« Application of Biotechnology to the Tomato Virus Crisis in West Africa: presenting the results and planning the activities of the second year »

Minutes of the meeting held at Samanko Research Station

Bamako, Mali

15 and 16 June 2006

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Workshop Program

Thursday, 15 June 2006

9:00 - 9:30	Welcoming remarks	
9:30 - 10:00	*Coffee break*	
10:00 – 10:45	Results presentation - AVRDC	Virginie Levasseur and Issoufou Kollo Abdourhamane
10:45 – 13:15	Country presentation	
	✓ -INRAB	Françoise Assogba-Komlan
	✓ ITRA	Aïssatou Ali Alfari
	✓ CRI	Offei Bonsu
	✓ CDH	Abdou Aziz M'Baye
	✓ INERA	Léonard Ouedraogo
13:15 – 14:30	*Lunch*	
14:30 – 15:30	Country presentation	
	✓ IER	Moussa Noussourou
	✓ INRAN	Habsatou Boukary
15:30 – 16:15	Results presentation – UC Davis	Robert Gilbertson
16:15 – 16:45	*Coffee break*	

Friday, 16 June 2006

9:00 – 10:00	Results presentation – Cornell University	Jeffrey Gordon
10:00 – 10:30	Results synthesis and summary of the discussion	Issoufou Kollo Abdourhamane
10:30 – 11:00	*Coffee break*	
11:00 – 13:30	Presentation of the activities for the second year : ✓ Funds ✓ Sample collection ✓ Variety trials	Virginie Levasseur
13:30 – 14:30	*Lunch*	
14:30 – 16:00	Synthesis of the actions for the second year ✓ Variety trials (cont'd)	Virginie Levasseur
16:00 – 16:30	*Coffee break*	
16:30 – 17:30	Closing remarks	

Welcoming Remarks

Dr. Virginie Levasseur (Associate Scientist – AVRDC West Africa)

After welcoming all partners, V. Levasseur mentioned that Dr. Harry Bottenberg from USAID West Africa Regional Program could not be present to our meeting because he needed to attend the Fertilizer Summit in Nigeria. She also mentioned that Dr. Ram Shetty and Mrs. Jean Harman from USAID Mali Mission would be present on the second day of the meeting.

Dr. Frank Shotkoski (Director of the Aid Biotechnology Support Project II)

In his remarks Dr Shotkoski, thanked the team for the good work. He mentioned he was pleased with the trials he visited in Benin, Togo and Ghana, when he visited them last February. He thinks our project is well supported, highlighting the good work being conducted by the NARES, UC Davis, Cornell University and AVRDC. Finally he mentioned he was looking forward to seeing the presentation of the results.

Dr. George Kuo (Director for International Cooperation – AVRDC)

Dr. Kuo thanked USAID and ABSPII for their support and collaboration with AVRDC and the NARES of West Africa. He pointed out that the support from USAID enabled AVRDC to transfer in West Africa some technologies and varieties developed by AVRDC, for the well being of West Africans. He welcomed the NARES partners who have been working hand by hand with AVRDC.

Results Presentation

AVRDC

A brief summary of the project objectives, the conclusions of the launching workshop held in Bamako in October 4-5, 2005 and the activities conducted at Samanko in 2005 and 2006 were presented briefly Virginie Levasseur. In her presentation she pointed out that the delays in planting of the trials and obtaining reports from the NARES partners were caused by a delay in project implementation. Instead of starting in September 2004 as it was initially planned, the project started in March 2005 which caused much delay in establishing the work plan, employing the project coordinator and holding the initial meeting with NARES partners. Hence, the contract between AVRDC and NARES partners for fund distribution was delayed considerably, which delayed consequently the possibilities for trial implementation. It is in order to avoid these delays that the meeting is held in June this year. She also mentioned the arrival of the ABSPII review team last February in Bamako.

After the introduction, Issoufou Kollo Abdourhamane presented the highlights of his missions to all the partners' experimental site and other locations where tomatoes are extensively grown. Then, the results obtained at Samanko Research station were presented. In Samanko, a total a total of 21 promising tomato varieties have been identified. Furthermore, the geminivirus pressure was quite high, allowing for a reliable differentiation of resistant vs. susceptible varieties.

In their questions, participants asked if the tomato varieties sent to them were hybrids or open pollinated, mentioning that it might be easier to work with open pollinated varieties for quick dissemination to tomato producers. It was mentioned that the CLN series coming from AVRDC were open pollinated

lines, as well as the 04 series coming from CIRAD – Guadeloupe. All others are hybrids. R. Gilbertson pointed out that the majority of currently available varieties with tomato leaf curl disease (TLCD) resistance are hybrids and that it would be difficult to find open pollinated varieties with equivalent levels of resistance. Indeed, these varieties are quite expensive to develop and the companies want to make sure the producers will buy their seeds each year. However, the worldwide trend in tomato production is toward the use of hybrids and it is hoped that the superior yields will offset the higher costs of seed.

