Published with Open Access at Journal BiNET
Vol. 20, Issue 01: 1664-1670
Journal of Bioscience and Agriculture Research
Journal Home: www.journalbinet.com/jbar-journal.html

Identification of beta satellite component in (*Hibiscus rosa-sinensis* L.) through PCR-RFLP from Faisalabad, Pakistan

Shahab Habib[1], Luqman Amrao[1], Muhammad Zeshan Ahmed[1], Maaz Ahmad[1], Shanam Naz[1], Muhammad Ahmad Zeshan[4], Hafiz Muhammad Asadullah[2], Abdul Manan Hamza[3] and Salman Ghuffar[1]

[1]Dept. of Plant Pathology, University of Agriculture Faisalabad, Pakistan
[2]Pulses Research Program, NARC Islamabad, Pakistan
[3]Dept. of Entomology, PMAS-Arid Agriculture University, Rawalpinidi, Pakistan
[4]Dept. of Plant Pathology, University of Sargodha, Sargodha, Pakistan.

✉ For any information: ask.author@journalbinet.com
Article received: 14.01.19; Revised: 04.02.19; First published online: 14 April 2019.

ABSTRACT

Shoe flower (Hibiscus rosa-sinensis L.*) is an ornamental plant belongs to the family Malvaceae. Due to its natural beauty and medicinal properties it is mainly grown in regions with tropical and subtropical environment. Shoe flower is known to infect by many diseases. Among all diseases Hibiscus leaf curl disease is caused by the begomovirus transmitted through Bemisia tabaci is very common and also an alternative host of cotton leaf curl disease due to the similar symptomology. In present study the identification of begomovirus in shoe flower was analyzed by using PCR-RFLP (Polymerase chain reaction- Restriction fragment length polymorphism). For this purpose, survey was conducted from seven different locations in university of agriculture Faisalabad. Plants were observed with virus symptoms of Hibiscus leaf curl disease such as upward curling of leaves, darkening and thickening of veins, stunting of plant size as well as leaf enation. For further molecular studies, DNA was isolated from all infected samples by using CTAB method and confirmed by Gel electrophoresis. PCR reaction amplified three DNA samples having beta satellite component, labeled (S3, S4 and S6) at 1.4 kbp. According to the (PCR-RFLP) analysis of three amplified samples, In BamH1 restriction enzyme, all the samples were restricted into 625 bp. In second restriction enzyme Pst1, DNA samples were restricted into 1.4 kbp. While in the case of ECOR1, fragment was restricted into 1.3 kbp.*

Key Words*: Shoe flower, DNA extraction, PCR, PFLP, Beta satellite and Begomovirus*

Cite Article: Habib, S., Amrao, L., Ahmed, M. Z., Ahmad, M., Naz, S., Zeshan, M. A., Asadullah, H. M., Hamza, A. M. and Ghuffar, S. (2019). Identification of beta satellite component in (*Hibiscus rosa-sinensis* L.) through PCR-RFLP from Faisalabad, Pakistan. Journal of Bioscience and Agriculture Research, 20(01), 1664-1670. **Crossref:** https://doi.org/10.18801/jbar.200119.202

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2019 The Authors, Research paper

I. Introduction

Shoe flower (*Hibiscus rosa-sinensis* L: family: *Malvaceae*) also known as hibiscus and China rose. It is mostly common in regions with such tropic and sub- tropic climate. Besides its natural beauty, shoe flower has also owns medicinal properties that are helpful for soothing of mucous membranes of the respiratory and digestive tracts (Sharma and Sultana, 2004). There are certain types of compounds present in Shoe flowers such as proantho cyanidins and flavonoids which are useful in spasmolytic (spasm inhibiting), analgesic (pain relieving) and good antipyretic (Rummel 2005). Shoe flower is known to infect many diseases due to several pathogens such as virus, bacteria and fungi. Among these shoe flower leaf curl disease due to viral attack is significantly common in tropical and sub-tropical areas. Advance technique to improve performance of flowering and other plants reported by several authors (Sultana et al. 2011; Siddique et al. 2007; Siddique et al. 2006; Islam et al. 2004) Due to this disease many physical changes occur in plants like leaf curling, leaf enation, darkening and thickening of veins (Lolita et al. 2014). It was first in central India (Mali 1980). Among all the families of Viruses, *Geminiviridae* is one of the major threats and causes serious problem for agriculture like cotton leaf curl disease, maize streak disease, tomato leaf curl disease and Cassava mosaic especially in tropic and sub-tropic regions (Varma and Malathi, 2003). In *Geminiviridae,* a genus begomoviruses are transmitted through white fly in persistence manner (Brown et al. 2012). Begomoviruses are the cause of this disease incident which transmission by whitefly, which indicated that cause of this disease, is begomovirus, further evidence obtained when shoe flower was found as natural or alternate host of begomovirus, and in Pakistan cotton leaf curl virus (Harrison et al. 1997). Briddon et al. (2001) studied the structure of the begomoviruses has ssDNA, typically bipartite and genomes containing all functions for its replication, it consists of two component DNA-A and DNA-B. The first component encoded to control expression of gene and second component for inter and intra-cellular movement genes. It also contains two types of satellite virus named as alpha satellite and beta satellite the first one is not involved in viral infection (Idris et al. 2011). Beta satellite plays an important role in disease symptoms because these viruses encoded specific types of virulence factors, which enhance the systemic spread of the virus characters (Briddon et al. 2003). In beta satellite βC1 gene is present in CLCuV and also responsible for disease symptoms induction (Qazi et al. 2007). Keeping in view the present study was expected to attain the objective of identification of beta satellite component through PCR-RFLP in shoe flower (*Hibiscusrosa-sinensis* L.) in Pakistan.

