

**Detection and partial molecular characterization of a Badnavirus isolate in Brazil****Detecção e caracterização molecular parcial de um isolado de Badnavírus no Brasil**

DOI:10.34117/bjdv6n7-794

Recebimento dos originais: 03/06/2020

Aceitação para publicação: 29/07/2020

**Sílvia Letícia da Paz Maich**

Doutora em Fitossanidade pela Universidade Federal de Pelotas  
Instituição: Universidade Federal de Pelotas  
Endereço: Av. Eliseu Maciel, s/n, Capão do Leão - RS, Brasil  
E-mail: silviapm\_2012@hotmail.com

**Carolina Garcia Neves**

Mestre em Fitossanidade pela Universidade Federal de Pelotas  
Instituição: Universidade Federal de Pelotas  
Endereço: Av. Eliseu Maciel, s/n, Capão do Leão - RS, Brasil  
E-mail: carolinagarcianeves@hotmail.com

**Ismail Teodoro de Souza Júnior**

Doutor em Fitotecnia pela Universidade Federal do Rio Grande do Sul  
Instituição: Universidade Federal de Pelotas  
Endereço: Av. Eliseu Maciel, s/n, Capão do Leão - RS, Brasil  
E-mail: agrojunior1@yahoo.com.br

**Monique Bezerra Nascimento**

Doutora em Fitossanidade pela Universidade Federal de Pelotas  
Instituição: Universidade Federal de Pelotas  
Endereço: Av. Eliseu Maciel, s/n, Capão do Leão - RS, Brasil  
E-mail: moniqueb.nascimento@hotmail.com

**Fábio Nascimento da Silva**

Doutor em Fitopatologia pela Universidade Federal de Viçosa  
Instituição: Universidade do Estado de Santa Catarina  
Endereço: Avenida Luiz de Camões, 2090, Lages – SC, Brasil  
E-mail: fabio.silva@udesc.br

**Danielle Ribeiro de Barros**

Doutora em Fitopatologia pela Universidade Federal de Viçosa  
Instituição: Universidade Federal de Pelotas  
Endereço: Av. Eliseu Maciel, s/n, Capão do Leão - RS, Brasil  
E-mail: danrbarros@hotmail.com

**RESUMO**

A batata doce é um dos vegetais mais importantes do mundo, sendo uma das principais fontes de nutrientes em alguns países. Apesar de ser considerada uma planta resistente, hospeda vários

patógenos, principalmente vírus. Anteriormente, a maioria dos vírus relatados na colheita tinha um genoma composto por RNA, no entanto, nos últimos anos, os vírus de DNA se destacaram. Entre eles estão as espécies pertencentes ao gênero *Badnavirus* (família *Caulimoviridae*). Portanto, o objetivo do presente estudo foi identificar a presença de espécies virais pertencentes ao gênero *Badnavirus* em amostras de folhas de batata-doce coletadas nas principais regiões produtoras da cidade de Pelotas - RS. Através da amplificação por PCR, foi possível detectar 48 amostras positivas entre as 303 amostras testadas, utilizando iniciadores degenerados e específicos para espécies do gênero. Uma amostra foi escolhida e usada para análise com outros pares de iniciadores específicos, a fim de obter sequências genômicas. No entanto, não foi possível obter a sequência completa do genoma, apenas parcial. Os fragmentos gerados mostraram uma identidade entre 72 a 87% quando comparados ao genoma completo dos isolados SPV-A (FJ560943.2) e SPV-B (FJ560944.1), demonstrando a grande variabilidade existente entre eles. Além disso, serão realizados estudos para obter a sequência completa do genoma, para entender melhor a ação do vírus na planta.

**Palavras chave:** doenças infecciosas, *Caulimoviridae*, genoma parcial, variabilidade genética.

### ABSTRACT

Sweet potato is one of the most important vegetables in the world, being one of the main sources of nutrients in some countries. Despite being considered a resistant plant, it hosts several pathogens, mainly viruses. Previously, most viruses reported in the crop had a genome composed of RNA, however, in recent years DNA viruses have been highlighted. Among them are the species belonging to the genus *Badnavirus* (family *Caulimoviridae*). Therefore, the aim of the present study was to identify the presence of viral species belonging to the genus *Badnavirus* in sweet potato leaf samples collected in the main producing regions of the city of Pelotas - RS. Through the PCR amplification it was possible to detect 48 positive samples among the 303 samples tested, using degenerate and specific primers for species of the genus. A chosen sample used for analysis with other pairs of specific primers, in order to achieve genome sequences. However, it was not possible to obtain the complete genome sequence, only partial. The generated fragments showed an identity between 72 to 87% when compared to the complete genome of the isolates SPV-A (FJ560943.2) and SPV-B (FJ560944.1), thus demonstrating the great variability that exists between them. Further, studies will be carried out in order to obtain the complete sequence of the genome, to better understand the action of the virus on the plant.

