Improving Sweetpotato Virus Diagnostics

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SASHA
Sweetpotato Action for Security and Health in Africa

Introduction

Virus infection, by a number of different types of viruses, is one of the most important constraints of sweetpotato production globally, especially in Sub-Saharan Africa (SSA). Among the more than 30 described viruses infecting sweetpotato. Sweet potato chlorotic stunt virus (SPCSV) and Sweet potato feathery mottle virus (SPFMV) are considered to be the most widespread and are particularly devastating when occurring in combination to cause the sweetpotato virus disease (SPVD). SPVD has been reported throughout SSA. However, beyond a few countries in East Africa, there is no clear information about the prevalence and distribution of different viruses and virus strains infecting sweetpotato. This information is essential to enable adequate control of the viruses in each region either through breeding for resistance to the appropriate viruses, production of planting material tested for the appropriate viruses and cultural methods preventing virus spread in the field. Diagnostic tests are not available for all viruses. Moreover currently, available tests are either not sensitive enough to reliably detect viruses directly from sweetpotato or require expensive laboratory equipment and a high level of experience to perform. Thus, improved and cheaper diagnostic methods are required.

Objectives

- Determine virus prevelance and diversity throughout African continent
- Develop one assay for simultanious detection of all sweetpotato viruses that can be use in standard laboratory in support of germplasm exchange
- Develop sensitive & cheap virus detection tool that can be used under field conditions in support of seed systems

Methods

- A continent wide sample collection was performed in Africa and analyzed using small RNA sequencing and assembly. New bio-informatics pipelines were developed to analyze the data and a database set up to house the data and results in an accessible format
- Data from the sweetpotato virus database mentioned above was used to develop specific molecular diagnostic tools including tube-arrays for the simultaneous detection of the most important sweetpotato viruses, and LAMP assays that could be used under field conditions.

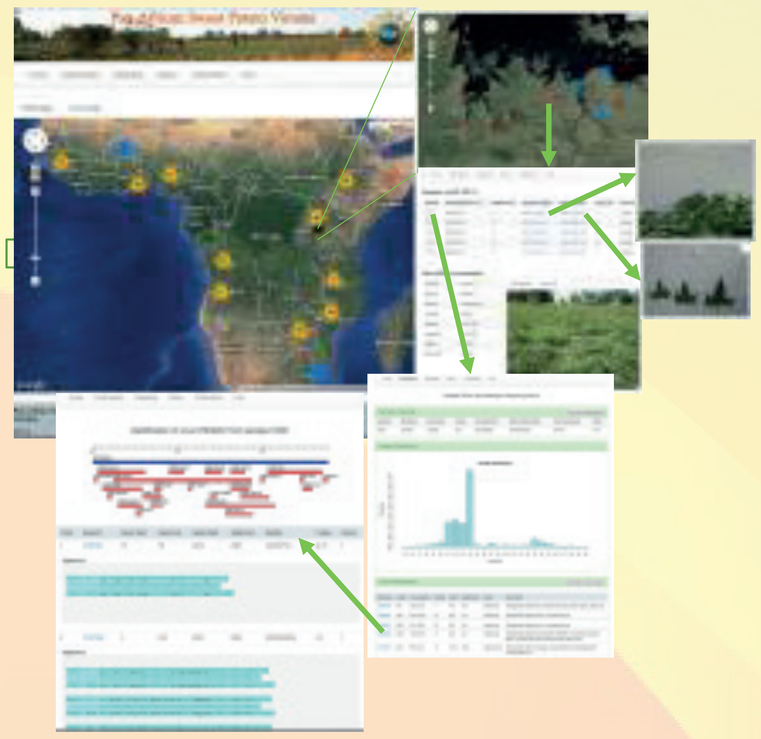
Results and discussion

The African sweetpotato virome

Continent wide survey was performed to determine the prevelance and diversity of viruses throughout Africa. More than 1600 geo-referenced samples were collected of which 500 have been sampled to date and the remaining will be finalized by the end of the year. The data has been deposited in the interactive African sweetpotato virome database (http://bioinfo.bti.cornell.edu/virome/index), a summary of the results to date are provided in the table below.

