11

Copenhagen Airport. have customs clearance

↓ ~~15 min~~. 10 Kr

By Bus

↓ 15 min

Flyboat Station } 4/6 Malmo-Airport- city

↓ 35 minutes } 4/6 30 kms

Malmöe } 40 kms

↓ 5 mins by Taxi

city centre

20 $

For 2
40 Kr
or
10 $

Hotel

a) Ehrenberg

b) Hakansson

A:

Mutagenic treatment

Seed irradiation was done with 25000 seeds in each millet variety unless otherwise mentioned. The seed was equilibrated over anhydrous $CaCl_2$ for 24 h, for uniform moisture, before mutagenic treatment. The seed was irradiated in a ^{60}Co gamma-cell(200-CANADA) of a strength of 900 Ci at the time of treatment with a delivery of 1.2krad/min. The doses used were 25 and 35 kR for millet and 10kR for chickpea. However, 35 kR was found to be too drastic for seedling survival and was therefore omitted for further studies.

The treated material was advanced by growing and testing in a range of latitudes (11^oN to 28^oN) in Delhi (28^oN), Hydrabad (17^oN), Vizianagaram (18^oN), Perumallapalle (15^o), Bangalore (13^oN) and Coimbatore (11^oN), by taking advantage of three crop seasons in ayear in different locations.

Sample size

A minimum of 20000 seeds was used in each treatment to ensure 10000 M_1 plants, which are space planted for maximum development in the M_1 generation.

Seed collection from M_1 plants

No selection is practised in M_1 generation except elimination of very weak plants of major cytological changes as reflected by abnormal appearance and/or poor seed set.

(a) Main tiller and two more spikes from other tillers are selfed in each M_1 plant. The seed of each spike is kept separately.

(b) In chickpea the seed is collected from the main stem and bulk of rest of the branches by taking 5 seeds/branch in each M_1 plant. The seed is kept separately for each branch also for future use.

Selection

The progenies showing field resistance are also screened for resistance in the laboratory. In addition, the M_2 generation is replicated in the field without any artificial inoculation, which will help preserve variation for non specific resistance.

Screening the material for disease resistance in the field

Screening of the material against downy mildew in the field at Delhi needed no artificial inoculation owing to a very heavy incidence of the disease in the field, equivalent in intensity to an artificially created disease plot. However, testing of progenies in the seedling stage was also done during the off-season. Only the normal looking survivors in the field with the requisite plant height and maturity were carried forward at every cycle. Each single plant was also tested for the maintenance of male sterility with the male sterile A-line to build up a corresponding A counterpart at each generation. It was also necessary to select for the complete restoration of fertility in the hybrids with the mutants from the pollen parents similarly treated for mutational ractification for resistance against downy mildew.

...2/-

1) Lowry, O.H, Rosebrough, N.J., Farr, A.L and Randall, R.J (1951). Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193: 265
2) Shannon, L.M., Kay, E and Lew, J.Y (1966). Peroxidase isozymes from horseradish roots. I. Isolation and physical properties. J. Biol. Chem. 241: 2166
3) Murty, B.R., S.C. Pokhriyal and S.L. Mehta (1975) Mutation breeding for resistance to downy mildew and ergot in Pennisetum and to Ascochyta in chickpea. In Induced mutations for disease resistance in crop plants (1975): IAEA 181: 165-181.
4) Murty, B.R. (1978) Disease Resistance Techniques in [illegible] Crop and Animal Production. Proc. Int. Symp. on Nuclear [illegible] Feb 78 New Delhi pp 1.

The race position is not clear in downy mildew and ergot but it is presumed that racial variation may be detected if suitable diffentials are available. In the case of Ascochyta, there is evidence for the presence of at least two races. The available information on these pathogens and the methods of creating artificial epiphyotics are summarized in Table I and II.

B:-

The main findings of the work done at the University of Mysore, in collaboration with this project to devise reliable and efficient inoculation techniques for screening against downy mildew used in this investigation, are summarized below:

(a) Downy mildew appears throughout the year on the plants grown in soil infected with oosporic material, irrespective of the date of planting. This has helped in testing three to four generations in one year.

(b) Artificial methods of inoculation with conidial/sporangial suspension in water are better than oospore inoculation, nearly 95% of the inoculated seelings being infected by the former methods.

(c) Foliar susceptibility of inoculated seedlings to conidial suspension can be detected as early as one week after inoculation while it will take at least four weeks to observe the first reliable symptoms of the disease in the field. This has shown that susceptibility of seedlings to conidial infection is an excellent tool for rapid evaluation of host resistance.

(d) There is great variation among different millet varieties for disease reaction as measured by the percentage of infected seedlings using the above method. This finding, along with the high repeatability of the tests, has permitted the detection and evaluation of genetic differences in the relative resistance of the irradiated progenies.

C:

Male steriles

The ractification of inbred 23B, the fertile counterpart of Tift 23A(male sterile) has been advanced from M_3 to M_6 generation. A total of 166 M_3 progenies were raised in 1971 in a plot heavily infected with downy mildew. The control (23B) and 23A, serving as checks and occuring at regular intervals, were completely wiped out by the disease in every block. Out of the survivors in these progenies (±66 000 plants in M_3 generation), 65 single plants which were agronomically good and free from disease in the field were selected for growing in the M_4 generation in the off-season nursery. Among these 65 M_4 progenies, 21 progenies showed complete freedom from mildew while 23A and B showed infection up to 50%. Eighteen of these 21 lines showed 90% maintenance of male sterility when crossed with the corresponding A-line and two of these had 100% maintenance of sterility (Table III). The above observations were also confirmed under inoculation in laboratory and glasshouse conditions in two independent tests in comparison with other male steriles and controls (Table IV).

