sugarcane produced from Apriasti *et al.* (2018) research, consist of B10.1,

B11.1, B10.2, B10.3, B11.3. Transgenic and Non Transgenic sugarcane was planted randomly in 4 blocks, each block had 6 plots consisting of 3 segments with 4 meters in length.

Viral infection on sugarcane infected using SAP method into 6 weeks plant (Apriasti *et al.,* 2018)

Observations were carried out on sugarcane growth variables, consisting of the number of plants, plant height, stalk diameter, middle internodes length, Brix, production weight (SAS Institute, 1996). The incidence of SCMV attacks was observed visually by inspection on the symptoms then followed by a PCR test. The results were statistically evaluated by Dunnett's test and t-test at $p < 0.05$.

Results

1.Trangenic sugarcane resistance towards SCMV infection

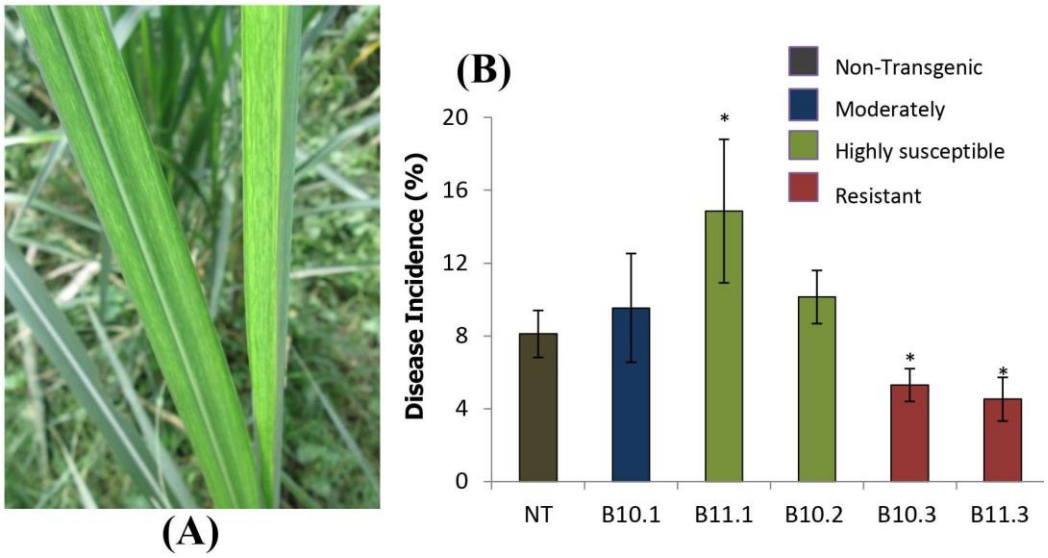


Figure 1. Figure 1. Gejala mosaic (A), dan insidensi penyakit pada tebu transgenik (B). NT: non-trangenic; B10.1, B11.1, B10.2, B10.3, B11.3: transgenic sugarcane. Values are means ±SD for four replication. Asterisks denote statistically significant differences (Dunnett's test: p≤0.05).

The resistance evaluation of transgenic was conducted by SCMV inoculation from PS864 symptomatic plant using a mechanical method on the second and third leaves from the top that has been fully open on 6 weeks after planting (Apriasti *et al.,* 2018). Based on the previous research, the incidence of disease was observed by measuring mosaic symptom percentage on the 1st and the 2nd leave area from the top of 3 months plant (Addy *et al.,* 2017). The result showed that the percentage of SCMV attacks online B10.3 and B11.3 were lower and significantly different compared to the NT plant. The response of lines B10.1, B11.1, and B10.2 towards SCMV infection was not significantly different if compared to the NT plant. This result was following the previous research that reported lines B10.3 and B11.3 were classified as resistant lines

(Hidayati *et al.,* 2021). Line B10.1 was classified as a moderate line, while B11.1 and B10. 2 were classified as highly susceptible lines toward SCMV attack if it was compared to the NT plant.