II. Materials and Methods

Collection of diseased samples

Identification of disease was done on the basis of symptomology such as upward curling of leaves, enation on lower sides of leaves, vein thickening and darkening, stunting the plants and Reduction of number of flowers. Infected young leaves were collected from seven different places in University of Agriculture Faisalabad (U.A.F). Samples were labeled as S1, S2, S3, S4, S5, S6 and S7 carefully placed them in ice box and those were brought to virology lab in Centre of Agricultural Biochemistry and Biotechnology (CABB) department and were stored at 80^0C in a freezer for further studies.

Isolation of total genomic DNA

After sample collection, according to (Doyle and Doyle, 1990) the total genomic DNA was isolated by using CTAB method. For removal of the sticky impurities in genomic DNA phenol- chloroform treatment was performed described by (Chomczynski and Sacchi, 2006).

Polymerase chain reaction (PCR) for Beta Amplification

PCR is used for the amplification of DNA followed by (Sunter et al. 1990). In PCR, universal primers were used for beta satellite component amplification named as beta 01 and beta 02.

Restriction fragment length polymorphism (RFLP)

PCR-RFLP analysis was done according to (Relman et al. 1996). At the end, samples were run at 1% agarose gel electrophorisis for 45 minutes for visualization.

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2019 The Authors, Research paper

III. Results and Discussion

Finally, the result illustrated that Shoe flower is the alternative host of begomo virus and closely matches with cotton leaf curl disease such as curling of leaf, thickening and darkening of veins, and leaf enation. Occasionally, Samples were collected on the basis of these symptoms shows in figure 01. Same results are also described by (Briddon and Markham, 2000) that is HLCuD is transmitted by white fly is a common vector of both HLCuD and CLCuD and also has similar symptomology.

Figure 01. Curling of leaves.

The DNA was extracted from seven samples exhibit the typical symptoms of shoe flower curl virus. Conformation of total DNA by gel picture as shown in Figure 02.

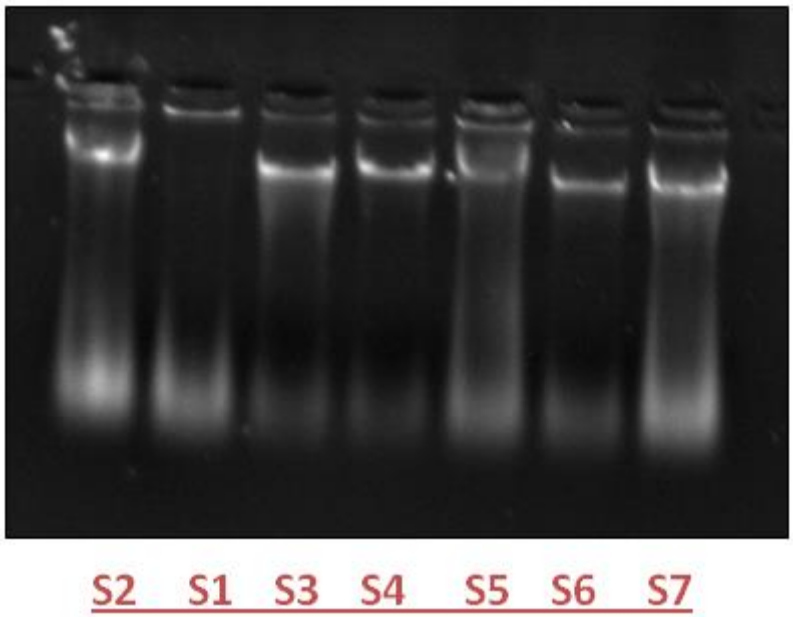

Figure 02. Gel picture of total genomic DNA.