Table 1: *Summary of results obtained to date*

AFRICA		No. samples	sequenced	Non-infected	% Infection	Badna and Mastre	% w/o badna & mastrevirus[2]		RNA viruses											DNA viruses					
									SPFMV	SPVG	SPVC	SPV2	SPVZ[3]	SPMMV	SPCSV	SPCFV	SPC6V	CMV viroid	Begomovirus	Alphasatellite	Mastrevirus	SPVCV	SPCV	Badnavirus
West Africa	Guinea	105	31	2	94	16	42		10	0	1	0	0	0	0	0	0	0 1	2	0	22	2	0	22
								%	32	0	3	0	0	0	0	0	0		6	0	71	6	0	71
	Nigeria	108	45	18	60	8	42		15	0	2	0	0	0	0	3	0	0	8	0	19	0	0	16
								%	33	0	4	0	0	0	0	7	0	0	18	0	42	0	0	36
	Benin	33	28	3	89	15	36		11	0	2	0	0	0	0	0	0	0	0	0	23	0	0	9
								%	39	0	7	0	0	0	0	0	0	0	0	0	82	0	0	32
	Ghana[1]	146	43	0	100	1	98		35	2	12	0	0	0	14	10	0	0	25	0	13	1	0	40
								%	81	5	28	0	0	0	33	23	0	0	58	0	30	2	0	93
	Burkina Faso[1]	10	4	0	100	0	100		3	0	0	0	0	0	3	0	0	0	4	2	2	0	0	3
								%	75	0	0	0	0	0	75	0	0	0	100	67	67	0	0	75
East Africa	Ethiopia	261	109	7	94	19	77		45	57	22	0	0	0	30	0	1	2	39	0	35	0	0	104
								%	41	50	20	0	0	0	28	0	1	2	36	0	32	0	0	95
	Tanzania	236	46	5	89	6	76		28	0	9	0	0	0	13	0	0	0	8	0	13	0	1	37
								%	61	0	20	0	0	0	28	0	0	0	17	0	28	0	2	80
	Uganda	200	96	0	100	5	95		64	1	44	0	0	0	48	9	0	0	53	0	45	0	17	78
								%	67	1	46	0	0	0	50	9	0	0	55	0	47	0	18	81
	Kenya	141	Not processed due to low quality RNA processing not yet started																						
	Rwanda[1]	53																							
Southern Africa	Malawi	129	30	3	92	2	87		24	7	4	3	1	0	12	11	0	0	2	0	14	1	9	26
								%	80	23	13	10	3	0	40	29	0	0	5	0	47	3	24	87
	Zimbabwe	156	50	3	94	2	90		46	3	12	1	1	0	14	0	0	0	1	0	26	0	0	32
								%	92	6	24	2	2	0	27	0	0	0	2	0	51	0	0	64
	Angola	179	44	2	95	12	68		16	0	1	0	15	0	3	0	0	1	6	0	30	5	1	38
								%	36	0	2	0	34	0	7	0	0	2	14	0	68	11	2	86
	Zambia	165	Samples stolen from DHL																						
	Mozambique[1]	58	35	3	91	0	91		31	15	21	12	8	15	18	1	0	0	17	2	17	0	0	31
								%	89	43	60	34	23	49	51	3	0	0	49	6	49	0	0	89
Total:		1980	561	46	92	86	85	%	64	17	24	4	5	3	27	7	0	1	30	1	44	2	6	78
Total under this project		1713[4]	479																						



The analysis has identified several new (but mostly not very common) viruses, but most importantly the variation, distribution and prevalence of known viruses. An enigmatic group of viruses belonging to the genus Badnavirus, which are not known to cause disease in sweetpotatoes was found most frequently, in more than three quarters of the samples. Furthermore sweet potato feathery mottle virus was confirmed as one of the most common viruses throughout the continent followed by begomoviruses and sweet potato chlorotic stunt virus. Clear geographical differences were however appreciated in presence and prevalence of different virus species as well as distinct strains, which are relevant to guide management, breeding and germplasm exchange. Also, the sequence data generated has been essential for improving the design of specific molecular diagnostic assays that can be used in support of germplasm exchange (sweetpotato tube-arrays) and seed systems (LAMP)

Key:
SPFMV, Sweet potato feathery mottle virus; SPVG, Sweet potato virus G; SPVC, Sweet potato virus C; SPV2, Sweet potato virus 2; SPVZ, Sweet potato virus Z; SPMMV, Sweet potato mild mottle virus; SPCSV, sweet potato chlorotic stunt virus; SPCFV, Sweet potato chlorotic fleck virus; SPC6V, Sweet potato C-6 virus; CMV, Cucumber mosaic virus; SPVCV, Sweet potato vein clearing virus; SPCV, Sweet potato collusive virus.

Notes:
[1] Samples collected and sequenced with support of SASHA, remaining with support of BREAD project.
[2] Because we currently consider Badna- and mastreviruses innocuous endophytes, we provide the value without counting them.
[3] Underlined (satellite) viruses are new species identified in this study, many new strains were discovered, but are not indicated in this table for simplicity.
[4] Including samples that can't be processed (Kenya) and lost samples (Zambia), without counting them the total is 1407, among which 479 were sequenced.

Conclusions

The African sweetpotato virome has provided us with an unprecedented insight into virus variability, distribution and prevalence over the continent which are relevant to guide management, breeding and germplasm exchange. It has enabled the development of more specific and easy to use molecular diagnostic tools that may in future be used for germplasm exchange or testing in seed systems. The prevalence and large variability of begomoviruses should be an alert to scientist to pay more attention to this relatively understudied group of viruses in sweetpotato.

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