2.The detection of SCMV infection on symptomatic sugarcane leaves

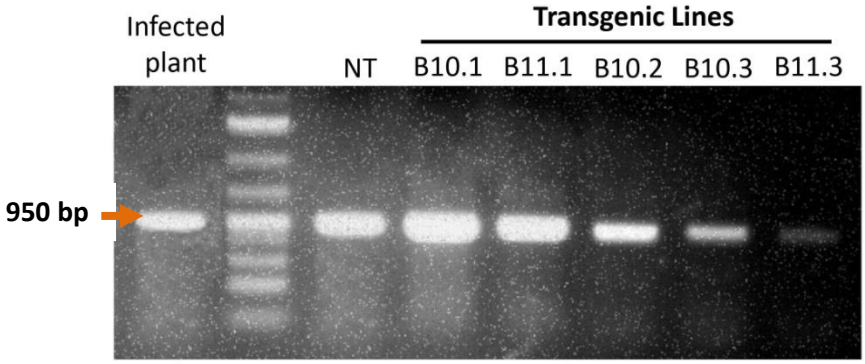


Figure 2. Diagnose of causative mosaic in symptomatic sugarcane leaf. RT-PCR result producing a single band with a size of 950 bp responsible for coat protein of SCMV. NT: non-trangenic; B10.1, B11.1, B10.2, B10.3, B11.3: transgenic sugarcane.

Mosaic symptom on sugarcane was indicated by yellowish and chlorosis leaves (Addy *et al.,* 2017), that caused by many viral agents in the plant included Sugarcane Streak Mosaic Virus (SCSMV), Sugarcane Mosaic Virus (SCMV), and Sorghum Mosaic Virus (SRMV) (Xu *et al.,* 2008). Therefore, molecular analysis was conducted to identify the causative viral agent that caused mosaic symptoms on transgenic sugarcane. The detection of SCMV infection after inoculation was conducted on transgenic sugarcane, NT sugarcane, and Infected sugarcane on PS864 varieties. The symptomatic leaves collected for RNA isolation and RT-PCR analysis for *SCMV-CP* gene detection with the fragment length of DNA was 950 bp using *SCMV-CP* primer pair. The amplification result of DNA showed that *the SCMV-CP* gene has detected in all symptomatic sugarcane, so the viral agent that infected all sugarcane lines was SCMV.

3.The evaluation of transgenic sugarcane agronomic character

Tabel 1. Agronomi traits of transgenic sugarcane at 9 month after planting.

ine	italk umber	Plant height cm)	italk diameter cm)	nternode ength (cm)	Cane yield ton/ha)	Brix(%)
NT	.09.25 d	92.43 d	2.45 d	13.98 c	96.81 c	13.37 b
B10.1	.27.00 b	300.88 bc	2.92 b	15.88 b	109.06 b	14.39 ab
B11.1	.12.25 d	97.75 bcd	2.80 c	15.39 b	77.56 d	13.71 b
B10.2	.19.50 c	99.30 cd	2.85 bc	15.60 b	93.44 c	14.30 ab
B10.3	.43.25 a	306.13 b	3.20 a	17.23 a	113.13 b	14.97 a
B11.3	.31.00 b	323.08 a	2.93 b	17.29 a	123.13 a	15.04 a

Sugarcane response toward SCMV showed mosaic symptoms on the leaves due to the virus capability on destroyed the chloroplast, inhibit photosynthesis, and decrease sucrose and production per hectare (Chauhan *et al.,* 2015). SCMV infection treatment conducted using the sap method, which was isolated from infected sugarcane, resulted that mosaic symptoms were found on some leaves especially the first and second leave, this result was in accordance with the observation that has been conducted by Addy *et al.* (2017). The agronomic observation was conducted on 9 months after planting the plant to observe stalk number, plant height, stalk diameter, internode length, stalk weight, and Brix to find out SCMV infection impact on plant growth and transgenic sugarcane production. The observation resulted that tiller total in line B10.1, B10.2, B10.3, and B11.3 were higher than NT plant. Plant height performance on transgenic sugarcane was increased compared to NT plant on line B10.1, B10.3, and B11.3. All transgenic line produced stalk diameter and internode length higher than NT plant. The combination between stalk number, plant height, and stalk diameter, affected the sugarcane productivity. Therefore, transgenic plants on B10.1, B10.2, B10.3, and B11.3, produced higher stalk weight than NT plants. Photosynthesis that has been blocked by the SCMV virus inhibited substrate production in sucrose formation. Brix measurement resulted that transgenic sugarcane line that classified as resistant plant resulted higher Brix and significantly different than NT plant. Agronomic character observation explained that growth and production inhibition on susceptible transgenic line and NT plant, while resistant transgenic plant had normal growth performance after infected by SCMV.

