CIMMYT

International Maize and Wheat Improvement Center

AMBIONET
Asian Maize Biotechnology Network

Report on the 2nd Annual Meeting
27 – 30 April 1999
Chinese Academy of Agricultural Sciences
Beijing, China

Prepared by:
Maria Luz George and Mireille Khairallah
May 1999

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Abbreviations

AFLP, Amplified Fragment Length Polymorphism
ASI, Anthesis Silking Interval
CIM, Composite Interval Mapping
DNA, Deoxyribonucleic Acid
DM, Downy Mildew
DMR, Downy Mildew Resistance
GxE, Genotype by Environment
ICIS, International Crop Information System
ITS, Internal Transcribed Spacer
MAS, Marker-Assisted Selection
MRDV, Maize Rough Dwarf Virus
PAGE, Polyacrylamide Gel Electrophoresis
PCR, Polymerase Chain Reaction
PGS, *Peronosclerospora*-genus specific
PIC, Polymorphic Information Content
PSS, *P. sorghi*-specific
QTL, Quantitative Trait Loci
RAPD, Randomly Amplified Polymorphic DNA
RFLP, Restriction Fragment Length Polymorphism
RIL, Recombinant Inbred Line
SCMV, Sugarcane Mosaic Virus
SSCP, Single Strand Conformation Polymorphism
SSR, Simple Sequence Repeats (microsatellites)

CHAPTER 1
Background of AMBIONET

The Asian Maize Biotechnology Network (AMBIONET) is a collaborative research and training network established in April 1998 that is aimed at building the biotechnology capacity of national maize programs in Asia. The focus of the Network is on the application of biotechnology tools to maize improvement and their integration into conventional breeding efforts in collaborative research programs. Through training and collaborative research, the Network aims to increase maize productivity through the development of improved cultivars with high yield potential, durable resistance to diseases and tolerance to abiotic stresses. Co-financed by the Asian Development Bank (ADB), CIMMYT, and the national research systems of partner countries, the AMBIONET facilitates interactions, advances communications, and promotes the sharing of information, technologies and germplasm within the network.

The Network was initiated with a start-up meeting between CIMMYT and China, Philippines and Thailand in Bangkok, Thailand in April 1998. Since then, India and Indonesia also joined the Network, bringing the number of partner countries to five.

CHAPTER 2
Overview of Meeting

The second annual meeting of AMBIONET was held in Beijing, China from April 27 – 30, 1999.

Members from each of the five NARS teams from China, India, Indonesia, Philippines and Thailand attended the meeting. A total of 11 NARS scientists, two each representing the AMBIONET teams from India, Indonesia, Philippines and Thailand, and three from China, attended the meeting. Representing CIMMYT were Dave Hoisington, Director of Applied Biotechnology Center and AMBIONET Project Manager; AMBIONET Coordinator Maria Luz George; and Resource Scientists Mireille Khairallah of the Applied Biotechnology Center, Daniel Jeffers of the Maize Program, and Surinder Vasal of the Asian Regional Maize Program. Also present were members of the AMBIONET Steering Committee made up of country representatives Dafang Huang of China, Mangala Rai of India, Djoko Damardjati of Indonesia, Edna Anit (representing William Dar) of the Philippines, and Sutat Sriwatanapongse of Thailand (See Attachment 1 for group photo and Attachment 2 for directory of participants).

This year's meeting was hosted by the Chinese Academy of Agricultural Sciences (CAAS). Logistical support was provided by the CIMMYT-China office and members of the China AMBIONET team.

Objectives

The meeting was a time to review activities, evaluate progress and revise workplans accordingly. Specifically, the objectives of the meeting were to:

- Review Year 1 activities and evaluate progress of Network and individual teams
- Identify Year 2 activities
- Generate ideas for the second phase of the project
- Interact and strengthen Network
- Demonstrate the PC-based MapData program, whose translation from Mac- to Window-based program was supported by AMBIONET

Meeting Sessions

As in the first annual meeting of AMBIONET, the meeting was intended to be participatory, encouraging a high level of involvement in a friendly and informal atmosphere. The program included brief presentations and allowed enough time for extensive discussions (see Attachment 3 for program), followed by agreements about issues raised.

Day 1

Session 1: OVERVIEW OF THE MEETING

The meeting started with welcome greetings from the hosting institute, represented by CAAS Vice President Dr. Wang Ren and Director of the Institute of Crop Breeding and Cultivation and CIMMYT Board of Trustees Member Dr. Xin Zhiyong.

The participants all introduced themselves, identified their expectations for the meeting (See Attachment 4 for a list of participants' expectations) and set clear objectives for the meeting.

Session 2: SUMMARY OF AMBIONET NETWORK-WIDE ACTIVITIES

AMBIONET Coordinator Luz George presented the milestones achieved by the Network as a whole during the first year of the project. Since April 1998, the following milestones were achieved:

MANAGEMENT OF THE PROJECT

- Coordinator on-board in July 1998
  - All five AMBIONET teams formed
  - Memoranda of Understanding (MOUs) for China, Philippines, Thailand signed
  - AMBIONET-India in August 1998, covered by existing MOU
  - AMBIONET-Indonesia in February 1999, covered by existing MOU
- Facilitation role of CIMMYT liaison offices in China and Thailand
- Establishment of CIMMYT office in IRRI October 1998
  - MOU between CIMMYT and IRRI
  - AMBIONET office now fully functional
  - IRRI administrative system for Coordinator, Philippines/India/ Indonesia
  - Facilitation role of IRRI liaison offices in Indonesia and India
- All Steering Committee members identified

CAPACITY-BUILDING

- First AMBIONET training course in CIMMYT, four weeks, November 1998
  - 4 trainees each from China, Philippines, Thailand
  - 3 from India
  - 2 from Indonesia
  - 2 guest trainees from Africa
- 3 exchange visitors to CIMMYT, one from the Philippines, two from China, three months each

ESTABLISHMENT OF COMMUNICATION CHANNELS

- Routine communications by email set up
  AMBIONET support for email connectivity in India, Indonesia, Thailand
- AMBIONET News
  - Prepared by the Coordinator, sent as hard copy and also placed in the AMBIONET homepage
  - First issue in January 1999, Second issue in April 1999
- AMBIONET homepage
  - Set up in November 1998
  - Updated and enhanced in February 1999
  - Mirror in Thailand being set up
  - Site to access resources/documents such as AMBIONET News and reports

REPORTING

- Report on the AMBIONET molecular marker course in November 1998
- First semi-annual progress report

NETWORKING

- List of on-site visits from April 1998 – April 1999

Place	When	Who	What was accomplished
1998			
China	May	Dave, Mireille	• met with CAAS collaborators
	Oct	Luz	• met with team & refined workplan
			• established links with CIMMYT-China
India	Aug	Luz	• met with team & developed workplan
Indonesia	Oct	Luz to RIMC	• met with CRIFC and RIMC administrators to establish links
Philippines	several	Luz to IPB/Des to IRRI	• various
Thailand	Aug	Luz to KU	• met with team & refined workplan
			• established links with CIMMYT-Thailand
CIMMYT, Mexico	Sept	Luz	• got introduced to CIMMYT
	Nov	Luz	• Training course
ADB	Dec	Luz	• updated Tony Perez on AMBIONET activities
			• delivered first progress report
1999			
Indonesia	Feb	Luz to RIFCB	• met with team and government administrators
			• developed workplan with team
	Apr	Mireille to RIFCB	• on-site visit
			• met with team to discuss workplan
			• presented a seminar
Philippines	Mar	Dave, Luz	• visited downy mildew trial at IPB
			• discussed on-going experiments
Thailand	Mar	Dave, Luz to DOA	• visited drought trials
			• met with team to discuss progress
CIMMYT, Mexico	Jan	Luz	• attended CIMMYT Annual Reporting Meeting
ADB	Mar	Dave, Luz	• met with Mandar Jayawant
China	Apr	Dave, Luz, Mireille, Dan, Sam, 8 NARS team members, 4 SC members	• 2nd AMBIONET Annual Planning Meeting

CAAS, Chinese Academy of Agricultural Sciences; CRIFC, Central Research Institute for Food Crops; RIMC, Research Institute for Maize and other Cereals; IPB, Institute of Plant Breeding; IRRI, International Rice Research Institute; KU, Kasetsart University; RIFCB, Research Institute for Food Crops Biotechnology; DOA, Department of Agriculture

Session 3: COUNTRY PROGRESS REPORTS

Each AMBIONET team summarized the outputs, as well as the problems encountered in their research activities.

CHINA

DEVELOPMENT OF MOLECULAR MARKERS FOR SCMV AND MRDV

- The cross Ye107 (S) × Huangzao 4 (R) was selected for mapping SCMV and MRDV
- F1 and F2 seeds of Ye107 × Huangzao4 were produced
- Symptom evaluation and mechanical inoculation methods for SCMV and MRDV established
- Some parental screening has been done: 31 polymorphic RFLP combinations of probe/enzyme and 19 polymorphic SSR primers identified between Ye107 and Huangzao 4
- Tangshan city & Beijing, Shanxi & Beijing as locations for SCMV and MRDV field evaluation, respectively

USE OF MOLECULAR MARKERS FOR HETEROTIC GROUPING OF CHINESE MAIZE GERMPLASM

- A diallel of 105 crosses between 15 inbred lines (from 5 heterotic groups) produced
- Field evaluation of 105 diallel crosses made in four locations (Fengyang, Anhui province; Yangling, Shanxi province, Zhengzhou, Henan province; Changping, Beijing), yield data presented
- A total of 105 RAPD primers were previously used for genetic diversity analysis of 15 inbred lines. The classification of 15 inbred lines with RAPD markers was consistent with morphological distance from SCA or mid-parental heterosis
- Forty five SSR primers and 49 RFLP probe/enzyme combinations produced stable and repeatable band profiles between 15 inbred lines. The classification of these inbred lines are in accordance with the grouping based on RAPD markers or available pedigree.
- Additional 100 maize inbred lines were purified and seed-increased in Hainan Island. These lines will be analyzed by RFLP and SSR markers in 1999.

