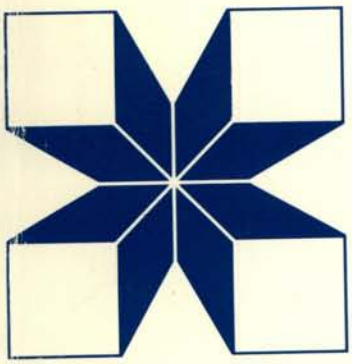
IDRC
CRDI
CIID



CANADA

OIL CROPS:
PROCEEDINGS OF THE THREE MEETINGS HELD AT PANTNAGAR AND HYDERABAD, INDIA, 4 – 17 JANUARY 1989

The International Development Research Centre is a public corporation created by the Parliament of Canada in 1970 to support research designed to adapt science and technology to the needs of developing countries. The Centre's activity is concentrated in six sectors: agriculture, food and nutrition sciences; health sciences; information sciences; social sciences; earth and engineering sciences; and communications. IDRC is financed solely by the Parliament of Canada; its policies, however, are set by an international Board of Governors. The Centre's headquarters are in Ottawa, Canada. Regional offices are located in Africa, Asia, Latin America, and the Middle East.

Le Centre de recherches pour le développement international, société publique créée en 1970 par une loi du Parlement canadien, a pour mission d'appuyer des recherches visant à adapter la science et la technologie aux besoins des pays en développement; il concentre son activité dans six secteurs : agriculture, alimentation et nutrition; information; santé; sciences sociales; sciences de la terre et du génie et communications. Le CRDI est financé entièrement par le Parlement canadien, mais c'est un Conseil des gouverneurs international qui en détermine l'orientation et les politiques. Établi à Ottawa (Canada), il a des bureaux régionaux en Afrique, en Asie, en Amérique latine et au Moyen-Orient.

El Centro Internacional de Investigaciones para el Desarrollo es una corporación pública creada en 1970 por el Parlamento de Canadá con el objeto de apoyar la investigación destinada a adaptar la ciencia y la tecnología a las necesidades de los países en desarrollo. Su actividad se concentra en seis sectores: ciencias agrícolas, alimentos y nutrición; ciencias de la salud; ciencias de la información; ciencias sociales; ciencias de la tierra e ingeniería; y comunicaciones. El Centro es financiado exclusivamente por el Parlamento de Canadá; sin embargo, sus políticas son trazadas por un Consejo de Gobernadores de carácter internacional. La sede del Centro está en Ottawa, Canadá, y sus oficinas regionales en América Latina, Africa, Asia y el Medio Oriente.

This series includes meeting documents, internal reports, and preliminary technical documents that may later form the basis of a formal publication. A Manuscript Report is given a small distribution to a highly specialized audience.

La présente série est réservée aux documents issus de colloques, aux rapports internes et aux documents techniques susceptibles d'être publiés plus tard dans une série de publications plus soignées. D'un tirage restreint, le rapport manuscrit est destiné à un public très spécialisé.

Esta serie incluye ponencias de reuniones, informes internos y documentos técnicos que pueden posteriormente conformar la base de una publicación formal. El informe recibe distribución limitada entre una audiencia altamente especializada.

IDRC-MR252e
February 1990

OIL CROPS:
PROCEEDINGS OF THE THREE MEETINGS HELD AT PANTNAGAR AND HYDERABAD, INDIA, 4-17 JANUARY 1989

1. The Brassica Subnetwork-II
2. The Other Oil Crops Subnetwork-I
3. The Oil Crops Network Steering Committee-I

Edited by

Abbas Omran
Technical Adviser, Oil Crops Network

Organized by

Indian Council of Agricultural Research, New Delhi, India
G.G. Pant University of Agriculture and Technology, Pantnagar, India
Directorate of Oilseeds Research, Hyderabad, India
International Development Research Centre, Ethiopia/Canada

CONTENTS

Part 2. Other Oilcrops Subnetwork-I

Part 3. Oilcrops Network Steering Committee-I

THE INTERINSTITUTIONAL COLLABORATIVE RESEARCH PROGRAM ON WHITE RUST (*ALBUGO CANDIDA*) BETWEEN INDIA (ICAR) AND CANADA (IDRC) FOR RAPESEED-MUSTARD IMPROVEMENT

P.R. Verma

Under the collaborative research program on white rust (*Albugo candida*) between Canada (IDRC) and India (ICAR) for rapeseed-mustard improvement, the research reported here was conducted during the six month period while I was in India mainly at the G.B. Pant University of Agriculture and Technology, Pantnagar; Oilseeds Plant Pathologist and Plant Breeders at Regional Research Station, Morena, were also involved in research and training.