Discussion

The mosaic symptom caused by viral infection was infected by environment, cultivar, growth stage on the infected plant (Rao *et al.,* 2006). Sugarcane at 1-2 months was very susceptible to SCMV infection with infection percentage reaching 80% in 15 days incubation (Balamuralikrishnan *et al.,* 2003). SCMV incubation in sugarcane until the symptom appear had a 4-15 day period after inoculation (Gemechu *et al.,* 2006). This research aimed to test the stability of endurance character on the 4th generation of transgenic sugarcane and its effect on sugarcane growth and production. SCMV inoculation was conducted on 1,5-month transgenic sugarcane and incubated until 45 dpi to reach clear symptoms on the 1st and 2nd new leave, after the leaves rolled, in accordance with the previous research (Hidayati *et al.,* 2021). The transgenic line that has been used in this research was chosen based on its endurance toward SCMV infection and classified into three groups, B10.1 (moderate), B11.1 and B10 (Highly susceptible), B10.3 and B11.3 (resistant) (Hidayati *et al.,* 2021). Symptom observation resulted that B10.3 and B11.3 were more resistant than the others with low incidence, 4% and 5% respectively. SCMV incidence in line B11.1 was higher than NT plant with an incidence percentage was 14% (Figure 1B). This result was in accordance with the endurance test in the previous generation (Apriasti *et al.* 2018; Hidayati *et al.* 2021) and the resistance character of transgenic sugarcane to SCMV virus was inherited to the next generation (the 4th generationThe Monocotyledonae plant with vegetative propagation produced stable expression in the fourth or fifth generation (Bettany et al. 1998), while the phenotypic expression in sugarcane remained stable in the third generation. The success of assembling transgenic

plants was determined based on the uniformity and stability of gene expression and characters introduced in natural environmental conditions (Joyce *et al*., 2014; Yao *et al*., 2017).

The resistance of transgenic sugarcane was confirmed by molecular analysis using the RT-PCR method with a capsid protein-based gene targeting to explain the symptoms caused by SCMV. . Capsid protein-based gene was often used for confirming the causative pathogen of mosaic in sugarcane (Haider *et al*. 2011; Darsono *et al*. 2018). The DNA fragment of capsid protein with a length of about 950 bp has been successfully amplified in all sugarcane lines, proving that the symptom that appeared on the leave was caused by SCMV. The appearance of mosaic symptoms on sugarcane leaves caused by SCMV infection was indicated by bright green or yellowish-green, especially on the leaf bones, resembling symptoms of nutritional deficiency, especially nitrogen, lack of water, accompanied by chlorosis that spreads to the entire leaf surface (Sholeh *et al.,* 2019). Specific molecular analysis was capable to detect the possible presence of specific viral RNA replication in infected plant tissues with specific primer pairs for viral capsid protein (Xu *et al.,* 2008; Addy *et al.,* 2017). Therefore, the RT-PCR method with a target capsid protein-based gene becomes an effective tool to diagnose viruses that cause mosaic symptoms in sugarcane leaves.