Polymerase chain reaction (PCR) for beta satellite amplification

Two universal primers such as β01 (CCGGTACCACTACGCTACGCAGCAGCC) and β02 (CCGGTACCTACCCTCCAGGGGTACAC) were used for PCR amplification of beta satellite viruses. While

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2019 The Authors, Research paper

beta satellite of CLCuV was used as positive control. Three samples of beta satellite such as S3, S4 and S6 shown in figure 03. In this figure by the comparing with 1.4 kb marker clearly shows that my PCR results were efficient. According to (Briddon et al. 2002) used extracted DNA from infected plant for amplification of beta satellites through PCR with the help of several universal primers β01 and β02 amplified the DNA at 1400 nucleotides bp. He also mentioned the annealing temperature for amplification of beta satellite component in the tomato and cotton plant is 50^{0}C and 57^{0}C.

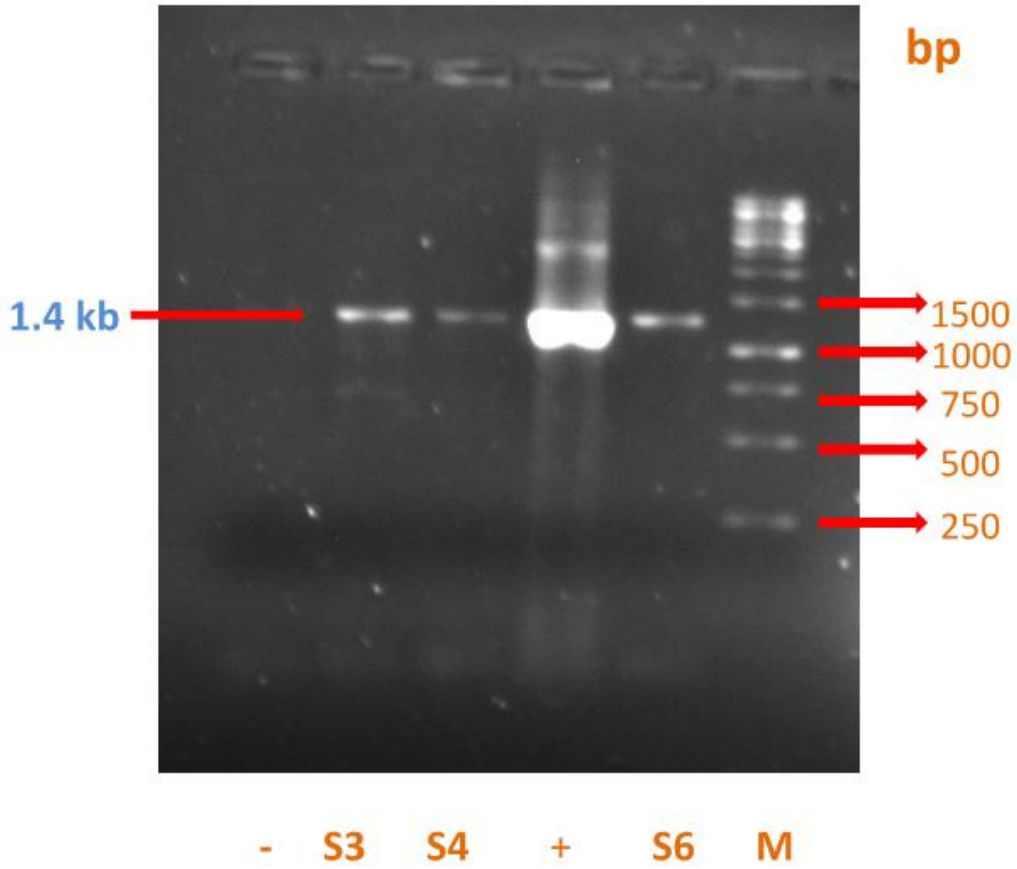

Figure 03. Gel picture of Polymerase chain reaction (PCR).