Another question was asked in relation to the different diseases I. K. Abdourhamane observed in the different countries. One disease that was mentioned in particular was bacterial canker (caused by *Clavibacter michiganensis* subsp. *michiganensis*). The question was asked as to whether there is anyway bacterial canker can be controlled? R. Gilbertson answered that this is a seedborne bacterium and that tomato seeds can be soaked in a solution of 1.27% solution of hydrochloric acid for 30 minutes to get rid of the pathogen from seed.

Institut National de Recherche Agronomique du Bénin (INRAB) - Benin

In Benin almost all the entries were showing susceptible reactions. In Benin, however, many other diseases and insect pests did attack tomato plants in the trial. This likely caused some problems to the staff evaluating the severity of geminiviruses there. And, indeed, the virus pressure might not have been so important because the susceptible check, Roma VF, had a mean severity index of 2.4, on a scale of 0 to 4.

In the questions and comments of the participants, it was asked to F. Shotkoski if the project would eventually take into account solving the other problems which constitute major constraints to tomato production in West Africa. Unfortunately, this ABSPII project for West Africa will not be able to tackle the other constraints within its already defined objectives. It was then emphasized that bacterial wilt, nematodes and possibly spider mites are the next big problems to be faced by tomato producers in West Africa once the geminiviruses problem will be solved.

Another question was asked about the reported yields, which seemed quite low in Benin. F. Assogba-Komlan answered that tomato production in Southern Benin is not usually conducted during the dry season, because of the geminivirus problems and other problems such as high night temperature. She said that yield of 4T/ha in the dry season is not bad at all for this part of Benin. This could be quite different, however, if the trial could be established in Northern Benin, where the climatic conditions are similar to the Sahelian conditions.

Institut Togolais de Recherche Agricole (ITRA) – Togo

In Togo, 5 promising varieties were identified. In Togo, as well as in Benin, the trial might not have been conducted at the ideal period to have high whitefly and virus pressure. Moreover, other problems like nematodes, fusarium wilt and the presence of the weeds has reduced the yield of all the varieties included in the trial.

During the comments and questions period, it was underlined the necessity to plant the trials when the whitefly population is very high to make sure that the disease is present. A. Ali Alfari then suggested to move the trial up in Northern Togo to get the ideal conditions of viruses infestation.

Crop Research Institute (CRI) – Ghana

In Ghana, two trials were conducted. The results of the second trial are more interesting as the virus pressure was higher. In the second trial, no variety has shown to be resistant to the geminiviruses present there and only four varieties were classified as moderately resistant. In his presentation, O. Bonsu mentioned that a weed was present in the trial which showed viruses-like symptoms, but it was not identified nor sampled for further analyses at UC Davis.

In the questions and comments by the participants, two points were raised. First was this issue of high flower abortion and severe fungal infection in both trials. O. Bonsu explained that high night temperatures are causing flower abortion. However, some participants thought that 23 ºC mean night temperature was not sufficient to cause flower abortion. Nevertheless, it seems that high night temperatures could contribute to lower tomato production in Center and Southern Ghana at this time of the year. As high night temperatures were mentioned quite often in Benin, Togo and Ghana, G. Kuo suggested that some of the heat tolerant tomato line be tested in those locations. If the different lines proved to be tolerant to these temperatures, maybe the resistance to TYLD could be bred into those varieties?

Regarding the severe fungal infection, O. Bonsu mentioned that constant rain during the trial created the perfect conditions for fungi development. It was mentioned again that it will be important for the trials to be conducted in 2006/2007 at the appropriate time of the year and that the other diseases or pests be controlled and properly identified in order to avoid mis-interpretation in TYCD scoring.

Centre pour le Développement de l'Horticulture (CDH) - Senegal

In Senegal several varieties were either disease free or had mild virus disease symptoms; the highest yields close to 60 tons/ha were recorded in Senegal.

The questions and comments from the participants were mainly about the level of infestation in Senegal and the incredibly high yields obtained there. A.A. M'Baye reported that geminivirus infection was very high. Indeed, their field was located close to another tomato field that had 100% TLCD. In their trial, the susceptible check had typical TLCD symptoms, although not 100% of the plants were infected. So one of the hypothesis is that the trial was planted a little bit late (December); when whitefly population were not as high as at the beginning of the dry season.

