

Summer School 2009: "RCA-based diagnosis of plant DNA viruses"

Stuttgart, Biologisches Institut
August 3rd-14th 2009

Report

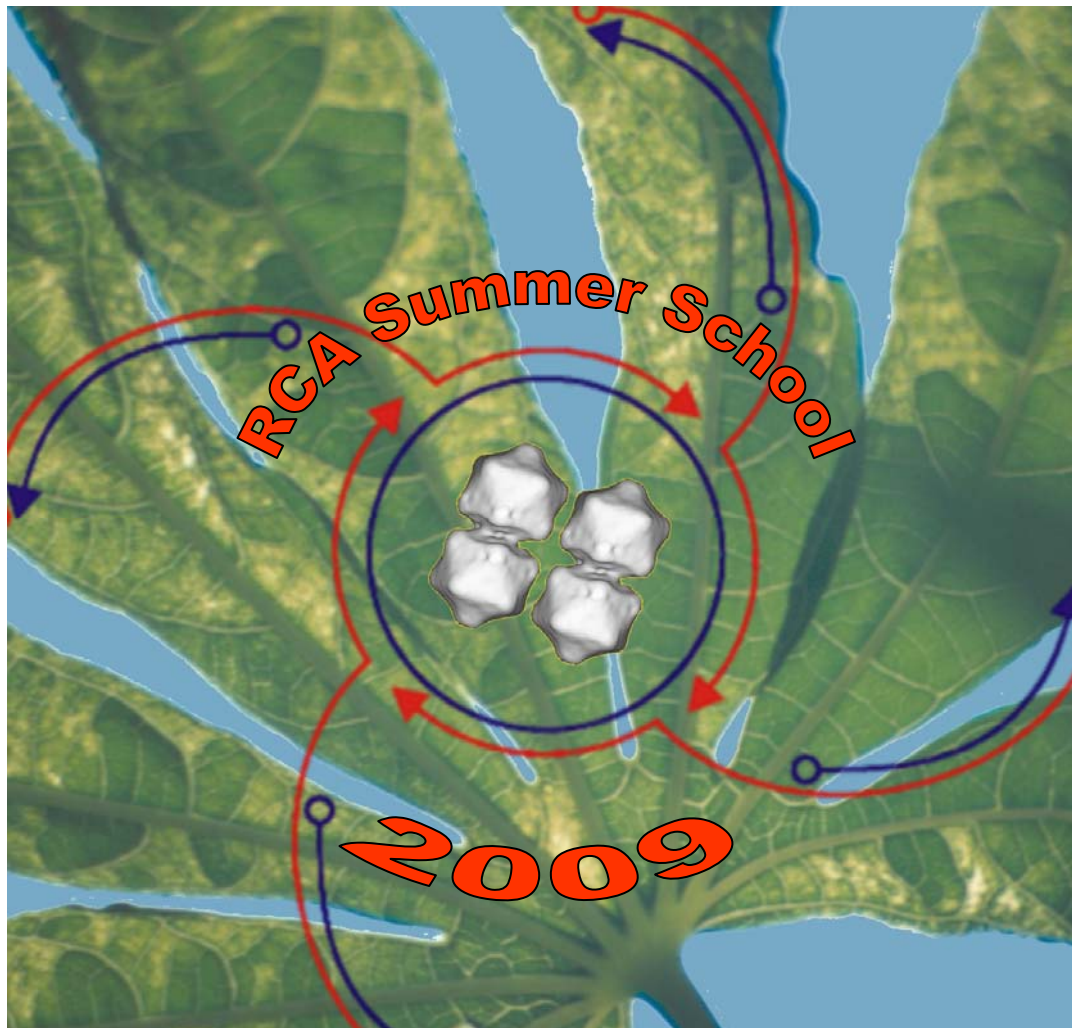


Fig. 1: Logo of the Summer School showing gemini particles, infected cassava leaf and the diagnostic tool of Rolling Circle amplification.



Fig. 2: Participants and supervisors of the International Summer School: Amit Sukal, Anika Rosa Dreilich, Huma Mumtaz, Isabel Moreno, Prof. Mohammed Ali-Shtayeh, Muna Zaid, Natalia Barboza, Rana Jamous, Rhasida Perveen, Thangarj Makesh Kumar, Dr. Yamila Martínez Zubiaur, and Prof. Dr. Holger Jeske, Dr. Björn Krenz, Dr. Anan Kadri, Judith Horn, Patricia Wyant, Benjamin Schäfer, Juan Alvaro Gomez Barrera PhD, respectively.

After the first Summer School in 2008, the second International Summer School on RCA technology was held with 11 participants in 2009. One of the selected people was not able to come and withdraw the acceptance for the invitation just before the start of the course. They came from Colombia, Costa Rica, Cuba, Fidschi Island, India, Pakistan, Palestine, and Germany, two were advanced scientists working at research institutes and plant protection agencies, nine were PhD or master students. Due to the generous support by private sponsors (Fig. 3), we were able to cover all costs for accommodation, local transportation, main meals, scripts and electronic documentations, and sight seeing tour. Except for one student who got a support for the airfare, all other participants were successful in acquiring independent grants for their travel expenses.