DEVELOPMENT OF MOLECULAR MARKERS FOR DROUGHT TOLERANCE

- Ten drought tolerance inbred lines from CIMMYT-ARMP (Asian Regional Maize Program) evaluated in Hainan Island
- The combination of Han23 (T) × TS 31 (S) selected for mapping drought responses, ASI (Anthesis Silking Interval) will be used
- F1 and F2 seeds of Han23 × TS 31 produced in Beijing and Hainan Island, respectively
- Ten polymorphic combinations of probe/enzyme out of 64 and 9 polymorphic SSR primers out of 71 identified between the parental lines Han23 and TS 31. This indicates a low level of polymorphism.
- Xinjiang and Guangxi provinces chosen as two locations for field evaluation for drought responses

TRAINING

- 4 trainees to AMBIONET molecular marker course
- 2 exchange visits to CIMMYT

TEAM MANAGEMENT ACTIVITIES

- Visits- 5 visits within the country, one visit from AMBIONET Coordinator Luz George
- Five team meetings for material selection, cross production, method analysis, equipment purchase
- Equipment purchases and reagents for AMBIONET

Item	Source of funds
Hybridization Incubator	AMBIONET
PTC-200 DNA Engine	AMBIONET
Sequi-Gen GT	AMBIONET
Beckman (AVANTI30) table refrigerated centrifuge	China Ministry of Agriculture
Genomyx DNA Sequencing/Differential Display Analyzer	China Ministry of Agriculture
Reagents	China Ministry of Agriculture

INDIA

ANALYSIS OF GENETIC DIVERSITY IN INDIAN MAIZE GERMPLASM:

- 37 inbred lines chosen, including 31 CM lines, 4 LM lines and two unreleased inbreds
- Several hybrid combinations generated at Hyderabad
  - 8 x 8 diallel set
  - 5 x 5 diallel set
  - Line x tester combinations (16 inbreds and 5 testers)
- Released maize hybrids (11) + Experimental hybrids (4) chosen for cultivar differentiation
- Morphological data for 19 characters (completion of data after harvest of material in May 1999)
- Leaf samples collected from the experimental set grown at Hyderabad
- DNA extraction in progress
- Selection of some released maize

DOWNY MILDEW (DM) STRESS MANAGEMENT

- DM susceptible/resistant Indian inbreds from Regional Maize Research Station at Karnataka
- CM series chosen for DM evaluation and for possible use as parents
- DM Fingerprinting trial: 30 inbreds from CIMMYT-ARMP for seed increase at Hyderabad, DM-infected leaf samples collected for DNA extraction
- Import permit obtained for Brewbaker's RILs
- Crossing program at Hyderabad to generate nearly 100 cross combinations involving NAI susceptible and resistant inbreds plus CM lines for genetic and molecular marker analysis of DM resistance; to be screened at Mandya and Udaipur
- Leaf samples collected from NAI lines (being grown at Hyderabad) for DNA extraction and polymorphism survey
- Preliminary screening of CM and CML lines for ASI, leaf wilting etc. identified some drought susceptible and tolerant inbreds – due to unseasonal climatic conditions at Hyderabad during the experiments, further experiments needed to verify the results
- Experiments planned for drought screening at Karimnagar (Kharif 99) and Hyderabad (Rabi 1999-2000) to identify promising lines and generate crosses

TEAM MANAGEMENT ACTIVITIES

- Three team members trained in the first AMBIONET Training Course held at CIMMYT
- AMBIONET Work Plan approval obtained from ICAR (Indian Council of Agricultural Research)
- Liaison with the IRRI-India Office established for procurement of local equipment; first batch of local equipment worth ~US$4000 received
- Foreign equipment needs identified and estimates obtained; orders being processed
- Approval obtained for lab rewiring to correct electrical deficiencies; scheduled for May-June 1999
- Internet Service Provider obtained for the Team Leader to facilitate day-to-day communications with AMBIONET Coordinator and Resource Personnel
- Approval obtained for telephone connection to the Maize Molecular Biology Lab, to facilitate internet connectivity to the lab
- AMBIONET team meetings held regularly (eight formal meetings during the reporting period), besides regular maize molecular biology lab meetings
- AMBIONET-India's activities for second year finalized

INDONESIA

ESTABLISHMENT OF AMBIONET-INDONESIA

- Collaboration between RIMC and RIFCB
- Four meetings between RIMC and RIFCB members held to build team
- AMBIONET members officially appointed by CRIFC Director
- Workplan officially approved in February 1999

RESEARCH PROGRAM

- Downy Mildew: Development of markers and MAS
  - Characterization of germplasm for DM reaction and DNA fingerprinting of DM pathogen
  - Phenotyping of Brewbaker's RILs
  - Developing and mapping of Indonesian F2/F3 population (GM15 x Mr15)
  - Preparation for MAS for DM
- Genetic Diversity and Heterotic Grouping

TEAM PROGRESS

- Training
  - Two members attended AMBIONET molecular marker training course in Mexico
  - Two members currently undergoing training in breeding (in-country)
- Visits
  - Luz, 1x to RIMC, 1x to RIFCB
  - Mireille, 1x to RIFCB
- Field preparations
  - Seed increase of identified local materials
  - Seed increase of CIMMYT-ARMP materials
- Communications and coordination
  - Purchase of computer
  - ISP supported by AMBIONET
- Problems encountered
  - Coordination of two institutes making up the team
  - Lack of trained people
- Advice from other teams sought for what makes a team work (See Attachment 5 for elements of teamwork as suggested by other AMBIONET teams)

PHILIPPINES

DEVELOPMENT OF MOLECULAR MARKERS FOR DOWNY MILDEW RESISTANCE (DMR)

- 105 S5 RILs of Ki 14 x Pi33 were seed increased and 20 S2 RILs were advanced to S3
- A total of 154 polymorphic SSR and RFLP markers were identified between Ki 14 and Pi33.
  - 47 SSRs/metaphor agarose
  - 58 SSRs/PAGE-silver stain
  - 49 RFLPs.
- Only 14 RFLP and 8 SSR markers were successfully mapped in 117 Ki 14 x Pi 33 RILs.
  - Three linkage groups formed
  - Mapping temporarily put on hold until the problems encountered were solved
  - Problems include a) high level of heterozygozity (20%) in the S4 RILs; (b) some segregating alleles not found in either parents; (c) polymorphic markers in the parents not segregating in the RILs; and (d) some alleles polymorphic between the parents not be found in the RILs.
- Phenotyping of Ki 14 x Pi 33 RILs were conducted in Koronadal, South Cotabato but QTL mapping of DMR was not accomplished because Ki 14 was found to be susceptible against the Koronadal DM pathogen population and no base map for Ki14 x Pi 33 was constructed

CHARACTERIZATION AND FINGERPRINTING OF DOWNY MILDEW ISOLATES

- Seed increase and DMR screening of 30 maize inbred lines in UPLB and Koronadal
- Based on percent incidence, resistant lines were identified
  - In UPLB, three lines were resistant, four moderately resistant and 16 susceptible
  - In Koronadal, two were resistant, seven moderately resistant and 19 susceptible
  - The most resistant lines identified in both locations are Nei 9008 and P 345C4S2B46-2-2-1-2-B-B-B and Ki3.
- Disease severity was positively correlated with disease incidence (r=0.77) but both of them were not correlated with incubation period.

- Differences were observed in conidial count and morphology between the UPLB and the Koronadal DM pathogen populations.
- Preliminary evaluation using *Peronosclerospora* ITS showed no variation among the different *P. philippinensis* isolates. However, the Philippine isolates were differentiated from the isolates from Thailand and Mexico using ITS and pCLY83. When *P. sorghi*-specific ITS primers were used, no amplification products were seen in the Philippine isolates.