Objectives: The main objectives of the visit to the participating institutes in India were as follows:

1. Collection of staghead from different *Brassica* crops.

A detailed survey of several mustard growing areas (districts) in M.P. and Rajasthan was carried out in 1988, and also to a limited extent in 1989. Stagheads from several fields of *Brassica juncea*, *B. campestris* var. Toria, *B. campestris* var. Yellow Sarson, *B. tornefortii* and *Eruca sativa* were collected. Staghead material has been air-dried, crushed and is being stored in refrigerator at 7-8^{0}C, both at G.B. Pant University, Pantnagar, and Saskatoon Research Station. The material will be used for germination of oospores for laboratory and field inoculation studies and for identification of biological races of *A. candida*.

2. Development of methods/ techniques.

The methods described here were developed by me at the Saskatoon Research Station and have already been reported in several scientific publications. These methods are essential for: i) determining conditions essential for production, survival and germination of sporangia and oospores; ii) initiation of disease in the laboratory, growth chamber, greenhouse, and field; iii) screening *Brassica* germplasm against *A. candida* and inheritance of resistance; iv) identification of biological races of *A. candida*, and v) determining effect of different environmental factors on progression of white rust.

These methods require specific temperature, humidity and lighting conditions and are already being used at the Saskatoon Research Station for several years. The main purpose of my visit to the participating institutes in India was to see if these methods could be made to work under less environment - controlled conditions in India.

a). Method for germination of sporangia

To obtain a suspension of sporangia, newly ruptured sori from infected leaves were gently dislodged with an artist's soft brush, a stainless steel spatula, or a razor blade, and allowed to fall into a petri plate containing sterilized distilled water. Using a small glass rod, the contents of the petri plate were gently stirred to disperse the sporangia. Plates were incubated at 10-12^{0}C in the refrigerator for 1-2 hours. During winter months (November-February), sporangia also germinated successfully even at room temperatures ranging from 16-19C.

Germination of sporangia (production of zoospores) was determined by examining sporangial suspension in petri plate under microspore. Plant Pathologists both at Pantnagar and Morena are now using this method successfully.

Suggestions:

i) Sporangia do not germinate in sterilized or unsterilized Pantnagar tap water.
ii) Unsterilized distilled water will also induce germination.
iii) While examining sporangial suspension in Petri plates under microscopes (10X) for germination of sporangia, more zoospores will be visible at the bottom of the plate than on the liquid surface.
iv) Avoid using sporangia from senesced infected leaves.
v) Avoid getting dirt of leaf peelings while collecting sporangia from leaves.
vi) Frozen sporangia on diseased leaves, or those in plastic vials will remain viable for 3-4 months.

b). Method for germination of oospores (resting spores)

Stagheads from several mustard fields in Rajasthan collected in March 1988 had been stored dry in the laboratory of Dr. J.S. Kolte, Department of Plant Pathology, Pantnagar. Hypertrophied tissue (staghead) was ground with a mortar and pestle, and the grindings were screened through a 60-mesh sieve to give a brown powder consisting largely of oospores of *Albugo candida*.

A small amount (0.3-0.5g) of oospore powder was placed in 50 ml sterilized distilled water in a 125 ml. Erlenmeyer flask (100-125 ml in 250 ml flask) and incubated at 150-200 rpm on a rotary shaker at 18-24^{0}C for 9-12 days. The spore suspension was then poured into a petri dish and kept stationary for 24-48h in a refrigerator at 10-13^{0}C. Spore suspension was examined under microscope for presence of zoospores.