During the discussion F. Shotkoski point out that emphasis should not be put on the yields, because the trial is not replicated. At this point, he reminded the participants that yields data should be viewed as preliminary and that further yield trials should be conducted to clearly say that these varieties are high yielding varieties or not.

Institut pour l'Environnement et la Recherche Agricole (INERA) - Burkina Faso

In Burkina Faso, the virus pressure was very high. Thus, no variety was classified as being highly resistant, although ten varieties were classified as being moderately resistant. The highest yield recorded in Burkina was of 10.2 T/ha and some participants were wondering if such low yields could be ever interesting for tomato producers.

Despite the fact that we should not put so much attention on yields at this stage, L. Ouedraogo mentioned that 10 t/ha was a very high yield for the dry season in Burkina Faso. He reminded the

participants how severe the TLCD crisis is in Burkina that all tomato producers are slowly moving south of the original tomato production area to try to escape from the TYLCV problem. Thus, a yield of 10 t/ha is potentially worth a lot of money in the local markets!

Institut d'Économie Rurale (IER) – Mali

IER conducted trials, in Baguineda, Cinzana, Sikasso and Sotuba. However, the trials were planted late and the virus pressure was low at some locations. The highest pressure was in Baguineda. Under these conditions, seventeen varieties were classified as resistant and another 14 varieties were classified as moderately resistant.

The main comments from the participants were on the importance of planting the trials at the appropriate time, that is, when the whitefly populations and the virus pressure are high. It seemed that everyone knew when this was in their different countries, so it was hoped that this could be accomplished in 2006-2007. It was also mentioned that the same scale used to score virus severity should be used at all locations to enable good comparisons between the sites. In that regard it was decided to go with the 0-4 rating scale for all locations.

Institut National de Recherche Agricole du Niger (INRAN) - Niger

In Niger virus pressure was almost inexistent. The results on yields were presented but since the trial is not replicated, we should not use them as evidence that one variety is better than another one. Once again, the importance of planting the trial at the appropriate time was emphasized.

University of California (UC Davis) – California, USA

The first part of the presentation of R. Gilbertson was a good lecture on viral diseases and their diagnosis. The second part concerned the identification of the viruses in tomatoes with virus symptoms collected from Burkina Faso, Ghana, Mali and Senegal during the 2006 survey trip. A total of 229 samples were collected and, in addition to tomatoes, these included weeds and some other crop species. The samples were initially analyzed using the squash blot technique and, if they were found positive for geminivirus infection, they were then analyzed with the polymerase chain reaction (PCR) tool to precisely identify which virus(es) were in the sample. Tomato and pepper were severely attacked by geminiviruses as most samples were positive for geminivirus infection. Okra plants showing leaf curl and crumple also were infected with geminiviruses, but preliminary evidence indicated that the geminivirus in okra was different than the ones in tomato and pepper.

Three new geminivirus species infecting tomato and pepper have been identified in Mali: *Tomato leaf curl Mali virus* (TLCMV), *Tomato yellow leaf curl Mali virus* (TYLCMV), and *Pepper yellow vein Mali virus* (PYVMV). A fourth one is being characterized. Furthermore, specific PCR-based tests have been developed that allow for the identification of TLCMV, TYLCMV and PYVMV. Thus, these tests were conducted with samples collected from Burkina Faso, Ghana Mali and Senegal. These viruses were also detected in samples from these different countries, consistent with their involvement in the TLCD complex in West Africa. However, the relative frequency of each virus varied among locations. In Ghana, for example, PYVMV was predominant, while in Senegal TYLCMV and TLCMV were prevalent. In Burkina and in Mali, however, mixed infections of 2 or even 3 of these viruses were found, which helps explain the severity of the symptoms in those countries. The samples of the weed species, Acanthospermum, collected in Burkina Faso, were negative for geminivirus infection. This weed was reported as a major reservoir for TYLCV. Further analysis will be conducted to confirm these results.

There were a few examples of samples with virus symptoms that were collected that were negative for geminivirus infection. The most significant was a rosette-type symptom observed on tomatoes in Senegal. This may be a different type of virus and will need to be identified.

The samples sent by NARES partners were all analyzed. But there is a need to discuss with each partner before summarizing these results and presenting them. R. Gilbertson hence invited each NARES representative to find a few minutes with him to discuss the results of their sampling.