TRANSFER OF DOWNY MILDEW RESISTANCE VIA MARKER-ASSISTED SELECTION
- Generated three F1 crosses with Ki14 by bulk pollination instead of plant to plant crossing.
- Because of the problems in the Ki14 x Pi33 materials, it was decided to generate new F1s

TRAINING
- One (1) exchange visit in CIMMYT-ABC
- Four (4) trainees to AMBIONET molecular training course in CIMMYT
- One (1) Graduate Student in IPB- UPLB - hands-on training on SSR analysis

TEAM MANAGEMENT ACTIVITIES
- Organizational meeting to establish the Philippine's AMBIONET team
- Collaboration with AgroSeeds for experimental set-up in the South Cotabato disease nursery
- Collaborative arrangements with Central Mindanao University-IPB station for seed increase
- Regular monthly team meetings to discuss research progress and other concerns
- Several site visits of AMBIONET Coordinator, Dr. Luz George.
- Visit of Dr. Hoisington and Dr. George with Philippines Team Leaders
- E-mail exchanges with Coordinator, CIMMYT scientists, other teams and collaborators

EQUIPMENT PURCHASE (AMBIONET funds)
Lyophilizer
Ultra pure (MilliQ) water purifier

THAILAND

MAPPING OF DOWNY MILDEW RESISTANCE
- Using the mapping population of Ki14 x Hi31 consisting of 110 original RILs with RFLP map, and 110 new RILs from Dr. Brewbaker of the University of Hawaii
- More than 110 SSRs selected for parental screening and 90% were polymorhic
- 90 SSRs screening in the 220 RILs were partially finished to date
- Preliminary analysis encountered potential problems in mapping construction:
  - The first 30 individual markers showed random distortion towards both Hi31 and Ki14.
  - Several RILs also showed heterozygosity.
- The RILs of Ki14 x Hi31 skewed towards susceptibility in both experiments. The opposite was true for Nar330 x Mo17 population in both experiments.
- Phenotyping of RILs was done in a humidified greenhouse where RILs were sprayed with spore suspension, five plants per entry, two replications were scored
- QTL mapping based on the RFLP on the original set did not find significant LOD score above the threshold. Field and screen-house screening will be conducted in the normal season in 1999

MOLECULAR FINGERPRINTING OF DM PATHOGEN POPULATION IN THAILAND
- ITS2-specific primers did not differentiate healthy plants (Ki14 and Hi31) from infected plants (16 DMF of Thailand and 2 DMF of Philippines). ITS2 primers amplified several DNA bands in healthy plants that corresponded to the pattern in infected plants.
- The ITS2 products were cleaved with 12 restriction endonucleases, DraI, KspI, EcoRI, EcoRV, BstEII, HindIII, HinfI, HaeIII, BamHI, XbaI, Tru9I and Bgl II. Only HaeIII and Tru9I generated polymorphic bands below 300 bp.
- The 300-bp ITS1 product was found both in the healthy and infected plants.

- For *Peronosclerospora*- genus specific primer (PGS), the 300 bp was observed among the differential set and *P. sorghi* Mexico but not from the healthy plants.
- Single Strand Conformation Polymorphism (SSCP) analysis on 5% acrylamide identified three patterns, each specific to three groups - *P.sorghi* (Mexico), *P.sorghi* (Thailand) and *P.philippinensis*.
- The inability of PGS primer to differentiate DM variation from the differential set grown in Thailand may be due to the following: a) low level of polymorphism generated by this primer, b) only one isolate may dominate the sampled location, or c) there is only single isolate in Thailand. Collection will be done on several locations in 1999 season.

USE OF MARKERS TO DETERMINE HETEROTIC GROUPING OF MAIZE INBREDS

- Sixty eight inbred lines consisting of 38 lines from KU (Ki series), 2 from DOA (Nei series), 7 from CIMMYT and 21 from private companies located in Thailand were used in the phylogenetic study based on 63 SSRs. For each primer used, alleles were annotated by comparing to Hind III-cleaved phiX-174. Unique alleles were selected to form standard allele assignment specific to each primer.
- Polymorphic Information Content (PIC) and allele frequencies were calculated for each primer. Most primers detected 3-4 alleles/primer and the maximum number was 11-12 alleles. PIC value and number of alleles/primer was highly correlated.
- Cluster analysis showed that the Ki series formed the largest group as expected. Inbred lines derived from the same cycles of recurrent selection tended to cluster. Inbred lines from private companies clearly separated away from the Ki series, reflecting the effort of private breeders in diversifying away from Suwan 1 population hoping for heterosis.
- Within the Ki series, each line still retained one or two rare, unique allele (s). Testcross and diallele cross will be formed by considering both %similarity and allelic frequencies.

MAPPING OF DROUGHT AND LOW NITROGEN TOLERANCE

- Field evaluation for 36 inbred lines including 2 checks, Nei 9202 and Nei 9008 was conducted in 1999
- The experiment were divided into 2 sets, with (60 kgN/ha) and without nitrogen fertilizer, alpha lattice design (9x4) with 4 replications in 2 rows of 5 m length with 75 cm spacing between rows and 20 cm between hills.
- The mean grain yield of the experiment under stress was 51 kg/rai (1rai = 6.25 ha) compared to 403 kg/rai of that under non-stress. There was highly significant difference in grain yield between the tested lines in the 2 sets of the experiment.
- Five tolerant lines: Nei 412014, Nei 412003, Nei 412015, Nei 412004 and Nei 412011, were selected based on their mean yield under stress and Low Nitrogen Index (LI). Under stress, the mean yield of the selected lines were in the range of 77 – 128 kg/rai with LI of 1.00 – 2.42.
- Four sensitive lines, Nei 412001, Nei 412029, Nei 412034 and Nei 412023 were also selected with their mean yield under stress 0 – 23 kg/rai and LI 0 – 0.44.
- Sixty four inbred lines (49 from DOA, 5 from KU and 10 from CIMMYT) including a check (Nei 9008) were evaluated for tolerance to drought under green house and field conditions
- Inbred lines were selected based on the data from both green house and field experiment. Four tolerant lines were selected including KK-DR(S)C4-153-3-7-1-1-3, Nei 412006, Nei 412005 and Nei 9202 while KS23(S)C2-285-2-1-1-1 and Nei 412035 were selected as sensitive lines.

Day 2

Session 1: DOWNY MILDEW DISCUSSION

- Goal is to map the resistance gene(s) to downy mildew in several mapping populations at several locations across the region using standard parameters so that comparisons can be drawn from the research, and the information obtained can be used in developing an efficient MAS program for the transfer of resistance
- Linkages:
  - USAID-funded project: Control of downy mildew in Egypt
  - Texas A & M University: Dick Frederiksen, Clint Magill
  - A.R./Crop Protection: Elhany El-Suity

- AMBIONET research related to downy mildew
  - DM pathogen fingerprinting/maize genotype reaction of standard set of inbred lines
  - Mapping resistance to DM in the region with several mapping populations (standardization of parameters measured)

PATHOGEN FINGERPRINTING

- Goal is to improve our understanding of the distribution and importance of the different *Peronosclerospora* species in the region
- Tools for fingerprinting the DM pathogen
  - PCR primers include *P. sorghi*-specific (PSS), *P.* genus specific (PGS), and fungal ITS sequences
  - RFLP probes from *P. maydis* library
  - PCLY83 differentiates well between species of *Peronosclerospora*, perhaps within species
  - Use the probe/enzyme combination PCLY83 + *Eco*RI for region-wide pathogen fingerprinting
  - SSCP analysis of amplified then digested products from PGS or ITS-2, Apichart offered to do all samples in his lab, other teams should send DNA from their infected samples
- Sampling of host/pathogen tissue: Use of inexpensive food dehydrator or oven at about 45°C to dry leaf tissue
- Lines for trapping the pathogen
  - Use less lines but more locations
  - Use susceptible but widely adaptable lines
  - Teams agreed to use 4 lines, #17 #23, #18, #24 (See Attachment 6 for list of germplasm)
  - China will also plant the lines and provide DNA
  - Use generic MTA (See Attachment 7 for draft of MTA, Materials Transfer Agreement) to agree on use of materials
- Locations: Minimum of 3 locations per country
- Sampling size
  - Plant a minimum of 10 plants per genotype per location
  - 2 leaf samples from separate plants for a total of 8 samples
- Inoculation and time of sampling
  - Use NATURAL infection!
  - Sample 3 weeks after planting or when there is good infection but before plants die

LINE EVALUATION

- Goal is to determine the reaction of a specific set of inbred lines to downy mildew for:
  - Possibly developing a set of differentials for downy mildew
  - Identifying sources of resistance useful in different regions
- For region-wide line evaluation, the teams agreed to use:
  - 30 lines (Sam Vasal's) + CML139 + few local lines of interest to each team
  - Test at a minimum of 2 locations per country
  - Use alpha lattice design, 2 reps per genotype per location,1 row/rep, min of 2.5 m per row
- Inoculation technique
- Use artificial inoculation
- Use spreader rows or whatever works to give enough and uniform pressure
  - Parameter to measure: % disease incidence
  - When to measure: 2X, at 12 and at 21 days post-emergence

Session 2. MAIZE FINGERPRINTING/DIVERSITY DISCUSSION

DIVERSITY STUDY

- Goals/objectives
  - Define heterotic groups
  - Understand genetic diversity of maize inbreds
  - Heterosis prediction
  - Develop more fingerprinting tools
  - Standardize methods

- Tools available
  - RFLP, SSR, AFLP
  - Level of genotyping defined by what we want from the data
  - Recommendation for teams to use SSRs principally and RFLPs if they wish so
  - For SSR markers, use of PAGE, internal lane and molecular weight standards to accurately size alleles of each SSR
  - For RFLPs, internal lane standards and molecular weight standards also necessary. No internal markers are necessary for AFLPs
  - Minimum number of markers- difficult to predict in advance; start with up to 50 and bootstrap to see if it is enough
- Scoring and storing data
  - Score conservatively- error of calling two identical bands different > calling two different bands identical
  - Connection with ICIS for possible storage of molecular data
- Sharing of fingerprinting data
  - Technical considerations make it very difficult to put together data from different sources.
  - Differences in the fingerprints of the same genotypes between labs could be due to differences in:
    - Amplification (cycling) parameters
    - Sources of Taq polymerase
    - Amplification (master mix) recipes
    - Type of gel, including agarose vs. PAGE; small PAGE vs. large PAGE gels; percent of acrylamide or ratio of bisacrylamide; manual vs. automatic sequencer gels
  - To make sharing possible:
    - use same markers
    - each group should always include the same set of 3-4 genotypes on all gels as reference gentoypes
    - use internal weight standards where it applies and molecular weights standards for best sizing of alleles
  - Can whole network share of data- do a common analysis?
    - Will decide later if feasible, priority for each team is to produce data for own purposes but use as much of the recommendations listed above as possible to make possible pooling of data feasible
    - Plan a workshop for optimizing procedures and comparing data among teams
    - Core set of 47 SSR primers for all teams to start with (these are common with the set that the Thailand team has used)
    - Use reference genotypes for each run (We have not defined those!!)
    - Scoring data- to be taken up in workshop
    - Data analysis- use same distance measures and clustering methods
- Data analysis
  - Molecular data: use bootstrapping to make sure you have a good data set
  - Multivariate analyses for clustering/grouping
  - Field data analysis in the case of heterosis prediction: regression of yield data with the genetic distances
  - For testers, use at least 2, depending on hybrid strategy and need