**Keywords:** Infectious diseases, *Caulimoviridae*, Partial genome, Genetic variability.

## 1 INTRODUCTION

The sweet potato (*Ipomoea potatoes* (L.) Lam.) is a species belonging to the family *Convolvulaceae*, originally from Central and South America (LOEBENSTEIN & THOTTAPPILLY, 2009). It is a perennial species, but it is cultivated as an annual crop, standing out for being a rustic plant, which presents good adaptation, resistance to water deficit and does not require innumerable care with cultural treatments (MONTES et al., 2008; CLARK et al., 2012). Sweet potato is the seventh most important food crop in the world and has a major caloric contribution to the human diet (SHEKHAR et al., 2015). In Brazil, it is one of the main vegetables consumed by the Northeastern population (OLIVEIRA et al., 2008).

Despite considered a rustic plant, its method of propagation and the lack of proper management, facilitate the dissemination of pathogens and mainly the appearance of diseases of viral nature (VALVERDE et al., 2007). Due to the characteristic of vegetatively propagated, the occurrence of viral infection tends to worsen during successive crops, resulting in the degeneration of the culture (KROTH et al., 2004), as well as the formation of viral complexes that act in synergism, potentiating the disease (UNTIVEROS et al., 2007). There are more than 30 viruses reported infecting sweet potato crops (CLARK et al., 2012). The largest number of viral species identified in sweet potatoes belongs to the *Geminiviridae* and *Potyviridae* families. Since species belonging to the *Geminiviridae* and *Caulimoviridae* families represent more than half of the viruses with a DNA genome (CLARK et al., 2012; WYLIE et al., 2017; ZERBINI et al., 2017). Some species of the genus *Badnavirus* (family *Caulimoviridae*) may be widely distributed in sweet potatoes, as they have already been identified on all continents, but their impact on culture is still unknown (CLARK et al., 2012; KREUZE et al., 2017). This genus includes plant viruses that have the genome formed by double-stranded DNA (dsDNA), depending on the species, the size varies between 7.2 to 9.2 kb, with bacilliform particles of 120 - 150 nm in length and 30 nm in diameter (BHAT et al., 2016).

Badnaviruses are classified as pararetroviruses, that is, they have the ability to integrate into the genome of the host plant, and are called endogenous parametrovirus sequences (Endogenous Pararetroviral Sequences- EPRVs) (STAGINNUS et al., 2009). They infect a wide range of hosts, and in some cases can cause significant losses in productivity and product quality (BHAT et al., 2016). Thus, the aim of the present study was to carry out the detection and molecular characterization of Badnavirus infecting sweet potato samples collected in southern Brazil.

## 2 MATERIAL AND METHODS

This research was carried out from material stored at -20°C, previously analyzed for the presence of viruses with a genome composed of DNA (MAICH, 2015). The collection of 303 symptomatic and asymptomatic sweet potato samples was carried out in 2013 and 2014 in the main producing districts of Pelotas. Leaf samples from different cultivars were collected, as yellow and chicken feet, being the most common. The samples were processed the next day of collection in the field.

The extraction of total DNA was performed for all samples collected individually from leaf discs about 1 cm in diameter according to Doyle & Doyle (1987) and served as a template for the PCR amplification reactions. The remaining of the leaf samples were stored in an ultra-freezer at -80°C.

To detect badnavirus, PCR reactions were performed in a total volume of 25  $\mu$ l, containing 5  $\mu$ l of GoTaq®Flexi Buffer, 25 mM MgCl<sub>2</sub>, 10 mM dNTPs mixture, 10 mM of each primer, using the primers Bad-FP (5-ATGCCITTYGGIITIAARAAYGCICC-3) and Bad-RP (5-CCAYTTRCAIACISCICCCCAICC-3) (YANG et al., 2003), and 1U of GoTaq® Flexi DNA Polymerase, completing the volume with water.