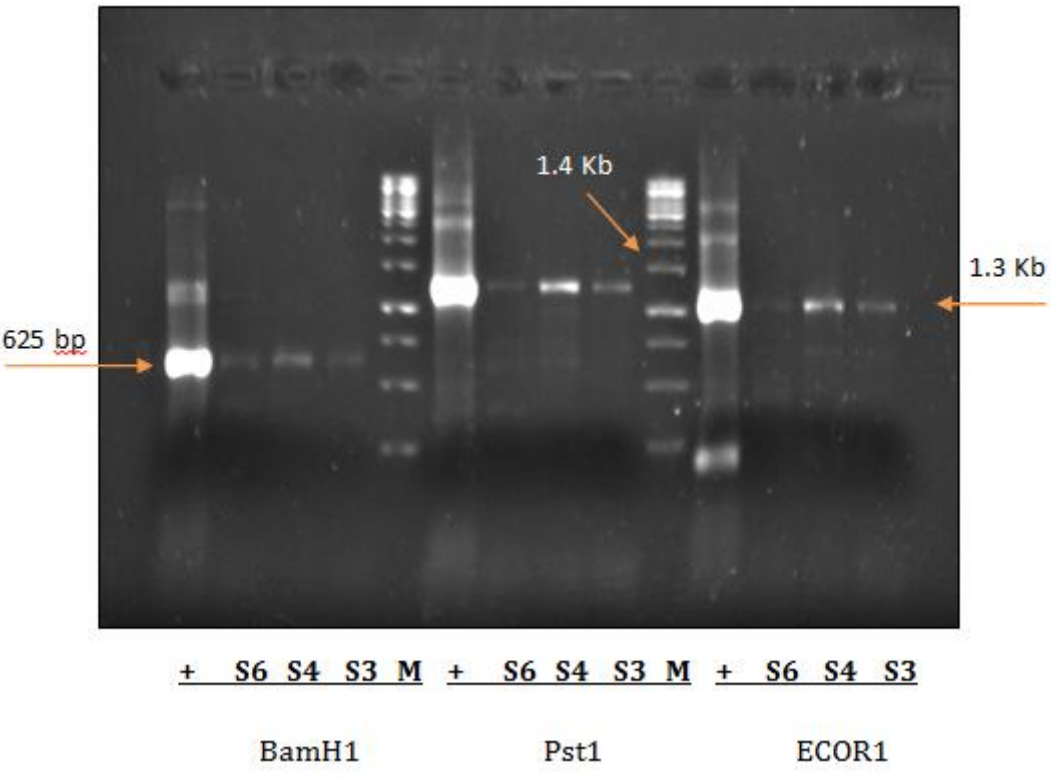

Figure 04. Restriction analyses in Gel Electrophoresis.

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2019 The Authors, Research paper

Restriction fragment length polymorphism (RFLP) analysis

For PCR- RFLP analysis, PCR amplified samples such as S3, S4 and S6 were restricted with common restriction enzymes named as (Pst1, BamH1 and EcoR1). Result showed that the diversity of beta satellite virus was presents in infected shoe flower plant with restriction enzyme Pst1 and BamH1 and ECOR I. Therefore, the results showed that during PCR-RFLP analysis there are different types of beta satellites responsible for prevailing in shoe flower infected with HLCuD as shown in Figure 04. In figure CLCuV was used as a positive control. Similar work was done by (Bertaccini et al. 2000) about PCR-RFLP analysis by using specific restriction, TaqI, TruI and AluI to identify the beta satellite viruses DNA infected by HLCuD.

IV. Conclusion

DNA was extracted from seven infected samples and confirmed by gel electrophoresis. Out of seven three DNA samples were amplified by PCR reaction having beta satellite component (S3, S4 and S6) at 1.4 Kbp. All three amplified samples were restricted by three different restriction enzymes (Bam H1, ECOR1 and Pst1) at different bp length.

Author Contribution

Authors SH, LA, HMS, AMH, SG developed the idea of this study, perform literature search, collected data and conducted experimental works. Authors MZA, MAZ, MA, SN performed statistical analysis and prepared the manuscript for submission. All authors read and approved the final manuscript.

References

[1]. Bertaccini, A. L., Carraro, D., Davies, D. A., Laimer, M., Camara, M., Martini, M., Paltrinieri, S. and Seemüller, E. (2000). Micropropagation of a collection of phytoplasma strains in periwinkle and other host plants, p. 101. In: 13thInternational Congress of IOM, July 14-19, Fukuoka, Japan.