Session 3. TRAIT MAPPING DISCUSSION

- Parental selection
  - Choose contrasting phenotype that is stable
  - Check the reaction in country, may want to even see F3 distribution for trait of interest before starting to map
  - Consider level of homozygosity (at least S4 level)
  - Level of polymorphism between the parents (usually not a concern for maize)
- Segregating populations
  - Several options: F2/F3, BC1F1/BC1F2, RILs

- F2 or BC1F1 for genotyping, F3 or BC1F2 for phenotyping
- RILs are genotyped and phenotyped
- F2/F3 vs RILs, consider the time factor in deciding which way to go
- For breeding applications, it is best to map in same population where you want to improve the trait to avoid mapping in one population then doing MAS in another
- How to develop populations: Do not bulk pollinate, always plant-to-plant, do not bulk seeds from different F1 ears
- Remember these are populations for genetic, not breeding, purposes, no selection should be applied
- 250 families is a good size for DM study

- Genotypic identification
  - Correct labeling (i.e. same number in field and lab) is critical
  - For markers, consider those that have a good distribution in the genome and good quality bands
  - Look at segregation ratios, discard any markers with bad or doubtful data
  - For teams working on same problem (downy mildew or drought), use common set of markers
  - Precise scoring of markers is as important as precise scoring in the field of the trait(s)
- Phenotypic evaluation
  - Measure the trait in the best way possible
  - To cover for GxE, use best experimental designs: alpha lattice design and at least two replications, large population sizes 200-250 families, and evaluate in more than 1 location
- Statistical analyses- CIM has advantages for QTL analysis over simple interval mapping
- Considerations for marker assisted selection:
  - A QTL map has to be made in every population where the transfer of the trait is to be achieved
  - BC-MAS is not therefore not very cost effective, cannot be routine application
  - CIMMYT considering alternative scheme (large-scale F2 approach), but still in concept stage
  - NOTE: Although MAS may not always be feasible for complex traits, the QTL mapping exercise provides basic information on the genetics of the trait

<u>DOWNY MILDEW</u>

- Populations for DM project
  - Indonesia and India are developing their own F2 populations for DM mapping
  - Thailand is using Brewbaker's set G RILs
  - Philippines will probably develop a new population rather than going with the one they previously chose
  - Egypt ATUT project: F2 populations of Dan and Mireille
  - Ki3xCML139 (AxB) cross of CIMMYT with broad-based insect resistance could be evaluated for downy mildew since Ki3 showed very good resistance in two locations in the Philippines (data from this network activities) and a linkage map already available
  - Brewbaker's set G RILs will be phenotyped in India, Indonesia and the Philippines
- Field evaluation: use same recommendations as in the downy mildew discussion, but it is recommended to inoculate by spore suspension in the whorl (instead of spreader rows) to ensure as uniform an infection as possible

<u>DROUGHT</u>

- Each team (China, India and Thailand) will develop its own population F2/F3 mapping population
- CIMMYT has populations (RILs) already mapped for drought tolerance
- Parameters to measure for drought tolerance: Teams agree on 3 criteria to look at
  - ASI
  - Recovery from wilting one month after emergence
  - Grain yield
  - Tassel blast and leaf firing will also be recorded
- Apply moderate and severe stress, whether to use normal irrigation as control will depend on availability of seeds
- Precision in experimental conditions critical in getting good data

Session 4. TRAINING

NEEDS ASSESSMENT

- Basic molecular marker course
  - Only Indonesia needs it
- Suggested advanced courses
  - Diversity analysis, QTL analysis
  - Crop information system
- Topic-specific short workshops
  - Maize and DM fingerprinting workshop
  - Drought- to be discussed in next planning meeting

TRAINING OPTIONS

- Group training: CIMMYT, regional, national
- Individual training
  - Exchange visitor at CIMMYT
  - Exchange visitor in region
  - Shuttle research within country
- NOTE: training in the region is desirable, demonstrates strengthening of NARS capacity

AGREEMENTS

- Thailand team will host a one week workshop on diversity analyses with SSR markers
  - Two molecular geneticists from all other teams will attend, preferably the project leader and the person who will be producing the diversity data
  - The course will include: lab exercises on silver staining PAGE on sequencing gels, proper scoring of SSR alleles, and analyses of data
- Philippines team agreed to host two trainees from Indonesia (RIFCB, RIMC) for two months to learn basic molecular genetics techniques and to generate data on parental screening, pathogen fingerprinting and go over DM trials with pathologist.

Evening Session 1. DEMONSTRATION OF MAPDATA FOR WINDOWS

HyperMapData is a Mac-based program for the entry, verification, preliminary analyses and export of segregating data. It was developed by David Hoisington at CIMMYT and is available free of charge. It has been a very helpful tool in our mapping efforts at CIMMYT. It was decided at the First AMBIONET meeting last year at Bangkok that AMBIONET funds should be allocated for the development of a PC-based equivalent of HyperMapData. This work has successfully proceeded and was done by programmers in CIMMYT's Software Development Group. MAPDATA version 1.0.0 was brought to Beijing and demonstrated to all participants. It is not 100% bug free and still lacks the feature of storing phenotypic data, but is very close to its original counterpart. The program was handed to each team along with a manual and team members were requested to try it out ASAP and send their comments back to CIMMYT for further improvements.

Participants have also asked that HyperMAS, another Mac-based program developed at CIMMYT to help us in MAS, be translated to a PC-version. This is now under study to llok at costs and timing.

Evening Session 2. STEERING COMMITTEE MEETING

The AMBIONET Steering Committee members convened their second meeting. All countries participating in the Network were represented. Mangala Rai, Deputy Director General of the Indian Council of Agricultural Research, was elected Chair of the AMBIONET Steering Committee, replacing Sutat Sriwatanapongse, who served as interim Chair for the first year. The minutes of the meeting are appended in Attachment 8.

Day 3

Session 1: TOUR OF BIOTECHNOLOGY FACILITIES AT CAAS

The morning session was spent visiting the biotechnology facilities at the CAAS, including the newly constructed building of the Institute of Plant Breeding and Cultivation. Dr. Xin Zhiyong and Dr. Dave Hoisington inaugurated the laboratory of the AMBIONET-China team with the unveiling of a plaque. Among the CAAS facilities visited were the Germplasm Bank, Plant Biotechnology Center, and others.

Session 2: FINANCES, IDEAS FOR PHASE 2

FINANCES

An amount of US$5,000 will be allocated to each team for inter-team interactions. Activities that may be funded from this amount may be travel of team leaders, co-team leaders or activity leaders to other teams. Specific activities such as consultation about research, laboratory set-ups, seminars to be presented by the traveler, etc. are suggested for the trip. A trip report will be required of the person who travels.

Teams may submit proposals for extra funding. Activities such as field evaluations or additional equipment may also be proposed.

IDEAS FOR PHASE 2

Additional countries, additional teams in the same country, and additional research themes are some of the possibilities mentioned for Phase 2 of AMBIONET. Specific suggestions included:

- Moving into applications, i.e. MAS for DM and drought
- Other traits: QPM (Quality Protein Maize), value-added traits, banded and leaf sheath blight, abiotic stresses
- More training
- Involvement with private sector, other advance research institutes
- Physical mapping and into cloning major virus resistance genes
- Gene discovery, functional genomics and reverse genetics
- Transgenics
- Apomictic maize

NEXT MEETING

Next year's meeting will be hosted by the India team in New Delhi and is tentatively scheduled for 6-10 March 2000.