Suggestions:

i) Collect only dried or almost-dried stagheads from fields. A large proportion of oospores from green stagheads are immature (absent or poorly developed central globule) and will not germinate successfully.
ii) Avoid collecting stagheads with downey mildew (*Peronospora parasitica*) mixed infection.
iii) Avoid grinding stagheads which have been infested with mites, nematodes or any other organisms.
iv) Oospores do not germinate in sterilized or unsterilized tap water from Pantnagar.
v) While incubating on the rotary shaker, a ring of oospore powder deposited around the neck of the flask above the water line should be removed (once or twice a day) and mixed back in the suspension.
vi) If possible, store air-dried stagheads in zero-degree room.

c). Detached-leaf culture technique for growing *A. candida*

In India, screening *Brassica* germplasm for resistance to *A. candida* has been restricted by a lack of controlled environment facilities. To overcome this problem, an attempt was made to determine if the detached-leaf culture technique, developed by me and used in several studies under controlled environmental conditions at Saskatoon Research Station, could also be used for *A. candida* infection studies at the Plant Pathology Department, G.B. Pant University of Agriculture and Technology, Pantnagar, where air

temperature, relative humidity and lights are not precisely controlled. The room temperatures (15-21^{0}C) during the winter months (November-February) were found favourable for obtaining infection on detached leaves/cotyledons.

Healthy leaves or cotyledons from 12-14 days old seedlings were detached and transferred to petri dishes containing 20-25 ml autoclaved benzyladenine (1 ppm) agar (1%) medium. Leaves were placed in the dishes with their lower (abaxial) surface in contact with the medium within one hour of detachment; the upper (adaxial) surface was inoculated. Four to five leaves were placed in a plate. Leaves were drop-inoculated (2-4 drops per leaf) with a zoospore suspension derived either from germinating sporangia or oospores. Control leaves were treated with distilled water. Petri dishes placed in a tray full of water were incubated in a room with a day-night temperatures of about 22-14^{0}C, respectively. Leaves were maintained under a 12 h day (lux not measured) and a mister (very coarse) was used 3-4 times a day during the first four days of the experiment. Observation on percent infected leaves were recorded 10-15 days after inoculation. A leaf with one or more visible pustules was considered as infected. Several tests were carried out using leaves/cotyledons of *B. juncea*, *B. campestris* var. Toria, *B. napus*, *B. nigra*, *B. carinata*, *Sinapis alba*, *Raphanus sativus*, *Eruca sativa*, *Sisymbrium officinale*, and *Capsella bursa-pastoris*. Inoculum from only *B. Campestris* var. Toria and *B. juncea* were used in all cross inoculation experiments. Inoculum from *B. campestris* var. Toria produced pustules only on B. campestris leaves; similarly, inoculum from *B. juncea* produced pustules only on *B. juncea*. Number of pustules per infected leaf and also size of pustules were considerably lower than in experiments conducted at Saskatoon Research Station under controlled environment conditions.

Suggestions:

i) To reduce contamination, detach leaves/cotyledons from plants grown in glasshouse.

ii) For obtaining large, thick cotyledons, remove apical meristems of glasshouse-grown plants every 3-4 days.

iii) On benzyladenine-agar medium, older leaves tend to senesce faster than younger leaves.

d). Inoculation technique for screening *Brassica* germplasm for resistance against *A.candida*

Using *B. juncea*, and *B. campestris* var. Toria as hosts, several inoculation experiments were carried out in the glasshouse and outside on plants grown in 12-15 cm diameter pots (4-5 plants/ pot). Only inoculum from *B. juncea* and/or *B. campestris* var. Toria were used in all inoculation experiments. Using a plastic bottle sprayer, 3-4 week old plants were inoculated with a zoospore suspension derived from germinating sporangia. Control plants were treated with distilled water. Pots were incubated over a cement pit (approximately 3'L X 2'W X 2' Deep) full of water under an angle iron misting chamber for 3-4 days. Plants were sprayed with distilled water once or twice a day for five days. Observation on percent infected plants were recorded 12-14 days after inoculation. Only *B. juncea* plants inoculated with *B. juncea* inoculum, and *B. campestris* plants inoculated with *B. campestris* var. Toria inoculum showed infection. Oilseeds Plant Pathologists are now using this method successfully for screening *Brassica* germplasm for resistance against *A. candida*.

Suggestions:

i) Grow only 4-5 plants per 12-15 cm pot.
ii) After germination of seeds in the glasshouse, transfer pots outside in order to get good sturdy plants; because of poor lights in the galsshouse, plants become very spindly and etioliated.
iii) In order to grow good plants in pots, Pantnagar soil should be mixed with farm yard manure and sand.
iv) Place pots in a metal tray and flood trays to water plants.