The reaction consisted of an initial denaturation of 94 ° C for 4 min, and then 35 cycles of the denaturation at 94 ° C for 30 s, pairing at 50 ° C for 30 s and extension at 72 ° C for 30 s, followed by a final extension at 72 ° C for 10 s min. The amplified products was analyzed by agarose gel electrophoresis (1% w/v), stained with GelRed™ (Biotium) and visualized through the L-Pix EX photo-documentation system.

Samples that showed fragments from amplification with the Bad-FP and Bad-RP primers were subjected to analysis by a second pair of primers, obtained from the Sweet potato virus B (SPBV-B) sequence, BadnaBKF (5 -CAAATTAGGAGGCAGATAAATG-3) and BadnaBSR (5-GGTCTTCTTATGTTCCACCTT-3) (MBANZIBWA et al., 2011). The reaction consisted of an initial denaturation of 94 ° C for 4 min, and then 35 cycles of the denaturation at 94 ° C for 60 s, pairing at 47 ° C for 60s and extension at 72 ° C for 60 s, followed by a final extension at 72 ° C for 10 s min. The amplified product was analyzed by agarose gel electrophoresis (1% w / v), stained with GelRed™ and visualized through the L-Pix EX photo-documentation system.

Subsequently, in order to sequence the viral genome, a sample was selected for amplification with specific primers for Badnavirus A and B obtained by Kreuze and collaborators (2009) (KREUZE et al., 2009). It was also necessary to design primers based on the sequences obtained previously to try to complete the genome (Table 1). The amplification products obtained were purified using the Wizard®SV Gel and PCR Clean-Up System kit (Promega), and cloned into the vector pGEM®-T Easy Vector System I (Promega).

**Table 1** – List of primers used in the study.

Primer	Sequence	Reference
SPBADNA2 1750F	5'-TCGAGGAATGGTAGGAAGATTATC-3'	Kreuze <i>et al.</i> , 2009
SPBADNA2 3150R	5'-GAGAGACATATGGTTCAGTGTAGTTG-3'	Kreuze <i>et al.</i> , 2009
SPBADNA2 3550F	5'-TGGAACCAAGATCAAGGAAGAA-3'	Kreuze <i>et al.</i> , 2009
SPBADNA 4600R	5'-TCCTGATGCCGATGATATGATCTG-3'	Kreuze <i>et al.</i> , 2009
SPBADNA 4600F	5'-CAGATCATATCATCGGCATCAGGA-3'	Kreuze <i>et al.</i> , 2009
6700F	5'-GTGCGAGTCCTACCCTTCAATCAG-3'	This study
1466R	5'-CTGTTGCGGCCCTGTTCTCCTC-3'	This study
3471F	5'-GGACGGAGGTCGTGTTGTTGTTCC-3'	This study
3863R	5'-CCATAGTACCCTTGAATCGGTATA-3'	This study
Bad-F	5-ATGCCITTYGGIITIAARAAYGCICC-3	Yang <i>et al.</i> , 2003
Bad-R	5-CCAYTTRCAIACISCICCCCAICC-3	Yang <i>et al.</i> , 2003
BadnaBKF	5-CAAATTAGGAGGCAGATAAATG-3	Mbanzibwa <i>et al.</i> , 2011
BadnaBSR	5-GGTCTTCTTATGTTCCACCTT-3	Mbanzibwa <i>et al.</i> , 2011

The product of the ligation reaction was used to transform *Escherichia coli* (DH5 $\alpha$ ) by the thermal shock method. Colonies containing the possible recombinant plasmids were peaked into liquid Luria Bertani (LB) medium and incubated at 37 °C for 12 h. After incubation, cultures were subjected to mini-preparation of plasmidial DNA by the alkaline lysis method (SAMBROOK & RUSSEL, 2001) and the resulting DNA analyzed on a 1% agarose gel stained with GelRed™.

Plasmidial DNA samples whose length approached 5000 bp, corresponding to the plasmidial vector linked to the viral genome, were subjected to cleavage with restriction enzymes EcoRI and NotI (Promega), and the electrophoretic pattern was analyzed on an agarose gel (1 %). The samples, in which the fragment was verified according to that obtained via PCR, were sent for sequencing using the Ludwig Biotecnologia service. After sequencing, the sequences obtained were analyzed on GenBank ([http // www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) to identify the isolates.