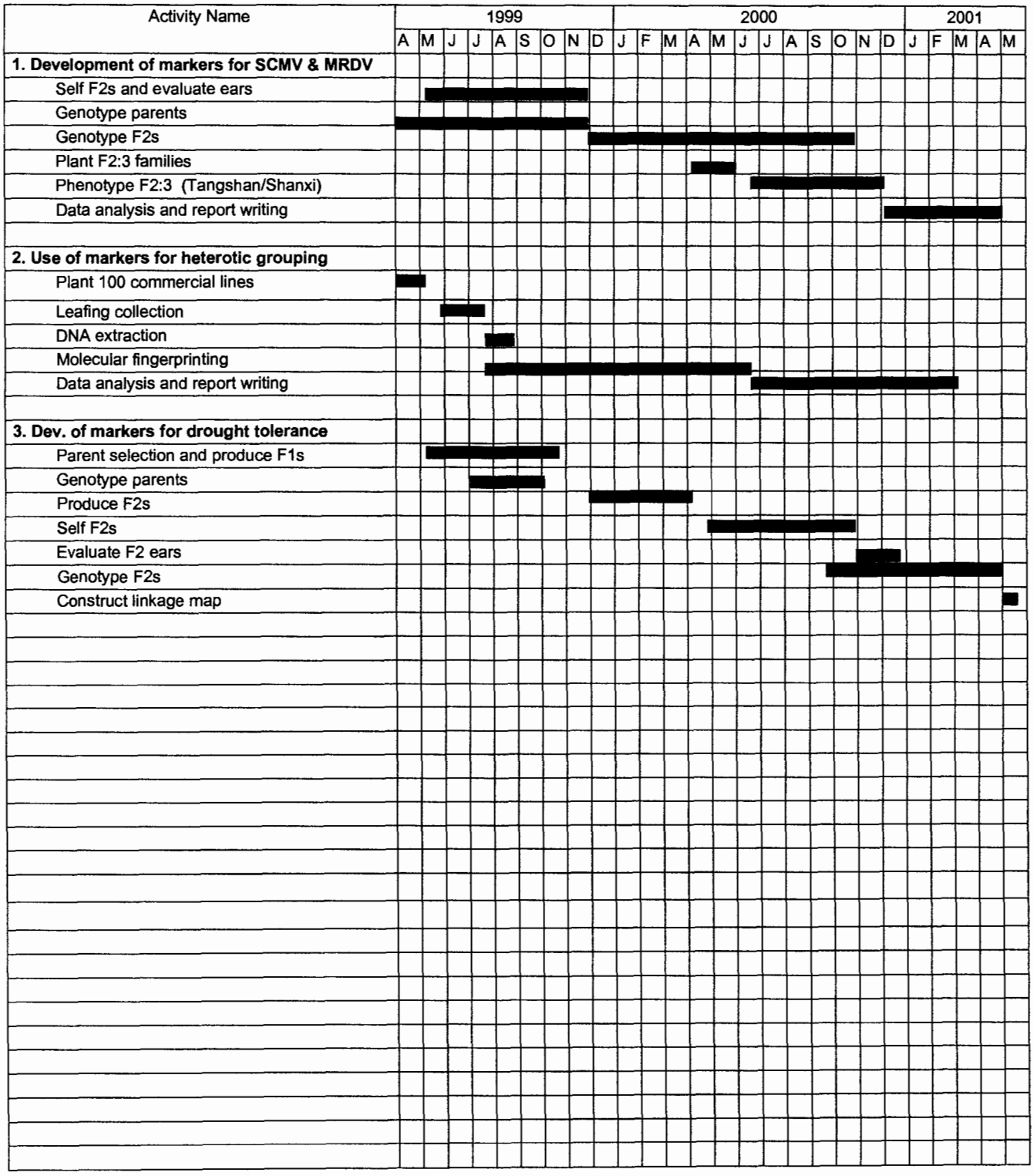
Session 3: WORKPLANS

After final revision of their workplans, the five teams presented their workplans and timelines for the second year to the group (Chapter 3).

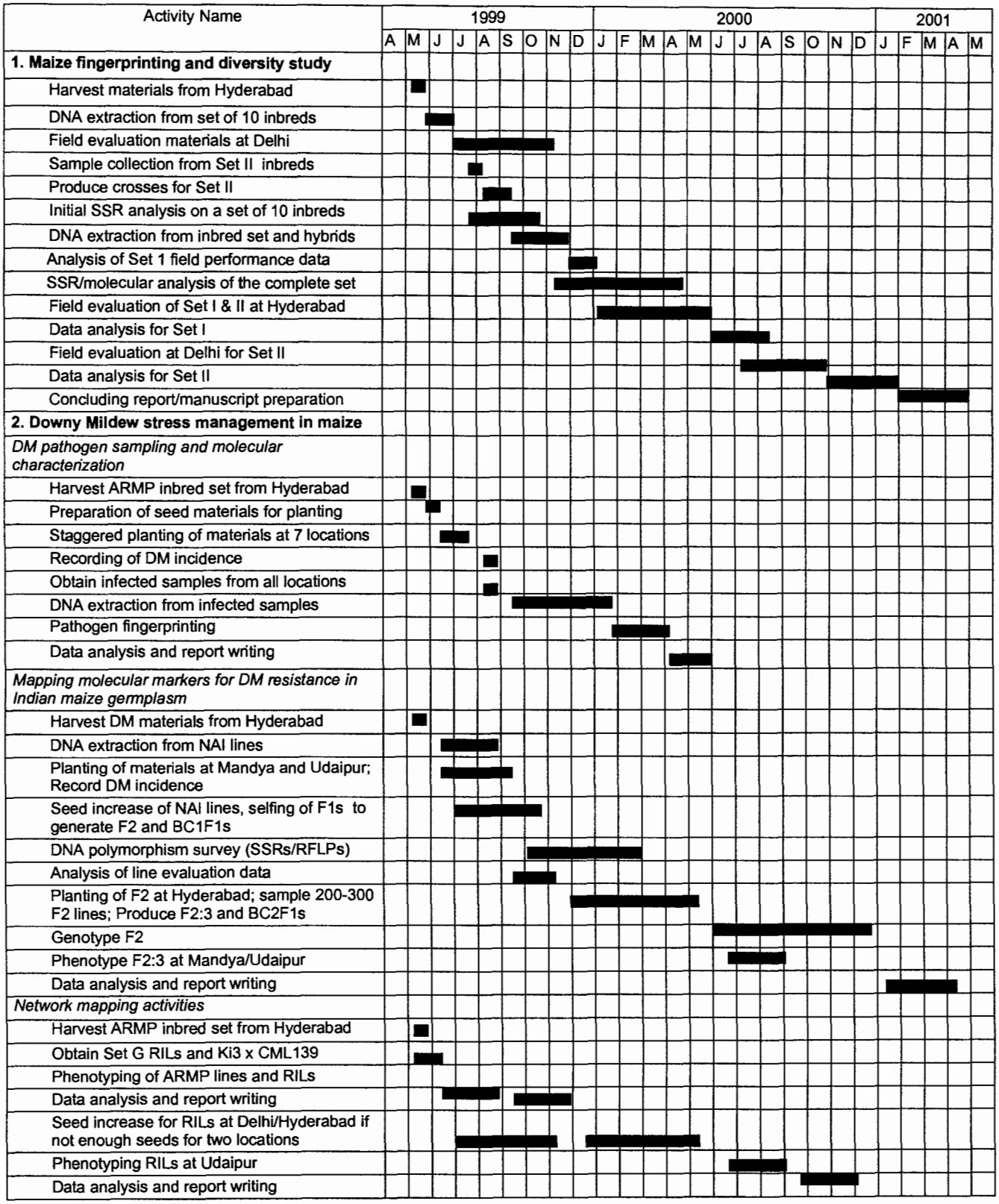
The participants evaluated the meeting after closing comments from Dave Hoisington (See Attachment 9 for the results of the meeting evaluation).

CHAPTER 3
Country Timelines

CHINA



INDIA


Activity Name
1999
2000
2001
A M J J A S O N D J F M A M J J A S O N D J F M A M
1. Maize fingerprinting and diversity study
Harvest materials from Hyderabad
DNA extraction from set of 10 inbreds
Field evaluation materials at Delhi
Sample collection from Set II inbreds
Produce crosses for Set II
Initial SSR analysis on a set of 10 inbreds
DNA extraction from inbred set and hybrids
Analysis of Set 1 field performance data
SSR/molecular analysis of the complete set
Field evaluation of Set I & II at Hyderabad
Data analysis for Set I
Field evaluation at Delhi for Set II
Data analysis for Set II
Concluding report/manuscript preparation
2. Downy Mildew stress management in maize
DM pathogen sampling and molecular characterization
Harvest ARMP inbred set from Hyderabad
Preparation of seed materials for planting
Staggered planting of materials at 7 locations
Recording of DM incidence
Obtain infected samples from all locations
DNA extraction from infected samples
Pathogen fingerprinting
Data analysis and report writing
Mapping molecular markers for DM resistance in Indian maize germplasm
Harvest DM materials from Hyderabad
DNA extraction from NAI lines
Planting of materials at Mandya and Udaipur; Record DM incidence
Seed increase of NAI lines, selfing of F1s to generate F2 and BC1F1s
DNA polymorphism survey (SSRs/RFLPs)
Analysis of line evaluation data
Planting of F2 at Hyderabad; sample 200-300 F2 lines; Produce F2:3 and BC2F1s
Genotype F2
Phenotype F2:3 at Mandya/Udaipur
Data analysis and report writing
Network mapping activities
Harvest ARMP inbred set from Hyderabad
Obtain Set G RILs and Ki3 x CML139
Phenotyping of ARMP lines and RILs
Data analysis and report writing
Seed increase for RILs at Delhi/Hyderabad if not enough seeds for two locations
Phenotyping RILs at Udaipur
Data analysis and report writing