[2]. Briddon, R. W. and Markham, P. G. (2000). Cotton leaf curls virus disease. Virus Research 71, 151-159. https://doi.org/10.1016/S0168-1702(00)00195-7

[3]. Briddon, R. W., Bull, S. E. Amin, I., Idris, A. M., Mansoor, S. I., Bedford, D., Dhawan, P., Rishi, N., Siwatch, S. S., Abdel-Salam, A. M., Brown, J. K., Zafar, Y. and Markham, P. G. (2002). Diversity of DNA β: a satellite molecule associated with some monopartite begomoviruses. Virol. 312, 106-121. https://doi.org/10.1016/S0042-6822(03)00200-9

[4]. Briddon, R. W., Mansoor, S. I., Bedford, D., Pinner, M. S., Saunders, K., Stanley, J., Zafar, Y., Malik, K. A. and Markham, P. G. (2003). Identification of DNA components required for induction of cotton leaf curl disease. Virol. 285, 234-243. https://doi.org/10.1006/viro.2001.0949

[5]. Brown, J. K., Fauquet, C. M., Briddon, R. W., Zerbini, F. M., Moriones, E. and Navas-Castillo, J. (2012). Family *Geminiviridae*. In Virus Taxonomy 9th Report of the International Committee on Taxonomy of Viruses, pp. 351–373. Edited by A. M. Q. King, M. J. Adams, E. B. Carstens and E. J. Lefkowitz. London, UK: Elsevier Academic Press.

[6]. Chomczynski, P. and Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on Nature Protocols 1, 581-585.

[7]. Doyle, J. J. and Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. Focus 12, 13-15.

[8]. Harrison, B. D., Liu, Y. L., Khalid, S., Hameed, S., Otim-Nape, G. W. and Robinson, D. J. (1997). Detection and relationships of cotton leaf curl virus and allied whitefly-transmitted geminiviruses occurring in Pakistan. Annals of Applied Biol. 130, 61–75. https://doi.org/10.1111/j.1744-7348.1997.tb05783.x

[9]. Idris, A. M., Shahid, M. S., Briddon, R. W., Khan, A. J., Zhu, J. K. and Brown, J. K. (2011). An unusual alpha satellite associated with monopartite begomoviruses attenuates symptoms and reduces beta satellite accumulation. J. Gen. Virol. 92, 706–717. https://doi.org/10.1099/vir.0.025288-0

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2019 The Authors, Research paper

[10]. Islam, M. M., Adhikary, S. K., Gain, P., Rahman, M. M. and Siddique, M. N. A. (2004). Effect of plant growth regulators on callus induction and plant regeneration in another culture of rice. Pakistan J. Biol. Sci., 7, 331-334. https://doi.org/10.3923/pjbs.2004.331.334

[11]. Lolita, M., Dolores, M., Gonzales, C. and Magdalita, P. M. (2014). Isolation and identification of a begomovirus associated with the leaf curl disease of Gumamela (*Hibiscus rosa-sinensis*) in the Philippine. Asian journal of agriculture and food sciences 2, 566-576.

[12]. Mali, V.R. (1980). Studies on leaf curl disease of China rose in Marathwada. Indian Journal of Mycol and Plant Pathol. 9, 119.

[13]. Mansoor, S., Khan, S. H., Bashir, A., Saeed, M., Zafar, Y., Malik, K. A., Briddon, R. W., Stanley, J. and Markham, P. G. (1999). Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. Virol. 259, 190-199. https://doi.org/10.1006/viro.1999.9766

[14]. Qazi, J., Amin, I., Mansoor, S., Iqbal, J. and Briddon, R. W. (2007). Contribution of the satellite encoded gene βC1 to cotton leaf curl disease symptoms. Virus Research128, 135-139. https://doi.org/10.1016/j.virusres.2007.04.002

[15]. Rajeshwar, R. R., Reddy, V. C., Maruthi, M., Colvin, N. J., Seal, S. E. and Muniyappa, V. (2005). Host range, vector relationships and sequence comparison of a begomovirus infecting hibiscus in India. Ann Appl Biol. 147, 15–25. https://doi.org/10.1111/j.1744-7348.2005.00005.x

[16]. Relman, D. A., Schmidt, T. M., Gajadhar, A., Sogin, M., Cross, J., Yoder, K., Sethabutr, O. and Echeverria, P. (1996). Molecular phylogenetic analysis of Cyclospora, the human intestinal pathogen, suggests that it is closely related to Eimeria species. J. Infect. Dis. 173, 440-445. https://doi.org/10.1093/infdis/173.2.440

[17]. Rummel, D. J. (2005). Botanical beauty book compendium of cosmetic uses. C and E Publishing, Inc. Quezon City. Philippines. p. 433.