All participants had already scientific experience with the molecular genetic analysis of geminiviruses, either in the applied field or in basic research. Therefore, an intense exchange of knowledge from various countries was possible and the basis of lively discussions. Particularly important was the experience from tropical and subtropical countries, which suffer most from geminivirus infection.

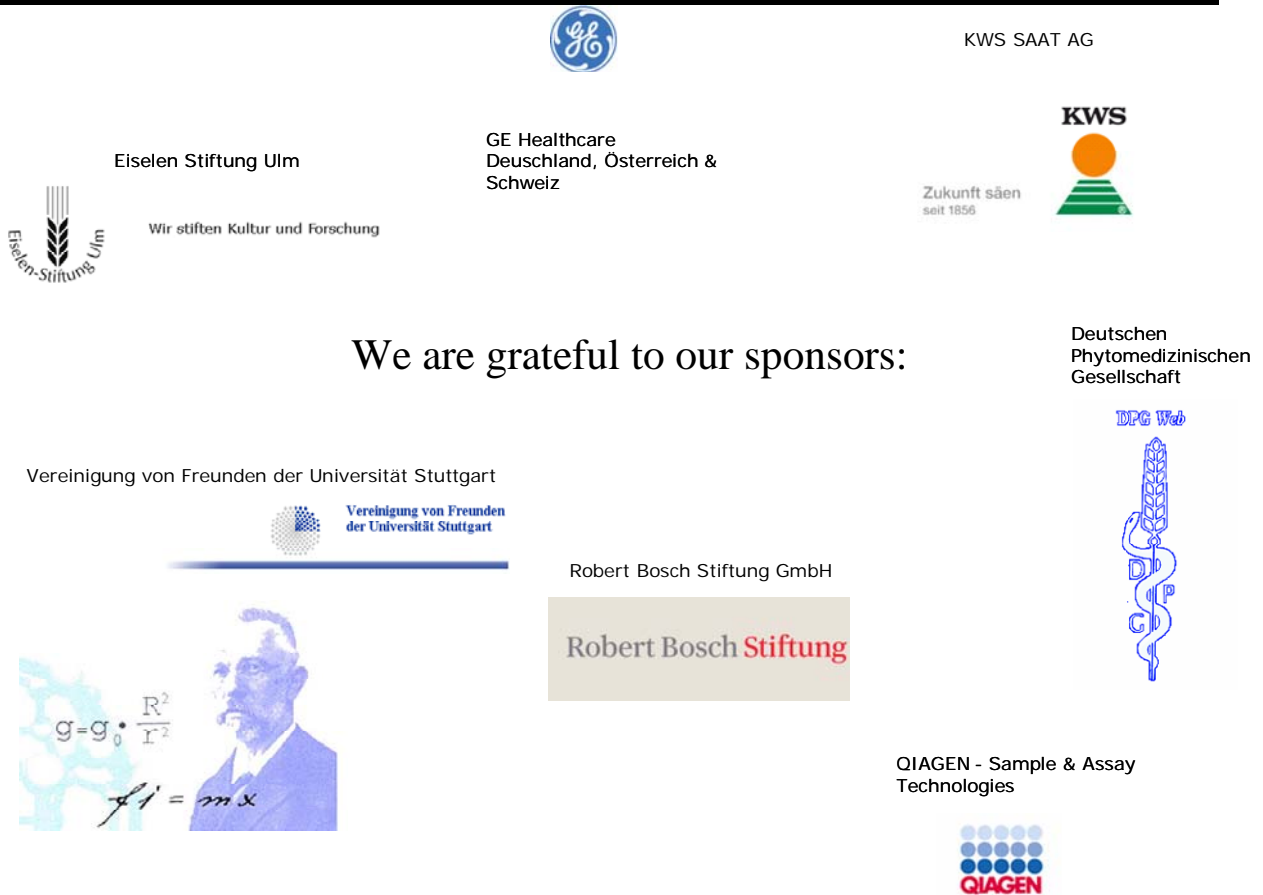


Fig. 3: Private sponsors of the International Summer School.

The primary aim of the course was an experimental training in Rolling Circle Amplification and Restriction Fragment Length Polyphormism for geminivirus diagnosis. Practical exercises at the bench were accompanied by morning lectures to present the background of the techniques and recent developments in geminivirus research. During the afternoon, the participants contributed with seminars about their current research and the situation of geminivirus infection in their countries. At the end of the course, the participants presented the results of their experiments of the two weeks in a small workshop and discussed the advantages and pitfalls of the techniques used. By learning from the first Summer School, we concentrated more on the mathematical evaluation of RFLPs, improved the capacities of computers and added a new module concerning the ligation of DNA fragments. Although a larger heterogeneity of the pre-knowledge of the participants was obvious in comparison with the first summer school, the aims of the course were completely reached due to the intense engagement of the supervisors.

The results of an evaluation form, which has been filled-in by the participants (see below), showed that most expectations were fulfilled and the acceptance of this course was again very high.