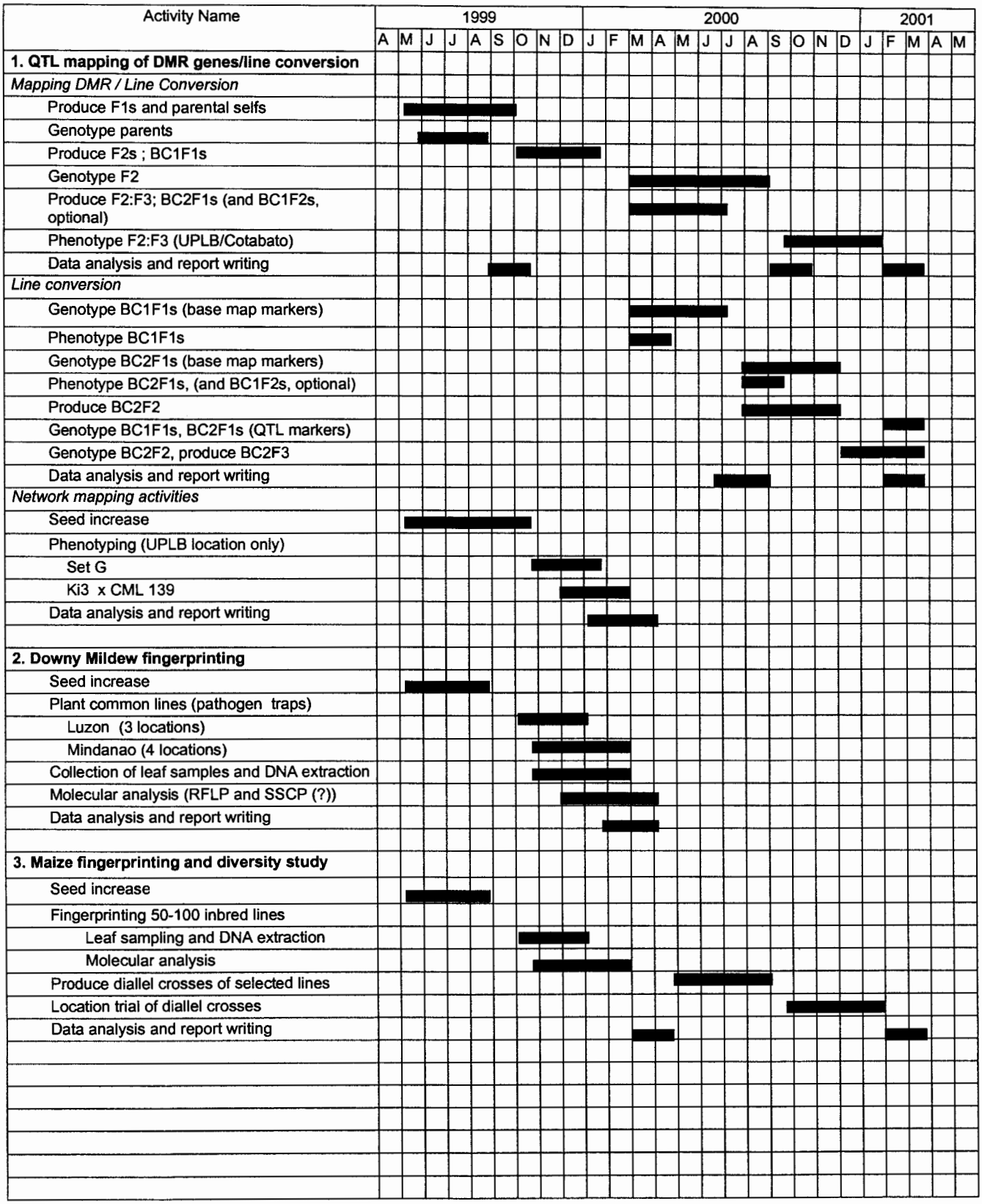
INDIA (Cont.)

Activity Name	1999									2000												2001				
	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
3. Mapping for drought tolerance in Indian maize germplasm																										
Obtain drought RIL set from CIMMYT (if seed is avaialble)																										
Planting of experimental set at Karimnagar (if possible) for screening inbreds/RILs for drought tolerance/susceptibility																										
Repeat experiment in winter season at Hyderabad to confirm parental lines for further analysis; Generate F1																										
Analysis of field evaluation data from Karimnagar and Hyderabad																										
Planting of parents and F1 at Delhi; generation of F2 and BC2F1s																										
DNA extraction from parental lines																										
DNA analysis for polymorphism																										
Continue Activity in Phase II (if possible) for mapping molecular markers for drought tolerance on the basis of materials and information generated from Phase I																										

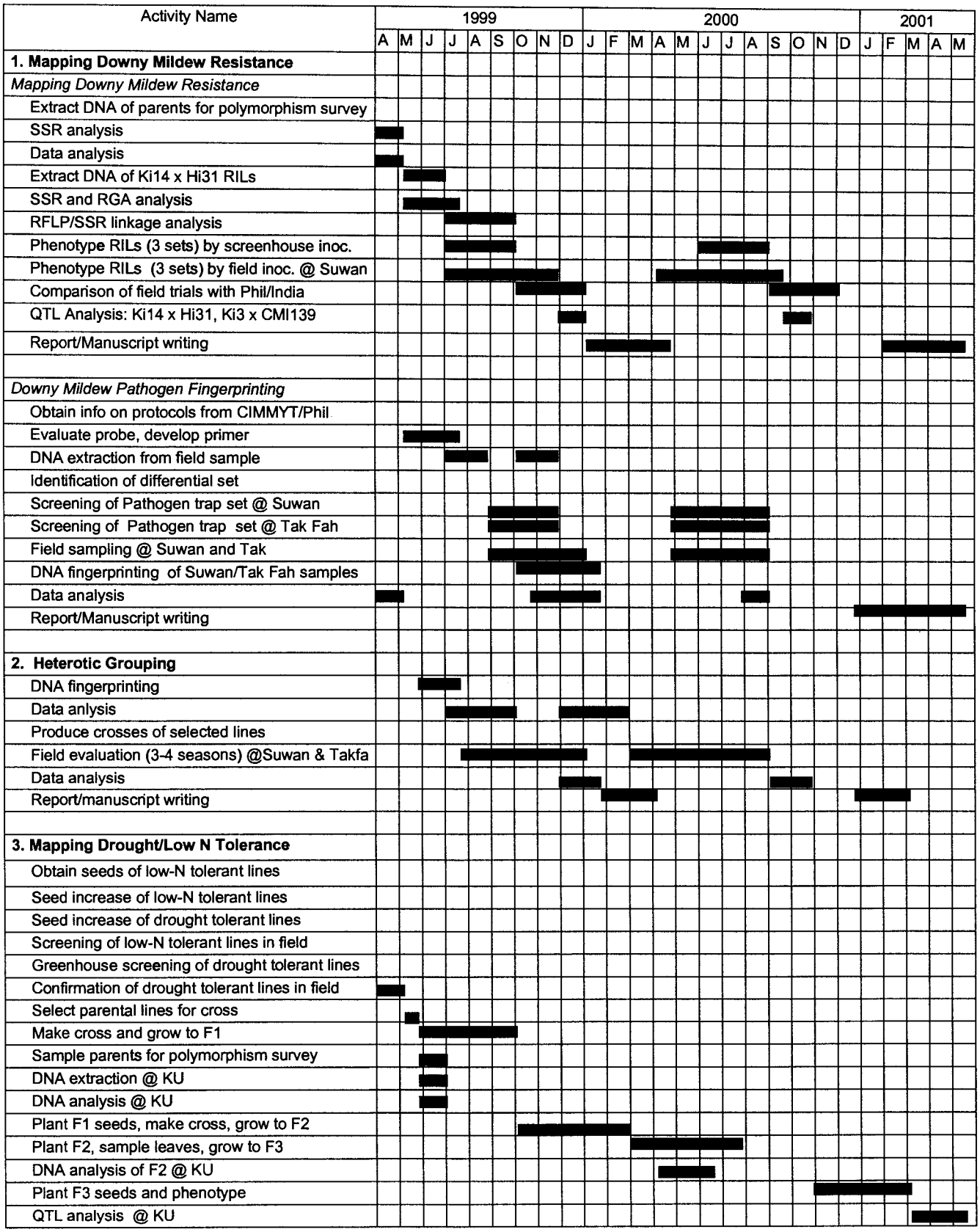
INDONESIA

Activity Name	1999									2000												2001				
	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M
1. MAS for DM resistance in Indonesian inbred																										
Characterization of germplasm																										
Identify materials/Seed Shipment *																										
Planting and Phenotyping :																										
Maros, S. Sulawesi																										
Bogor, West Java																										
Lampung, Sumatra																										
Pathogen characterization																										
Tissue sampling **																										
Maros, S. Sulawesi																										
Bogor, West Java																										
Lampung, Sumatra																										
DNA extraction																										
Pathogen fingerprinting																										
Phenotyping of common RIL for DM																										
Seed Shipment																										
Field evaluation, Maros, S. Sulawesi																										
Mapping of team F2:3 Population																										
Parental survey																										
Cross																										
Advanced F1 → F2																										
Advanced F2 → F3																										
Segregation analysis :																										
By Molecular marker																										
By Phenotype (Bogor & Maros)																										
QTL analysis																										
Preparation of MAS for DM resistance in Indonesian hybrids																										
Cross																										
Develop Backross (BC1)																										
Advanced BC1F1 → BC2F1																										
Advanced BC2F1 → BC2F2																										
Molecular selection																										
Phenotypic selection																										
2. Genetic diversity and hybrid performance																										
Select lines (46 lines)																										
Grow materials (Bogor)																										
Collect leaf samples																										
Extract DNA																										
DNA analysis																										
Make diallel cross																										
Harvest material																										
Plant single crosses, parental lines																										
Harvest single cross trial																										
Data analysis																										
Report writing																										