Another amplification method used in this study was the RCA (Rolling circle amplification), in order to differentiate between virus sequences and episomal sequence, as well as the amplification of the complete genome. The reaction consisted in use a mix consisting of a template DNA, a mixture of dNTPs, thioprotected primers, specific BadnaBKF primer, bacteriophage phi 29 DNA polymerase enzyme and a buffer solution suitable for enzymatic activity and DNA stability, which was subjected to the isothermal reaction at 30°C for 20 hours and 10 min at 65°C to inactivate the enzyme.

### 3 RESULTS AND DISCUSSION

According to the data observed in this study, in the evaluation with degenerate primers for the identification of badnavirus, of the 303 sweet potato samples analyzed, 48 were positive, presenting an amplified fragment of approximately 600 bp. The positive samples were subjected to amplification with a second pair of primers, specific for SPBV-B, and all of them amplified a fragment of approximately 800 bp.

Despite the relatively expressive number of positive samples, the generated fragment is small to confirm the presence of the virus, since they can also be endogenous pararetroviral sequences (EPRVs). These sequences can be integrated into the plant's genome through illegitimate recombination, and their presence is not necessarily associated with infection (GEERING et al., 2005). In addition, there are cases in which these sequences, under specific stress conditions, can become pathogenic, resulting in the reconstitution and activation of the viral genome (NDOWORA et al., 1999).

Kreuze and collaborators (2009) identified two badnavirus isolates in the sweet potato crop in Peru, known as Sweet potato Badnavirus A (SPV-A) and Sweet potato Badnavirus B (SPV-B), collectively called Sweet potato pakakuy virus (SPPV) (KREUZE et al., 2009). Some primers used

by the authors made it possible for us to obtain larger fragments of the genome, so that we could then verify the presence of the virus in the plant and not the EPRVs.

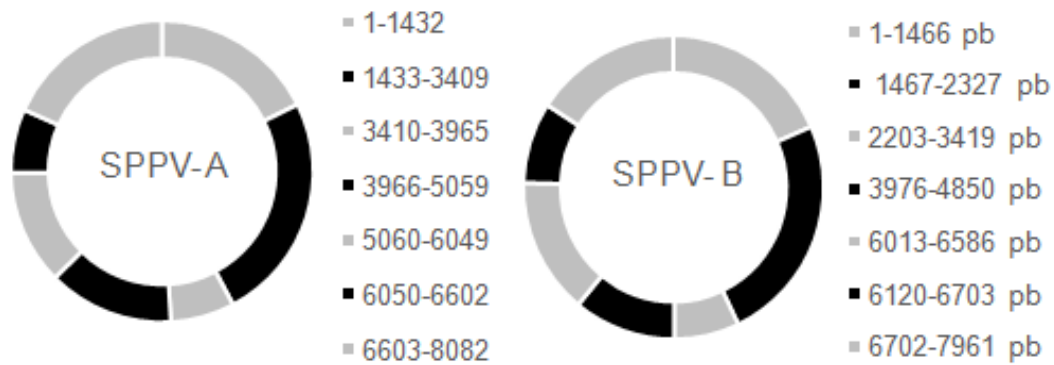
For viral species belonging to the genus Badnavirus, the standard limit established by the ICTV (International Committee of Taxonomy of Virus) is >80% nucleotide identity in the RT/RNaseH region (GEERING & HULL, 2012). However, when performing the alignment of the sequences obtained with those deposited on the GenBank, a great variability was observed between the fragments. As for example, the sequence obtained by the primer BadnaBKF and BadnaBSR, compared to other sequences deposited in the GenBank, presented 98% identity with the isolated SPVV-A isolated partial sequence (KT448750.1), staying in position 1 to 861 in the genome, also presented 88% identity with the Hunan-184 isolate (MK052981.1) in position 1433 to 2271 in the genome.

In this way, when comparing the fragment with the sequences that present greater identities, the positions of the fragment in the genome are inserted. Thus, we tried to compare only with the complete sequences contained in the database. When the obtained fragments were compared with the complete sequences of Sweet potato badnavirus A (SPV-A) (FJ560943.2) and SPV-B (FJ560944.1), deposited in GenBank, the identity of the fragments varied between 72 to 87%, thus demonstrating the high variability between them (Table 2, Figure 1).