[18]. Sharma, S. and Sultana, S. (2004). Effect of *Hibiscus rosa-sinensis* extract on hyper proliferation and oxidative damage caused by benzoyl peroxide and ultraviolet radiation in mouse skin. Basic Clin Pharmacol Toxicol 95(5), 220-225. https://doi.org/10.1111/j.1742-7843.2004.pto950504.x

[19]. Sunter, G., Hartitz, M. D., Hormuzdi, S. G., Brough, C. L. and Bisaro, D. M. (1990). Genetic analysis of tomato golden mosaic virus: ORF AL2 is required for coat protein accumulation while ORF AL3 is necessary for efficient DNA replication. Virol. 179, 69-77. https://doi.org/10.1016/0042-6822(90)90275-V

[20]. Sultana, J., Sutlana, N., Siddique, M. N. A., Islam, A. K. M. A., Hossain, M. M. and Hossain, T. (2011). In vitro bulb production in Hippeastrum (Hippeastrum hybridum). Journal of Central European Agriculture, 11(4), 469-474. http://dx.doi.org/10.5513/jcea.v11i4.849

[21]. Siddique, M. N. A., Sultana, J., Sultana, N. and Hossain, M. (2007). Effect of planting dates on growth and flowering of Hippeastrum (*Hippeastrum hybridum*). Int. J. Sustain. Crop. Prod. 2(5), 12-14.

[22]. Siddique, M. N. A., Sultana, N., Haque, M. A., Hossain, M. M. and Ahmed, J. U. (2006). Effects of twin scale size and hormones on in vitro propagation of Hippeastrum (Hippeastrum hybridum). Plant Tissue Culture and Biotechnology, 16(2), 105-110. http://dx.doi.org/10.3329/ptcb.v16i2.1111

[23]. Varma, A. and Malathi, V. G. (2003). Emerging geminivirus problems: A serious threat to crop production. Annals of Applied Biology 142, 145-164. https://doi.org/10.1111/j.1744-7348.2003.tb00240.x

[24]. Vasudeva, R. S., Varma, P. M. and Capoor, S. P. (1953). Some whitefly (*Bemisia tabaci*) transmitted viruses in India. In: 6th International Congress of Microbiology, Rome 5, 520–521.

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2019 The Authors, Research paper

HOW TO CITE THIS ARTICLE?

Crossref: https://doi.org/10.18801/jbar.200119.202

MLA

Habib, et al. "Identification of beta satellite component in (*Hibiscus rosa-sinensis* L.) through PCR-RFLP from Faisalabad, Pakistan." Journal of Bioscience and Agriculture Research 20(01) (2019): 1664-1670.

APA

Habib, S., Amrao, L., Ahmed, M. Z., Ahmad, M., Naz, S., Zeshan, M. A., Asadullah, H. M., Hamza, A. M. and Ghuffar, S. (2019). Identification of beta satellite component in (*Hibiscus rosa-sinensis* L.) through PCR-RFLP from Faisalabad, Pakistan. Journal of Bioscience and Agriculture Research, 20(01), 1664-1670.

Chicago

Habib, S., Amrao, L., Ahmed, M. Z., Ahmad, M., Naz, S., Zeshan, M. A., Asadullah, H. M., Hamza, A. M. and Ghuffar, S. "Identification of beta satellite component in (*Hibiscus rosa-sinensis* L.) through PCR-RFLP from Faisalabad, Pakistan." Journal of Bioscience and Agriculture Research 20(01) (2019): 1664-1670.

Harvard

Habib, S., Amrao, L., Ahmed, M. Z., Ahmad, M., Naz, S., Zeshan, M. A., Asadullah, H. M., Hamza, A. M. and Ghuffar, S. 2019. Identification of beta satellite component in (*Hibiscus rosa-sinensis* L.) through PCR-RFLP from Faisalabad, Pakistan. Journal of Bioscience and Agriculture Research, 20(01), pp. 1664-1670.

Vancouver

Habib S, Amrao L, Ahmed MZ, Ahmad M, Naz S, Zeshan MA, Asadullah HM, Hamza AM and Ghuffar S. Identification of beta satellite component in (*Hibiscus rosa-sinensis* L.) through PCR-RFLP from Faisalabad, Pakistan. Journal of Bioscience and Agriculture Research. 2019 April 20(01): 1664-1670.

Published with open access at journalbinet.com
EISSN: 2312-7945, © 2019 The Authors, Research paper