PHILIPPINES



THAILAND


Activity Name
1999
2000
2001
A M J J A S O N D J F M A M J J A S O N D J F M A M
1. Mapping Downy Mildew Resistance
Mapping Downy Mildew Resistance
Extract DNA of parents for polymorphism survey
SSR analysis
Data analysis
Extract DNA of Ki14 x Hi31 RILs
SSR and RGA analysis
RFLP/SSR linkage analysis
Phenotype RILs (3 sets) by screenhouse inoc.
Phenotype RILs (3 sets) by field inoc. @ Suwan
Comparison of field trials with Phil/India
QTL Analysis: Ki14 x Hi31, Ki3 x CMI139
Report/Manuscript writing
Downy Mildew Pathogen Fingerprinting
Obtain info on protocols from CIMMYT/Phil
Evaluate probe, develop primer
DNA extraction from field sample
Identification of differential set
Screening of Pathogen trap set @ Suwan
Screening of Pathogen trap set @ Tak Fah
Field sampling @ Suwan and Tak
DNA fingerprinting of Suwan/Tak Fah samples
Data analysis
Report/Manuscript writing
2. Heterotic Grouping
DNA fingerprinting
Data anlysis
Produce crosses of selected lines
Field evaluation (3-4 seasons) @Suwan & Takfa
Data analysis
Report/manuscript writing
3. Mapping Drought/Low N Tolerance
Obtain seeds of low-N tolerant lines
Seed increase of low-N tolerant lines
Seed increase of drought tolerant lines
Screening of low-N tolerant lines in field
Greenhouse screening of drought tolerant lines
Confirmation of drought tolerant lines in field
Select parental lines for cross
Make cross and grow to F1
Sample parents for polymorphism survey
DNA extraction @ KU
DNA analysis @ KU
Plant F1 seeds, make cross, grow to F2
Plant F2, sample leaves, grow to F3
DNA analysis of F2 @ KU
Plant F3 seeds and phenotype
QTL analysis @ KU

CHAPTER 4
Action Steps

SUMMARY OF ACTION STEPS

Action	When	Responsible person
Research Program Action Steps		
1. Send SSCP PCR protocol to Mireille	ASAP	Apichart
2. Do the alpha lattice design and send to all teams	ASAP	Dan
3. Provide detailed activity plans to resource scientists	ASAP	Team/Activity Leaders
4. Check with Dave Bergvinson regarding availability of seeds (AxB population)	ASAP	Mireille
5. Send 20 seeds/line of Brewbaker's RILs seeds to Sam	When the seeds arrive from Hawaii	Luz
6. Seed increase of Brewbaker's RILs seeds	When the seeds arrive from the Philippines	Sam
7. Provide genotype data of Brewbaker's RILs to teams	ASAP	Apichart
8. Send protocol on recommended inoculation method to teams	ASAP	Dan
9. Check the availability of seeds for drought study and send info to teams	ASAP	Dave
10. Send DM RFLP probes to China	ASAP	Mireille
Network Management Action Steps		
10. Send estimated cost of Thailand workshop to Luz	ASAP	Apichart
11. Send proposals of team activity for extra funds to Luz/Dave	May	Team Leaders
12. Prepare meeting report	May	Luz
13. Report to ADB	May	Dave and Luz

ATTACHMENTS

Attachment 1

Group photo of Meeting Participants

Participants in the 2nd AMBIONET Annual Planning Meeting in Beijing, China, 27 – 30 May 1999. L to R, seated: S. Sriwatanapongse, M. Khairallah, D. Hautea, M.L. George, E. Anit, Fu Junhua, He Zhonghu, Han Nanping; L to R, standing: Li Xinhai, M. Dahlan, Y. Chantachume, Shihuang Zhang, Xin Zhiyong, A. Vanavichit, D. Jeffers, Wang Ren, D. Hoisington, D. Damardjati, M. Rai, F. Kasim, B. M. Prasanna, S. Vasal, A. Salazar, N.N. Singh, Dafang Huang.

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Meeting Program

27 APRIL, TUESDAY

8:30 – 8:45	**WELCOME** Ren Wang, Xin Zhiyong
8:45 – 9:00	**WELCOME/OVERVIEW OF MEETING/OBJECTIVES/AGENDA** Dave/Luz
9:00 – 9:30	**INTRODUCTIONS**
9:30 – 10:00	**SUMMARY OF AMBIONET ACTIVITIES (Year 1)** Luz
10:00 – 10:30	**Tea/Coffee Break**
10:30 – 11:30	**COUNTRY PROGRESS REPORT: China** S Zhang/ X Li
11:30 – 12:30	**COUNTRY PROGRESS REPORT: India** NN Singh/Prasanna
12:30 – 1:30	**Lunch**
1:30 – 2:30	**COUNTRY PROGRESS REPORT: Indonesia** Firdaus/Sugiono
2:30 – 3:30	**COUNTRY PROGRESS REPORT: Philippines** Des/Art
3:30 – 4:00	**Tea/Coffee Break**
4:00 – 5:00	**COUNTRY PROGRESS REPORT: Thailand** Apichart/Yod
5:00 – 5:10	**REVIEW OF DAY/OVERVIEW OF WEDNESDAY** Luz
evening	**DEMO OF PC-BASED HYPERMAP DATA** Mireille
evening	**SC MEETING** Sutat/Dave

28 APRIL, WEDNESDAY

8:30 – 8:45	**OVERVIEW OF DAY** Luz
8:45 – 9:30	**FINANCIAL ISSUES** Luz/Dave
9:30 – 10:30	**DM FINGERPRINTING DISCUSSION** **Techniques, Future Plans, Etc.** Dan

Meeting Program (Cont.)

10:30 – 11:00	**Tea/Coffee Break**
11:00 – 12:30	**MAIZE FINGERPRINTING DISCUSSION** **Germplasm, Methods, Data Storage/Analysis, Etc.** Mireille
12:30 – 1:30	**Lunch**
1:30 – 3:00	**TRAIT MAPPING DISCUSSION** **Populations, Field Work, Markers, Strategies, Data Analysis, Etc.** Mireille
3:00 – 3:30	**Tea/Coffee Break**
3:30 – 4:30	**TRAINING** **Needs Assessment, Options, Year 2 Activities** Luz/Mireille
4:30 – 5:30	**TEAM MEETINGS: Revise Workplans**
5:30 – 5:40	**REVIEW OF DAY/OVERVIEW OF THURSDAY** Luz
evening	**TEAM MEETINGS: Finalize Workplans**

29 APRIL, THURSDAY

9:00 – 12:00	**TOUR OF BIOTECHNOLOGY LABS IN CAAS** S. Zhang
12:00	**Lunch**
1:00 – 3:00	**YEAR 2 WORKPLAN PRESENTATION (15-20 Min Per Team)** Luz/Teams
3:00 – 3:30	**Tea/Coffee Break**
3:30 – 4:30	**BUDGET (Revisit)** Luz/Dave
4:30 – 5:00	**FEEDBACK ON SECOND PHASE IDEAS** Dave
5:00 – 5:30	**NEXT STEPS/EVALUATION OF MEETING** Luz
5:30 – 6:00	**CLOSING COMMENTS** Luz/Dave
evening	**CLOSING DINNER**

List of Participant Expectations

- Interactions among members leading to synergies in the Network
- Good scientific discussions
- Review of team activities- how much has each team done?
- Lessons learned from each team: successes, failures, solutions
- What do we still have to do?
- Discuss products of network, challenge of issues such as germplasm exchange
- Learn from other countries- how to coordinate different components of a team
- Learn new technology used by others, such as their applications in hybrid corn
- Learn what realistic technology can be applied to complex traits such as drought tolerance
- Link up conventional breeding with biotechnology
- Develop friendships
- Get new ideas on hybrid breeding, virus resistance
- Discuss new technology such as transgenic research
- Explore possibility of enlarging project
- Hike the great wall!
- Further strengthen collaboration by sharing materials and ideas
- Stronger working relationships between countries
- Learn about China experience- big country, agricultural productivity
- Show impact of training courses- example: CIMMYT's graduates Des and Prasanna
- Permeability of ideas
- Breeders and biotechnologies- complementarities and common ground
- Find speedier solutions to complex problems through the network
- Form new linkages
- Review, reflect, learn
- Leave with a clear sense of commitment

Elements of Team Work: Suggestions from AMBIONET Teams

- Clear definition of responsibilities
- Strengthen links in country, get support from administrators, aim for national good
- Look at lessons learned in facing challenges as outputs
- Setting reasonable expectations
- Commitment, less concern about who gets the credit and put the good of the country first
- Communications, especially face to face communication
- Develop goodwill and trust in the team
- Openness- willingness to accept changes, compromise (do not resist!)
- Breeders and biotechnologists work together by complementation and sharing resources
- Respect for each person in the team
- Get people involved to develop a sense of belonging: each team member is important
- Support from the top
- Spend time in each other's fields/lab
- Mutual accountability, do not blame
- Overcome geographical barriers and mental barriers

List of Inbred Lines for Downy Mildew Study

No.	Inbred line
1	Pi21
2	Pi31
3	Nei9008
4	Nei9203
5	Nei9204
6	Ki3
7	Ki14
8	AMATLCOHS115-2-3-3-1-2-B-B
9	AMATLCOHS233-1-1-1-1-2-2-B-B-B
10	P345C3S3B-40-8-1-1-2-2-B
11	AMATLCOHS9-1-1-1-1-1-2-B
12	AMATLCOHS245-1-1-1-2-1-1-B-B
13	P345C4S2B46-2-2-1-2-B-B-B
15	IPB9204-1-3-1-2-4-B
16	24STE-5*24STE-17)-BBBB###-B-1-B-2-B-B-B
17	24STE-5*24STE-17)-BBBB###-B-5-B-4-B-B-B
18	SIN.AM.TSR-76-1-1-B-1-BBBB-5-##-BBBBBBBBB
20	P24(STE)C2-29-BBBB-#-3-BBBBBBB
21	G26 C25 HS45-3-4-1-6-BBBB
22	CML20
23	CML270
24	CML289
25	CML272
26	P8
27	P12
28	Pi23
29	Pi27
30	Pi35
31	CML 139

Materials Transfer Agreement

Research Results, Products, Germplasm and Intellectual Property Rights

Any intellectual property which a party contributes for use in the research activities of AMBIONET will remain the property of the original party. Material Transfer Agreements (MTA) or Licenses will be signed by the receiving parties prior to exchange of any protected material.