Participants were pleased with these results and surprised to learn that they are dealing with so many viruses. The question was then asked how difficult their task will be to identify some resistance. R. Gilbertson explained that it might be very difficult to find one variety which is resistant to all the geminiviruses in West Africa. There are, however, some varieties that are resistant to more than one virus. For example, GemPride is resistant to two of the three viruses identified thus far in West Africa. This will be a starting point for future selection program. Agroinoculation screening of the different varieties will help us precisely identify which varieties are resistant to which viruses.

Cornell University – Ithaca, USA

Jeff Gordon presented the progress made at Cornell University in the collection of TYLCV-resistant tomato cultivars for the variety trials and in the development of a transgenic tomato using the *pvr1* potyvirus-resistance gene from pepper. The presentation additionally offered a good review of the molecular biological techniques used in the breeding program for the new transgenic tomato variety.

The breeding program at Cornell is working to introgress the *pvr1* gene into the background of the TYLCV-resistant tomato cultivar GemPride, a hybrid produced by Seminis. The gene was first moved into the laboratory tomato cultivar MicroTom, and a backcrossing program is under way to move the gene into GemPride while retaining as many of the agronomic characteristics of GemPride as possible.

During the debate, R. Gilbertson justified working with potyvirus resistance by pointing out that potyviruses have been reported in Burkina Faso, in peppers, and that they can cause significant damage on pepper and tomato in the region. The question of the copy number of *pvr1* in the transgenic MicroTom was addressed by F. Shotkoski, who pointed out that Southern blotting had previously been used to confirm the presence of just one single copy of the gene.

There were some questions on how the resistance to TYLCV was bred into existing resistant varieties and if there is a marker for TYLCV resistance. J. Gordon answered that the resistance currently used in existing varieties was introgressed from a number of *solanum* species, including most prominently *Solanum chilense*. There are currently no high-resolution publicly available markers for TYLCV-resistance genes. However, a goal of the research conducted by J. Gordon is to find markers for the TYLCV-resistance genes used in each of the cultivars tested in the variety trials. Once these markers are identified, it will ease the process of breeding for resistance to TYLCV.

Planning of the activities for the second year

Fund distribution

This year, there is a slight increase in the budget given to the NARES from AVRDC. Instead of 3,999.50 USD, a total of 4,200 USD will be distributed to each NARES. Then some NARES representatives wondered if they could have access to more funds since they were asked to conduct the trials in other location, thus increasing the costs of running the trial. F. Shotkoski then announced that Cornell University was ready to increase the budget of the NARES since the work conducted so far was very impressive. Hence, each NARES partner will receive an additional amount of 3,000 USD to conduct their activities. Everyone was pleased and thanked Cornell University.

Sample collection

R. Gilbertson mentioned that he was very pleased with all the samples he received from the NARES. Now that all the facilities have been established at UC Davis, he mentioned that he would be ready to receive 48 different samples from each NARES partner this year. Gilbertson indicated that he will bring the sampling kits to Samanko during a visit to Mali in early September 2006.

Answering the questions of the participants, R. Gilbertson mentioned that some weed samples could be collected and sampled. However, collectors should be very careful and take only the weeds which are showing severe virus symptoms. This is because some weeds might not have enough virus in them for the squash blot analyses to detect them. As these nylon membranes and there analyses are resource consuming, he would prefer to have samples which are clearly infected with geminiviruses.

R. Gilbertson announced that he was so proud of all the work being done by the partners that he was ready to send each one of them copies of two tomato disease identification guides: one from Seminis Seed Company and the other the Compendium of Tomato Disease published by the American Phytopathological Society. He said that these should be used by the partners to help identify the different problems occurring in their tomato trials and make sure that when they score for TLCD symptoms, they are not scoring symptoms caused by other diseases or pests. All participants thanked R. Gilbertson for this initiative.

Variety trials

For the 2006/2007 cropping season, it was suggested that each NARES conduct a replicated variety trials with a reduced number of lines. AVRDC would conduct the same trial in addition to another screening trial with new tomato varieties gathered over the year by Cornell and AVRDC would be screened. NARES representatives insisted, however, to conduct the replicated variety trial and the screening trial. It was then decided that included in the variety trials, there shouldn't be more than 13 varieties and 20 to 25 varieties would be included in the screening trial.

To choose the varieties to be included in the variety trial, each NARES picked up the top ten promising varieties for their country. Table 1 (Appendix 1) shows the choice of each NARES and the total number of time each variety was selected. It was then decided that the varieties which were chosen by six, five and four countries would be selected as the core list of varieties to be included in the trial. These are F1 3019 Galina (6), GemPride (6), Thoriya (6), Bybal (5), Chenoa (5), Industry DR 10403 (5), Lety F1 (5), Realeza (5), Attack (4), Ponchita (4) and Yosra (4). If, for some reason, one of seeds of anyone of these

varieties is not available in sufficient quantity, the other varieties being selected by three or by two countries will be included in the list.