SCMV virus infection in plants reduced chlorophyll content due to changes in chloroplast structure and chlorophyll synthesis (Pazarlar *et al.,* 2013; Zhao *et al.,* 2016), causing a decrease in photosynthesis product (Pan *et al.* 2001; Addy *et al*. 2017). SCMV attack on sugarcane caused the inhibition on growth (viswanathan and Balamuralikrishnan, 2005; Bagyalakshmi et al. 2019), producing shorter internodes, shorter stalk height, reduce stalk yield and sucrose content (Singh *et al.,* 2003; Yao *et al.,* 2017). In addition, SCMV infection reduces the activity of the enzyme Sucrose Phosphate Synthase, which plays a role in the synthesis and accumulation of sucrose, which affects the growth and sugarcane yield (Addy *et al.,* 2017). This research showed that line B10.3 and B11.3 had better growth performance compared to the other transgenic line, Cane yield and sucrose content were also high and concluded that this line was resistant to SCMV infection and had genetic stability. On the other hand, the hight incidence percentage in line B11.1 and B10.2 did not affect the growth and production. The possibility of recovery mechanism from SCMV infection occurred in transgenic sugarcane lines B11.1 and B10.2 during the grand growth phase after infection resulted a normal growth performance. Incidence observation in transgenic sugarcane was conducted in 3-month sugarcane on the first and second leaves. The possibility of recovery mechanism in the line B11.1 and B10.2 during the grand growth phase produced a similar growth performance with moderate line and NT plants at lower incidence. The recovery phase of the infected plant can occur even when the symptom still appeared in the infected tissue, while the next plant development will happen normally. During the fast development phase, The DNA of the virus decreased in the infected tissue due to the viral particle migration to the new tissue near epical tissue, so the viral replication decreased in the infected tissue. Furthermore, the viral DNA remained in the new tissue but did not indicate any symptoms because the virus escaped from the plant resistance mechanism to maintain the subliminal (Carrillo-Tripp *et al.,* 2006). The recovery mechanism in transgenic sugarcane using pathogen-derived resistance approaches need to be proved with experimental research.

References

Addy, H.S., Nurmalasari, Wahyudi, A.H.S., Sholeh, A., Anugrah, C., Iriyanto, F.E.S., Darmanto, W., Sugiharto, B. 2017. Detection and Response of Sugarcane Against The Infection of Sugarcane Mozaic Virus (SCMV) in Indonesia, Agronomy, Vol 7 (50):1-11.

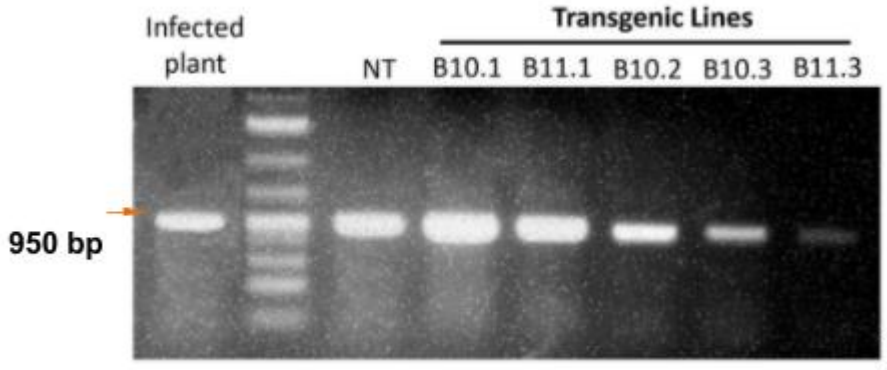
Aslam, U., Tabassum, B., Nasir, I.A., Khan, A., Husnian, T. 2018. A Virus-Derived Short Hairpin RNA Confers Resistance Against Sugarcane Mosaic Virus in Transgenic Sugarcane, Transgenic Research. Vol 27:203-210

Balamuralikrishnan, M., Doraisamy, S., Ganapathy, T. and Viswanathan, R., 2003. Sugarcane mosaic virus infection progress in relation to age of sugarcane.

Baulcombe, D.C. 1996. Mechanism of Pathogen-Derived Resistance to Virus in Transgenic Plants. American Society of Plant Physiologists, Vol 8: 1833-1844.

Bettany, A.J., Dalton, S.J., Timms, E. and Morris, P., 1998. Stability of transgene expression during vegetative propagation of protoplast-derived tall fescue (Festuca arundinacea Schreb.) plants. Journal of experimental botany, 49(328), pp.1797-1804.

Carrillo-Tripp, J., Lozoya-Gloria, E. and Rivera-Bustamante, R.F., 2007. Symptom remission and specific resistance of pepper plants after infection by Pepper golden mosaic virus.



Phytopathology, 97(1), pp.51-59.