All germplasm exchanges between AMBIONET participants will be done under an MTA, which will clearly state the rights, if any, the recipient has to the respective germplasm.

As all results and projects from AMBIONET research are considered an international public good, little if any protection of intellectual property is anticipated. If the participants mutually agree that protection is necessary to meet the goals of AMBIONET and to assure that the material remains in the public sector, all issues related to ownership, filing and costs will be decided by mutual consent prior to seeking any form of protection.

Attachment 8

Minutes of Steering Committee Meeting

AMBIONET Steering Committee, Meeting of 27 April 1999, Beijing, China

Present were: David Hoisington (CIMMYT), Dafang Huang (China), Mangala Rai (India), Djoko Damardjati (Indonesia), Edna Anit (representing William Dar, Philippines), Sutat Sriwatanapongse (Thailand), Maria Luz George (AMBIONET Coordinator, secretary)

Apologies from: Private sector representatives Brent Zehr and Manny Logrono

AGENDA

1. Minutes of last meeting
2. Election of SC Chairman
3. Others

I. Approval of Minutes of Last Meeting

Item 1: Roles and responsibilities of the Steering Committee
Approved.

Item 2: Membership of Steering Committee
Approved.
Clarification:
Philippine country representative William Dar is no longer connected to PCARRD. The selection of who could best represent the Philippines will be a country decision.

Item 3: Meeting schedule
Approved.
Suggestion: to add a qualifier, to "once a year, preferably, coinciding with the annual project review meeting"

Item 4: New member countries
Approved.
Suggestion: to rephrase sentence to "Member countries will be encouraged to join in the second phase".

II. Election of the Chairman

India Country Representative Dr. Mangala Rai was elected Chairman of the Steering Committee, replacing interim Chairman Dr. Sutat Sriwatanapongse. It was agreed that the Chairmanship would be rotated, with a new Chairman elected each year.

III. Others

A. Review of Network progress

- There was general agreement that the AMBIONET in now working in a network mode, and that the planning process has been satisfactory.
- The Network activities are on a sound scientific footing and are expected to yield tangible results.
- The first three years of the Network will accomplish capacity building, develop teamwork, and generate enough information on heterosis and downy mildew disease.
- Thus, the coming year will be critical for the next phase, must have enough results to land a second phase.
- The country representatives in the Steering Committee have a critical role to support and facilitate the country team so that they can accomplish their goals.

Minutes of Steering Committee Meeting (Cont.)

B. Sustainability of Network

- Next year, the Network should focus on identifying donors to approach to put the Network in a sustainable state
- The Network should consider other donors or the governments of the partner countries as other sources of funds
- SC members should report AMBIONET progress to higher authorities

Philippines: PCARRD is informed of the progress of Philippine team and additional funds were given to the AMBIONET-Philippine team

Thailand: Making the government aware of biotechnology activities by inviting government administrators to visit the KU lab. Thrust towards stronger interaction with the private sector, initially on biosafety and IPR issues, leading to interactions in more issues.

China: Agricultural biotechnology gets very high government priority, the government pays attention to the transgenic approach. Support for maize lags behind that of other crops. It is difficult to interact with the private sector on the research level.

CIMMYT: There is agreement on the management level to submit a second-phase proposal to ADB.

C. Phase 2 of project- How can SC help to get extension of the project?

- Extension will depend in performance, the synergy that is developed in the Network.
- Critical role of SC is to help in determining what a second phase will be.
- Possible second phase options are the addition of other countries, other institutes in a country, integrate genetic engineering into project, etc.
- The coming year will answer a lot of the questions regarding IP issues/ownership issues. Before second phase, some of the issues will be clarified and will put the Network in a sound footing.

D. Capacity-building and the issue of personnel turnover

- The issue of team members who undergo training and leave AMBIONET. It was suggested to explore the possibility of requiring a bond as a prerequisite for training.

E. Third AMBIONET meeting

- India will be the host.

Results of Meeting Evaluation

1. **What are your comments about the meeting in terms of:**

A. Content (agenda):

- Just enough to be covered in a 4-day period
- Just right
- Comprehensive, realistic
- OK
- OK, good
- Good (5)
- Good, but there should have been a paper from each country.
- Very Good
- Adequate
- The agenda was very well drafted with adequate time allotted
- Well set and properly executed

B. Process (facilitation)

- It was an excellent one
- There were some who "participated" more... but that's understandable
- Excellent!
- Excellent, establishing a friendly and open atmosphere on the first day was critical.
- Good (5)
- Good, also need improvement
- Very Good (2)
- Very smooth
- Lively facilitation encouraged all to participate
- A little bit too much details at some points, but in overall, it was good

C. Participant interactions:

- There was an active interaction; every idea was made clear without hesitation.
- Very great
- Excellent!
- Excellent. All contributed.
- Excellent, but very little time for full interaction with all members from all teams due to hectic schedule.
- With small number of participants, it is very good.
- Considerable, efficient, effective but still there is vast room to improve. It must be wide. It is hoped that the foundation laid would ensure in the next meeting further greater interactions.
- OK
- Not very actively for me
- General ?
- Very good
- Good (3)
- Good, we learn some good ideas from each other

C. Arrangements (coordination of travel, logistics):

- It was something to be commended.
- The hotel deposit for long distance was unexpected. We didn't know the arrangement for dinner when we arrived.
- Everything (invitations, tickets) arranged on time with frequent follow-ups and reminders
- Very good (3)

Results of Meeting Evaluation (Cont.)

- Good (5)
- Excellent, very fast action for late participants
- Reasonable
- Very good, enough time was given to get the Government clearance and informed from time to time by the Coordinator
- Absolutely fine

C. Length of meeting

- Just right (3)
- OK (6)
- Fair
- Adequate
- Maybe 1 day more, if possible
- Quite ?
- Not very long
- Not very long, some people need one more day in Beijing

2. What did you find most useful about the meeting?

- Interactions
- Interaction, fellowship, useful comments to improve our work
- Networking within or among the groups
- Discussion and exchange of ideas between countries
- Good discussion on downy mildew/other diseases and genetic diversity
- In general, entire meeting was quite good. Interactions and discussions were excellent.
- Interactions with Resource Scientists and other teams
- It is now moving in the network mode. Discussions have brought far more clarity and commitments to the cause and the programme. Now there is greater understanding and ---- to the activities in perspective.
- Discussion and planning up to the minutest details.
- Interactions with other teams and resource personnel; thorough discussion about methodology
- Interaction and attention during the meetings
- Discussions
- The important consideration about diversity analysis, discussion about DM and drought tolerance
- Exchange ideas and modify workplan
- Most of all ?

3. Were your expectations met? If not, what expectations were not met?

- Yes (7)
- Yes, they were met.
- Yes, I like being asked about my expectations.
- Yes, with the 4-day long association (officially and non-officially), it is evident that strong collaboration among member countries exits.
- Most expectations were very well met.
- Satisfied
- Yes, we have to go a long way before addressing problems concerning abiotic stressess e.g traits responsible for drought tolerance, etc.
- Mostly, not yet met is hiking the Great Wall (slated for tomorrow).
- Yes, but I wish we can get more assistance in transgenic maize research.
- Yes, we should think more what we will do in phase II.

Results of Meeting Evaluation (Cont.)

4. **What could have been done better?**
   - None
   - Nothing
   - Clearer instruction even before departure (thru email) on travel, dining and financial arrangement upon arrival at the venue
   - In the initial presentations, targets should have been brought about and reporting should have been with respect to the target for each of the activities.
   - Presentation of a research summary report before the presentation made by individual countries
   - Possibility of visiting maize fields (if maize is being grown now); more interactions with maize workers in China.
   - It may be more convenient if the meeting place is at or close to the hotel.
   - Food is not good enough.
   - Arrange first or early and work together
   - Exchange information

5. **Are there any outstanding issues that still need to be resolved? What are they and how should they be resolved?**
   - No (2)
   - None
   - Nothing
   - Nothing I could think of.
   - MAS for drought-tolerance selection, parameters and mapping
   - Within country budget management and report
   - Supply of equipments
   - Germplasm exchange
   - Some issues such as biosafety and IPR need to be discussed further
   - Details with respect to field trial procedures
   - Information communication is a problem. We should strengthen the internet communication
   - Drought work needs more discussion

6. **What comments do you have about the meeting facilities/hotel accommodations, and arrangements?**
   - None
   - Spartan, but comfortable enough.
   - It was a good hotel.
   - Not very bad
   - Excellent and congratulations to CIMMYT group and the CAAS staff as host.
   - Everything is very good. For Muslim participants, participants have been assigned to a special table.
   - Good.
   - Very good
   - Overall, very good
   - Excellent (2)
   - Excellent. Chinese friends are very kind- Thanks for hospitality.
   - Quite good and satisfactory
   - Leave one evening to participants for shopping