The protocol for the variety trial was extensively discussed. Here are the main points which will be included in the protocol:

- ✓ A randomized complete block design with 3 repetitions;
- ✓ Each plot will have thirty six plants, in 3 rows;
- ✓ Only the inside row will be used for observations (only 12 plants);
- ✓ Tomato plants will be individually scored for TYLCV, using the same scale proposed by Dr. Gilbertson (from 0 to 4);
- ✓ Tomato plants will be scored three times: at flowering stage, fruiting stage and after the first harvest;
- ✓ Yield will be calculated by counting the number of fruits per plant and the number fruits per cluster on three individual plant randomly chosen in each plot;
- ✓ All pesticides can be used to control all other diseases, pathogens and pests other than whiteflies;
- ✓ Tomato plants are not staked, according to the usual practices used by tomato producers.

A standardized protocol will be prepared by AVRDC and send to each partner along with the seeds. It is very important that each NARES strictly apply the protocol and plant the trial in time.

Closing remarks

Mrs. Jean Harman (Leader of the Economic Growth Team – USAID Mali Mission)

Mrs. Harman was extremely happy with what she saw and heard during the meeting. She highlighted the importance of such multidisciplinary project with diverse institutions as the NARES, AVRDC and two renowned US Universities. She mentioned to all participants that their role in this project goes far behind their simple presence in Bamako and they should all continue their good work and collaboration. She is quite confident that by solving this severe constraint to tomato production, each collaborating country will gain a lot from an increase in tomato production. She wishes everyone good luck for the activities planed this year!

Dr. Robert Gilbertson (Professor of Plant Pathology – UC Davis, California, USA)

Dr. Gilbertson thanked all NARES partners, he his very proud of the results everyone gathered this year and he his very confident the trials to be conducted next year will bring very promising data to solve the virus crisis in West Africa. He then thanked AVRDC team in Mali for their coordination of the meeting and the work being done with NARES partners. Finally, he sincerely thanked USAID Mali Mission and West Africa Regional Program for the support and making this project a reality.

Dr. Frank Shotkoski (Director of the Aid Biotechnology Support Project II)

Dr. Shotkoski thanked USAID Mali Mission and USAID West Africa Regional Program for their support. He really thinks this project will have great impact for all tomato producers in West Africa within a few years. He has been extremely happy with the results and the lively discussion that went on during this meeting.

Dr. George Kuo (Director for International Cooperation – AVRDC)

Dr. Kuo thanked everyone for their presence in Bamako and their active participation during the meeting. He warmly thanked the donor for their assistance and presence during the meeting. Dr. Kuo especially thanked NARES partners for their constant collaboration with AVRDC, encouraging them to continue in this way. He finally wished everyone a very good and productive year!

Annex 1. Selection of the varieties for the advanced yield trial

Variety	Be	BF	Gh	Ma 1	Ma 2	Ni	Se	To	Frequency
F1 3019 Galina	X	X		X	X	X		X	6
GermPride	X	X	X	X			X	X	6
Thoriya	X			X	X	X	X	X	6
Bybal		X		X	X	X	X		5
Chenoa	X			X	X	X	X		5
Industry DR 10403		X		X	X	X		X	5
Lety F1	X				X	X	X	X	5
Realeza				X	X	X	X	X	5
Atak				X	X	X	X		4
Ponchita				X	X	X	X		4
Yosra	X		X	X			X		4
FTC 6236		X	X				X		3
04 495	X					X		X	3
Cheyenne E448		X			X				2
HA 3060	X							X	2
HMX 4810		X				X			2
Nadira	X					X			2
Nirouz TH99806	X	X							2
TY 75		X					X		2
Yassamen TH99802		X					X		2
CLN 2460E	X								1
Favi 9							X		1
FTC 6231							X		1
FTC 7127	X								1
FTC 7351			X						1
04 498								X	1
PS 43316	X								1
Sasya 0202 F1								X	1
TLCV 15	X								1
CLN 2123A									0
CLN 2468A									0
CLN 2498E									0
CLN 2545A									0
CLN 2545B									0
FTC 7088									0
FTC 7483									0
PT 4722A									0
04 108									0
04 240									0
04 501									0

Be = Benin
BF = Burkina Faso
Gh = Ghana
Ma 1 = Mali (Baguineda)
Ma 2 = Mali (Samanko)
Ni = Niger
Se = Senegal
To = Togo

Annex 2. List of the participants, 15-16 June 2006, Bamako, Mali

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