**Table 2** – Fragments obtained from isolates SPV-A and SPV-B.

<b>Primer</b>	<b>Identity SPV-A/ Position in the genome</b>	<b>Identity SPV-B / Position in the genome</b>
Bad-FP/ Bad-RP	-	76% / 6120-6703
BadnaBKF/ BadnaBSR	87% / 1433-2263	80% / 1467-2327
1750F/3150R	72% / 2152-3409	72% / 2203-3419
3550F/4600R	80% / 3966-5059	77% / 3976-5067
4600F/ Bad-R	75% / 6050-6602	75% / 6013-6586

Fig. 1 Schematic representation of the *Badnavirus* sequences obtained in the genome. In black are the sequences obtained.



This high genetic variability previously reported in the complex of viral species responsible for the disease Banana streak viruses (BSVs) in banana plants (*Musa* spp.) (HARPER & HULL, 1998; HARPER et al., 2005; FIGUEIREDO et al., 2006), as well as for the virus *Dioscorea bacilliform* AL virus (DBALV) in the yam crop (*Dioscorea* spp.) (LIMA et al., 2013). In the sweet potato crop, in addition to the low SPPV concentration, an expressive sequence variation in different genotypes has also been reported, as well as infection by more than one species of the virus in several genotypes, suggesting an active evolution of the SPPV virus (ZERBINI et al., 2017).

However, primers designed to close some gaps in the genome amplified the plant's genome, making it impossible to obtain the complete genome. The identification of badnavirus species is still a challenge, because one of the most widely used amplification techniques is PCR, which makes it difficult to differentiate between episomal and integrated forms of the virus (BOUSALEM et al., 2009). EPRVs are present in different host species, considered the most abundant class of endogenous viral sequences (GAYRAL P ISKRA-CARUANA, 2009).

RCA is a method that has been used frequently lately, due to its high efficiency to differentiate endogenous and episomal sequences from badnavirus, making it possible to overcome the diagnosis deficiency via PCR (JAMES et al., 2011). Therefore, the same sample submitted to the PCR technique was also used in this method, and it was possible to obtain a fragment of approximately 8000 bp, thus reinforcing the presence of the virus in the host.

Both SPV-A and SPV-B have already been identified in Honduras, Guatemala (KASHIF et al., 2012), and Tanzania (MBANZIBWA et al., 2014). In China, out of 200 samples collected in several provinces, in 34 of them the presence of Sweet potato badnavirus A (SPBV-A) was reported (QIN et al., 2016). In Brazil, this is the first report of Badnavirus in the sweet potato crop, requiring in-depth studies regarding the damage caused by this pathogen in the sweet potato crop. New

sequences are being provided in order to obtain larger fragments for the assembly of the complete genome of the isolate.

Thus, the need to acquire or maintain propagating material of good sanitary quality and thus guarantee productivity is reinforced, this information is also important for the development of control strategies and understanding of the diversity of viruses present in the culture for the sampled region.

### ACKNOWLEDGEMENTS

The authors would like to thank the CNPQ - National Council for Scientific and Technological Development - for their financial support.