Chauhan, R.P., Rajakaruna, P. and Verchot, J., 2015. Complete genome sequence of nine isolates of canna yellow streak virus reveals its relationship to the sugarcane mosaic virus (SCMV) subgroup of potyviruses. Archives of virology, 160(3), pp.837-844.

Darsono, N., Azizah, N.N., Putranty, K.M., Astuti, N.T., Addy, H.S., Darmanto, W., Sugiharto, B. 2018. Production of a Polyclonal Antibody against the Recombinant Coat Protein of the Sugarcane Mosaic Virus and Its Application in the Immunodiagnostic of Sugarcane. Agronomy, Vol 8 (93): 2-13.

Dewanti, P., Widuri, L.I., Ainiyati,C., Okviandari, P., Maisaro, Sugiharto, B. 2016. Elimination of SCMV (Sugarcane Mozaik Virus) and Rapid Propagation of Virus-free Sugarcane (Saccharum officinarum L) Using Somatic Embryogenesis, Procedia Chemistry, Vol 18: 96-102.

Dirjenbun. (2020) 'Statistik Perkebunan Indonesia 2018-2020, Tebu', in Statistik Perkebunan Indonesia 2018-2020, pp. 1–68. Available at: www.ditjenbun.pertanian.go.id.

Haider, M.S., Afghan, S., Riaz, H.A.R.O.O.N., Tahir, M., Javed, M.A., Rashid, N.A.E.E.M. and Iqbal, J., 2011. Identification of two Sugarcane mosaic virus (SCMV) variants from naturally infected sugarcane crop in Pakistan. Pak. J. Bot, 43(2), pp.1157-1162.

Joyce, P., S. Hermann, A. O'Connell, Q. Dinh, L. Shumbe, and P. Lakshmanan. 2014. Field performance of transgenic sugarcane produced using Agrobacterium and biolistics methods. Plant Biotechnology Journal 12: 411–424.

Pan, D.R.; Ping, X.L.; Luo, J.; Ying, F.H. Improvement of photosynthetic characteristics and yield of Sugarcane mosaic virus-free chewing cane. J. Fujian Agric. For. Univ. 2001, 30, 320–323.

Pazarlar, S., Gümüş, M.U.S.T.A.F.A. and ÖZTEKİN, G.B., 2013. The effects of tobacco mosaic virus infection on growth and physiological parameters in some pepper varieties (Capsicum annuum L.). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 41(2), pp.427-433.

Putra, A.D., Sudiarso, dan Islami, T. 2013. Pengaruh Komposisi Media Tanam Pada Teknik Bud Chip Tiga Varietas Tebu (Saccharum officinarum L.). Jurnal Produksi Tanam, Vol 1 (1):16-23

Rao, G.R.; Chatenet, M.; Girard, J.G.; Rott, P. Distribution of Sugarcane mosaic and Sugarcane streak mosaic virus in India. Sugar Tech 2006, 8, 79–81.

SAS Institute Inc (1996). Forecasting Examples for Business and Economics Using the SAS System. Cary, NC: SAS Institute Inc.

Sholeh, A., Sugiharto, B. and Addy, H.S., 2019. Monitoring Sugarcane mosaic virus (SCMV) on recent sugarcane varieties in East Java, Indonesia.

Singh, V.; Sinha, O.K.; Kumar, R. Progressive decline in yield and quality of sugarcane due to Sugarcane mosaic virus. Indian Phytopathol. 2003, 56, 500–502.

Viswanathan, R.; Balamuralikrishnan, M. Impact of mosaic infection on growth and yield of sugarcane. Sugar Tech 2005, 7, 61–65.

Xu, D.L., Park, J.W., Mirkov, T.E. and Zhou, G.H., 2008. Viruses causing mosaic disease in sugarcane and their genetic diversity in southern China. Archives of Virology, 153(6), pp.1031-1039.

Yao, W., M. Ruan, L. Qin, C. Yang, R. Chen, B. Chen, and M. Zhang. 2017. Field Performance of Transgenic Sugarcane Lines Resistant to Sugarcane Mosaic Virus. Frontiers in Plant Science 8 (104): 1-9.

Zhao, J.; Zhang, X.; Hong, Y.; Liu, Y. Chloroplast in plant-virus interaction. Front Microbiol. 2016, 7, 1565.