Programme International Summer School on RCA Diagnosis 2009 (August 3rd-14th 2009)

Week 1	Mo, 03.08.	Tu, 04.08.	We, 05.08.	Th, 06.08.	Fr, 07.08.
Lecture	Holger Jeske: RCA introduction; Safety regulations	Holger Jeske: Restriction analysis	Holger Jeske: Gel electrophoresis	Holger Jeske: Geminiviral DNA intermediates	Holger Jeske: Plant sample preparation and ligation theory
Experiment I	AG (dilutions) 3x Midi RCA	RFLP (<i>Hpa</i> II) fragment size determination -AG 3xMidi -PAGE 6x	Computer-assisted identification of viral RFLP pattern	Cloning of geminiviral genomes: Digestion and ligation	Transformation
Experiment II			Elution of viral NA from FTA cards	RCA on FTA card preparation	RFLP (<i>Hpa</i> II) and fragment size determination 1x Midi
Experiment III		Biolistic particle delivery			
Experiment IV				Total NA preparation by CTAB method RCA 1x Midi	RFLP (<i>Hpa</i> II) and fragment size determination 2x Midi 6x PAGE
Seminar:	Amit S.: Virus elimination		Makeshkumar: Gemini- and Nanovirus problems in tropical crops	Natalia B.: Geographic distribution of begomoviruses in Costa Rica	Rashida P.: Detection of CLCuV in asymptomatic weeds

Programme International Summer School on RCA Diagnosis 2009 (August 3rd-14th 2009)

Week 2	Mo, 10.08.	Tu, 11.08.	We, 12.08.	Th, 13.08.	Fr, 14.08.
Lecture	Björn Krenz: AbMV and gene silencing	Tatjana Kleinow: I like to move it..... How geminiviral proteins facilitate transport of viral DNA within a plant	Christina Wege: Mixed Infections of Geminiviruses and Unrelated RNA Viruses or Viroids: A Multitude of Effects with a Highly Probable Impact on Epidemiology and Agriculture	Mohamed Hassan: Preliminary Survey of Geminiviruses in Egypt Using Rolling Circle Amplification	Holger Jeske: Summary
Experiment I	Colony RCA 1x Midi	RFLP analysis of Colony RCA products 2x Midi	Sequencing of Colony RCA products	Sequencing and BLAST search	Presentation of the course result. Discussion of perspectives Further collaboration
Experiment II	Computer-assisted identification of viral RFLP pattern				
Experiment III			Total nucleic acid preparation by CTAB method; RCA 1x Midi	RFLP (Hpa II) analysis and fragment size determination 1x Midi	
Experiment IV	Computer-assisted identification of viral RFLP pattern				
Seminar:	Anika D.: Phylogenetic studies to the Vanilloideae esp. in the Caribbean area	Yamila Z.: Advance in geminivirus studies in Cuba Huma M.: Genetic diversity and molecular characterization of dicot-infecting Mastreviruses in Pakistan	Isabel M.: Virus resistance in cassava	Rana J. & Mohamed A.: Hc-pro as a tool to study suppressor-mediated induction of disease symptoms in plants Muna Z.: Use PCR in diagnosis animal disease	

International Reference Centre for the Genomics and Diagnosis of Viruses with Small Circular DNA

Consortium: Prof. Dr. H. Jeske, Universität Stuttgart, Biologisches Institut; Prof. Dr. E.R. Bejarano, Universidad de Malaga, Dpt. Cellular Biology, Genetics and Physiology; Dr. B. Gronenborn, Centre National de la Recherche Scientifique, Institut des Sciences du Végétal;
Dr. S. Ullmann, Qiagen GmbH, Nucleic Acid Preparation Research

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Evaluation:

Please tick the boxes where appropriate:

The overall quality of the course was

high			low		
1	2	3	4	5	6
7	2	1			

The programme fulfilled my expectations

yes			no		
1	2	3	4	5	6
7	2	2			

The practical expertise of the supervisors was

high			low		
1	2	3	4	5	6
8	3				

I can recommend the course for PhD students:

yes			no		
1	2	3	4	5	6
8		3			

The quality of the lectures was

high			low		
1	2	3	4	5	6
8	2	1			

I can recommend the course for advanced scientists in the plant protection field:

yes			no		
1	2	3	4	5	6
6	1	2	1		

The quality of the course script was

high			low		
1	2	3	4	5	6
7	3	1			

The following percentage of the course-content was completely new for me:

100	80	60	40	20	<20
	4	5	1	1	

The understandability of the explanations was

high			low		
1	2	3	4	5	6
4	7				

The following percentage of the course-content was already known for me, but repetitions and extensions were welcome:

100	80	60	40	20	<20
1	2	3	2	3	

I felt free to ask questions

yes			no		
1	2	3	4	5	6
9	2				

The following percentage of the course-content was already known for me, and therefore repetitions and extensions were not necessary:

100	80	60	40	20	<20
			1	4	6

I got appropriate answers to my questions

yes			no		
1	2	3	4	5	6
7	2	2			

The value of the course for my scientific career is

high			low		
1	2	3	4	5	6
7	2	1	1		

Comments and suggestions:
(you may also use the back page....)

The value of the course for practical applications in agriculture is

high			low		
1	2	3	4	5	6
5	4	2			