### REFERENCES

- BHAT, A.; HOHN, T.; SELVARAJAN, R. Badnaviruses: The Current Global Scenario. *Viruses* v.8, p.177. 2016.
- BOUSALEM, M.; DURAND, O.; SCARCELLI, N.; LEBAS, B.S.M.; KENYON, J.L.; MARCHAND, L.; LEFORT, F.; SEAL, S.E. Dilemmas caused by endogenous pararetroviruses regarding the taxonomy and diagnosis of yam (*Dioscorea* spp.) badnaviruses: analyses to support safe germplasm movement. *Archives of Virology* v.154, p.297–314. 2009.
- CLARK, C.A.; DAVIS, J.A.; ABAD, J.A.; CUELLAR, W.J.; FUENTES, S.; KREUZE, J.F.; GIBSON, R.W.; MUKASA, S.B.; TUGUME, A.K.; TAIRO, F.D.; VALKONEN, J.P.T. Sweet potato Viruses: 15 Years of Progress on Understanding and Managing Complex Diseases. *Plant Disease*, Saint Paul, v. 96, p.168-185. 2012.
- DOYLE, J.J. & DOYLE, J.L. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytoch Bull* v.19, p.11–15. 1987.
- FIGUEIREDO, D.V.; MEISSNER FILHO, P.E.; SILVA NETO, S.P.; BRIOSO, P.S.T. Detecção e análise da variabilidade de sequências do *Banana streakvirus* (BSV) em bananeiras no Brasil. *Summa Phytopathologica* v.32, p.118-23. 2006.
- GAYRAL, P. & ISKRA-CARUANA, M. Phylogeny of *Banana streak virus* reveals recent and repetitive endogenization in the genome of its banana host (*Musa* sp.). *Journal of Molecular Evolution* v.69, p.65-80. 2009.
- GEERING, A.D.W. & HULL, R. Family *Caulimoviridae*, in: KING AMQ, ADAMS MJ, CARSTENS EB, LEFKOWITZ E J (Eds.), *Virus Taxonomy*. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, Amsterdam, The Netherlands, pp 429–443. 2012.
- GEERING, A.D.W.; POOGGIN, M.M.; OLSZEWSKI, N.E.; LOCKHART, B.E.L.; THOMAS, J.E. Characterisation of banana streak mysore virus and evidence that its DNA is integrated in the B genome of cultivated *Musa*. *Archives of Virology* v.150, p.787- 796. 2005.



HARPER, G. & HULL, R. Cloning and sequence analysis of *Banana Streak Virus* DNA. *Virus Genes* v.17, p.271-278. 1998.

HARPER, G.; HART, D.; MOULT, S.; HULL, R.; GEERING, A.; THOMAS, J. The diversity of *Banana streak virus* isolates in Uganda. *Archives of Virology* v.150, p.2407–2420. 2005.

JAMES, A.P.; GEIJSKES, R.J.; DALE, J.L.; HARDING, R.M. Development of a novel rolling-circle amplification technique to detect *Banana streak virus* that also discriminates between integrated and episomal virus sequences. *Plant Disease* v.95, p.57-62. 2011.

KASHIF, M.; PIETILÄ, S.; ARTOLA, K.; JONES, R.A.C.; TUGUME, A.K.; MÄKINEN, V.; VALKONEN, J.P.T. Detection of viruses in sweet potato from Honduras and Guatemala augmented by deep-sequencing of small-RNAs. *Plant Dis* v.96, p.1430-1437. 2012.

KREUZE, J.F.; PEREZ, A.; UNTIVEROS, M.; QUISPE, D.; FUENTES, S.; BARKER, I.; SIMON, R. Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for discovery and sequencing of viruses. *Virology* v.388, p.1–7. 2009.

KREUZE, J.F.; PEREZA GALVEZA, M.; CUELLARB, W.J. Badnaviruses of sweet potato: symptomless co-inhabitants on a global scale. *BioRxiv*. 2017. Doi: <http://dx.doi.org/10.1101/140517>  
KROTH, L.L.; DANIELS, J.; PIEROBOM, C.R. Degenerescência da batata-doce no Rio Grande do Sul. *Revista Brasileira Agrociência* v.10, p.79-82. 2004.

LIMA, J.S.; LIMA, A.T.M.; CASTILLO-URQUIZA, G.P.; SILVA, S.J.C.; ASSUNÇÃO, I.P.; MICHEREFF, S.J.; ZERBINI, F.M.; LIMA, G.S.A. Variabilidade genética de isolados de badnavírus infectando inhame (*Dioscorea* spp.) no nordeste do Brasil. *Tropical Plant Pathology* v.38, p.349-353. 2013.

LOEBENSTEIN, G. & THOTTAPPILLY, G. The Sweet potato. Dordrecht. The Netherlands, Springer Sciences Business Media BV. 2009.

MAICH, S.L.S.P. Levantamento e caracterização molecular parcial de begomovírus infectando batata-doce nos principais distritos produtores da região de Pelotas- RS. 44f. Dissertação (Mestrado) – Programa de Pós-Graduação em Fitossanidade. Universidade Federal de Pelotas. Pelotas, RS, Brasil. 2015.