3. Establish disease nursery in the field

For screening *B. juncea* introductions in the field, a disease nursery has been established both at Pantnagar and Morena. In order to build up the inoculum in this particular patch of land, plant pathologists have been advised to add every year a large amount of ground, sieved staghead powder (oospores) along with the seed. Only stagheads collected from *B. juncea* plants are to be added. High soil moisture needs to be maintained for germination of oospores and early infection. The overhead sprinkler irrigation system proposed both for Pantnagar and Morena will be for this disease nursery.

At Morena Research Station, the incidence and severity of white rust was significantly higher on *B. juncea* plants grown in the oospore-added disease nursery than anywhere else at the farm. Also, the plants in the disease nursery were the first one to show white rust symptoms. The plant pathologist and plant breeders were very much pleased with this success.

At Pantnagar, the incidence and severity of white rust on plants in the disease nursery was similar to those grown in areas without oospores. The most probable reason was that the nursery did not get irrigation until one month after seeding.

4. Determine if oospores are also produced in infected leaves

In 1983, we reported for the first time that the production of *A. candida* oospores in detached naturally infected *B. campestris* leaves after inoculation and 14 days of incubation at temperatures of 9-24°C; higher temperatures were more favourable for oospore production.

At Pantnagar, white rust infected, senesced leaves of both *B. juncea* and *B. campestris* var. Toria were examined late in the season, and were found to contain a large number of mature oospores. This is the first report of the production of *A. candida* oospores in naturally infected leaves.

Until now, it has been assumed that in rapeseed and mustard *A. candida* produced oospores mainly in hypertrophied inflorescences (stagheads) and to a smaller extent, in stem blisters. In light of our 1983, and this finding, we now know that the naturally-infected *Brassica* leaves are also important for adding oospores, the primary source of infection, in the soil.

5. Identification of biological races of *Albugo candida* on different *Brassica* crops in India.

Amongst the several fungal diseases affecting oilseed crops in India, white rust caused by *Albugo candida* is considered most destructive. Although, a large number of accessions in the genus *Brassica* and its allies have/are being evaluated since 1979, the progress in finding sources of resistance

against *A. candida* has been rather less than satisfactory.

The progress in breeding for resistance against white rust hinges mainly in identifying biological races of *A. candida* affecting various *Brassica* crops. At present, eight biological races of *A. candida* have been reported from North America. To our knowledge, presently, there is no information regarding biological races of *A. candida* on various *Brassica* crops in India.

Seeds of various known differential hosts of reported biological races were taken from my lab. at the Saskatoon Research Station to G.B. Pant University of Agriculture and Technology, Pantnagar, India, for cross-inoculation studies to be carried out for identification of biological races of the white rust fungus, *A. candida*. Following is the list of seeds taken to India:

1. *Raphanus sativus* (radish) cv. Comet
2. *R. sativus* (radish) cv. Cherry Belle
3. *Brassica juncea* cv. Domo
4. *B. juncea* cv. Commercial Brown
5. *B. juncea cv.* Southern Giant Curled
6. *B. campestris* cv. Torch
7. *B. campestris* cv. Candle
8. *B. campestris* cv. Tobin (1986 Re-Sel, Seidel)
9. *B. campestris* cv. Tobin 1
10. *B. campestris* cv. Tobin 2
11. *B. campestris* cv. Tobin 3
12. *B. napus* cv. Regent
13. *Sinapis alba* cv. Gisilba
14. *Capsella bursa-pastoris*
15. *Sisymbrium officinale*
16. *Roripa islandica*

Results of several preliminary inoculation studies, both on detached leaves in the laboratory and on intact plants in pots in the glasshouse, suggest that in India, the race of *A. candida* attacking *B. juncea* is different from the race attacking *B. campestris* var. Toria. This appears to be similar to what has been reported from North America. This, however, needs to be confirmed with more detailed experiments involving differential hosts. Also, need to be determined are the races attacking *B. nigra, B. carinata, B. torrnefortii, B. campestris* var. Yellow sarson, *Eruca sativa* (taramira) and other Cruciferae hosts grown in India.

The reason I was not able to conduct detailed experiments for identification of biological races using various differential hosts was lack of time, and equipments including defreeze, humidifiers, light and temperature controlled incubating room.

Having worked out the methods for gemination of sporangia, and oospores, detach-leaf culture technique, and inoculation method in the glasshouse, I can probably complete identification of biological races of *A. candida* infecting various Cruciferae crops in India in four months (November-February) provided the equipments mentioned above are in place at Pantnagar.