MBANZIBWA, D.R.; TAIRO, F.; GWANDU, C.; KULLAYA, A.; VALKONEN, J.P.T. First report of *Sweet potato symptomless virus 1* and *Sweet potato virus A* in sweet potatoes in Tanzania. *Plant Disease* v.95, p.224. 2011.

MBANZIBWA, D.R.; TUGUME, A.K.; CHIUNGA, E.; MARK, D.; TAIRO, F.D. Small RNA deep sequencing-based detection and further evidence of DNA viruses infecting sweet potato plants in Tanzania. *Ann Appl Biol* v.165, p.329–339. 2014.

MONTES, S.M.N.M.; FIRETTI, R.; GOLLA, A.R.; TARSITANO, M.A.A. Custos e rentabilidade da batata-doce (*Ipomoea batatas* L.) na região oeste do estado de São Paulo: estudo de caso. *Infobibos*. 2008.

NDOWORA, T.D.G.; DAHAL, G.; LAFLEUR, D.; HARPER, G.; HULL, R.; OLSZEWSKI, N.E.; LOCKHART, B. Evidence that badnavirus infection in *Musa* can originate from integrated pararetroviral sequences. *Virology* v.255, p.214-220. 1999.

OLIVEIRA, M.K.T.; NETO, F.B, CÂMARA FA, DOMBROSKI JLD, FREITAS RMO (2008) Multiplicação *in vitro* de batata-doce (*Ipomoea batatas* Lam). *Revista Caatinga* v.21, p.129-134. 2008.

SAMBROOK, J. & RUSSEL, D. *Molecular Cloning - A Laboratory Manual* (3 ed.). Cold Spring Harbor, NY. Cold Spring Harbor Laboratory Press. 2001.

QIN, Y.H.; LI, X.C.; ZHANG, Z.C.; QIAO, Q.; ZHANG, D.S.; WANG, Y.J.; TIAN, Y.T.; WANG, S. First Report of *Sweet potato badnavirus A* in China. *Plant Dis* v.100, p.865. 2016.

SHEKHAR, S.; MISHRA, D.; BURAGOHAİN, A.K.; CHAKRABORTY, S.; CHAKRABORTY, N. Comparative analysis of phytochemicals and nutrient availability in two contrasting cultivars of sweet potato (*Ipomoea batatas* L.). *Food Chemistry* 173: 957-965.

Doi.org/10.1016/j.foodchem.2014.09.172. 2015.

STAGINNUS, C.; ISKRA-CARUANA, M.L.; LOCKHART, B.; HOHN, T.; RICHERT-PÖGGELER, K.R. Suggestions for a nomenclature of endogenous pararetroviral sequences in plants. *Archives of Virology* v.154, p.1189–1193. 2009.

UNTIVEROS, M.; FUENTES, S.; SALAZAR, L.F. Synergistic interaction of *Sweet potato chlorotic stunt virus* (Crinivirus) with Carla-, Cucumo-, Ipomo-, and Potyviruses infecting sweet potato. *Plant Disease* v.91, p.669-676. 2007.

VALVERDE, R.A.; CLARK, C.A.; VALKONEN, J.P.T. Viruses and virus disease complexes of sweet potato. *Plant Viruses* v.1, p.116-126. 2007.

WYLIE, S.J.; ADAMS, M.; CHALAM, C.; KREUZE, J.; LÓPEZ-MOYA, J.J.; OHSHIMA, K.; PRAVEEN, S.; RABENSTEIN, F.; STENGER, D.; WANG, A.; ZERBINI, F.M. & ICTV REPORT CONSORTIUM. ICTV Virus Taxonomy Profile: *Potyviridae*. *Journal of General Virology* v.98, p.352-354. 2017.

YANG, I.C.; HAFNER, G.J.; REVILL, P.A.; DALE, J.L.; HARDING, R.M. Sequence diversity of South Pacific isolates of taro bacilliform virus and the development of a PCR-based diagnostic test. *Arch Virol* v.148, p.1957- 1968. 2003.

ZERBINI, F.M.; BRIDDON, R.W.; IDRIS, A.; MARTIN, D.P.; MORIONES, E.; NAVAS-CASTILLO, J.; RIVERA-BUSTAMANTE, R.; ROUMAGNAC, P.; VARSANI, A. and ICTV Report Consortium ICTV Virus Taxonomy Profile: *Geminiviridae*, *Journal of General Virology* v.98, p.131–133. 2017.