The M_5 generation consisted of 143 singel-plant progenies for the 18 M_4 progenies, which combined both resistance to mildew and maintenance of male sterility with Tift 23A. Actually 7 to 10 plants were selected in each of the 18 M_4 progeny of which 143 were finally available to be carried forward to M_5.

...3/-

LMS

The above 143 progenies of 23B were grown again during 1972-73 and screened under intense incidence of disease in the field. The test under artificial inoculation was felt ~~necessary~~ unnecessary at this stage since disease incidence was so severe that the controls 23A and 23B were wiped out within three weeks after sowing and most of them within a fortnight. One or two tillers on isolated plants lingered on but were badly affected without any seed set. There was not even one plant in the controls which was free from the disease.

Among the 143 M_5 progenies of 23B, eight were found to be resistant but segregated for varying degrees of maintainer relationship while three more progenies have shown 0% to only traces of infection with complete maintenance of male sterility. These three progenies were carried forward to M_6 by the selection of 35 to 40 individual plants in each progency and their corresponding counterparts of the A-line and will be harvested by June 1973. The above single-part progenies now in the M_6 generation represent the third backcross and will again be tested for segregation and residual variability, if any, for resistance. Another cycle of selection in A and B will be continued, to reach the 4th backcross after which the line may be considered stable.

Each plant in each progeny will be checked for the maintenance of sterility with the A-line and for disease resistance under artificial inoculation in the laboratory. Segregation for photosensitivity under the long days in summer will also be checked. It is expected that uniform single plants in each progeny can be bulked after this test and will be ready to replace Tift 23A and B.

Cytoplasms Tift 23A — 1962 released

A_1 (13) — 23A, 101A, 102A, 104A, 105A, 106A, 107A, 110A, 111A, 201A, 202A, 204A, Selection Tift 23A

A_2 (4) — 66A, 103A, 239B2A, 301A

A_3 (4) — 67A, 108A, 109A, 401A

D:

Biochemical Assay:

Extraction of soluble protein:

Shoots were ground in chilled pestel and mortar with 50 mM Tris-cl buffer (pH 7.6) (1.4w^{t}/vol). The supernatant obtained by centrifugation at 10,000 x g for 10 min. was used for peroxidase assay.

Peroxidase Assay:

Peroxidase activity was assayed according to the method of Shannon et al (1966) with slight modifications. The reaction mixture in a final volume of three ml contained. O-dianisidine, 1-69 u Moles; H_2O_2, 10.0 µ Moles; Na-acetate buffer pH 4.5, 171.0 µ Moles, Enzyme preparation 0.1 ml. Change in absorbance was recorded at 460 nm in spectrophotometer. One unit of enzyme activity has been defined as a change in one optical density (O.D) per minute and specific activity as Δ O.D./min/mg Protein. Protein was estimated according to the method described by Lowry et al (1951)

E:

Biochemical Analysis:

A biochemical analysis for theperoxidase activities of the spectrum of male steriles obtained by mutational breeding and also back-cross derivatives was undertaken in laboratory conditions. The results indicate wide differences in the peroxidase activity particularly the component with high electrophoretic mobility. It was also observed that such differences were maximum in the early seedling stage (Table). These diffences can be reduced in subsequent stages of growth. Differences due to fertile cytoplasm vs. sterile are also evident. The resistant counterparts derived from Nigerian parents appear to have characteristically different degree of this activity.

Biochemical analysis of the peroxidase activity in shoots was carried out under controlled condition at 24°C in B.O.D. incubators at 72 hours and 6 days growth after sowing by. Dr. S.L. Mehta and his associates a-t the Nuclear Research Laboratory. A similar analysis was also carried out in shoots at 10 days after sowing in the field. The resutls also indicate differences between resistant lines and their susceptible counterparts with substantial increased activity in the resistant male steriles. The differences were pronounced in the 72 hourrold and 6 day old material under the controlled conditions. However, there was rapid narrowing down of the difference when the material was grown in the field and tested at 10-day old level. Another interesting feature of the differences is the consistent higher activity in material related to the Nigerian stocks(Table).

The mutant lines were compared woth other resistant derivatives which did not reveal any significant difference among the resistant counterparts whether derived by mutational rectification or back-crossing. There were small but consistent differences in the activity between A & B lines; the latter having slightly increased peroxidase activity. This difference indicates that plasmic differences namely sterile vs. fertile have a role to play in the observed results.

The analysis was done on thebasis of activity per fresh gm weight. When it was also estimated on the basis of per mg. of protein there were no differences between

A & B lines. On the other hand there was a slight reversal of the magnitude of activity.

The peroxidase activity in the Maiwa lines, 126D_2A, and 5141A in comparison to the checks 23A and 23D_2A in 10-day old seedlings is not significant while the activity is higher in the mutational counterpart 5071A. Since most of the infection takes place in the early seedling stage and may manifest in a much later stage due to the systemic nature of the disease, the large magnitude of peroxidase activity in the resistant counterparts in seedlings upto 6 days should be considered as critical regarding the infection. However, other parameters of isozyme activity are also being estimated.

The presence of considerable differences in variety x age of seedling and varieties x A vs. B lines of the male steriles suggest that care should be exercised in interpreting the association of specific isozyme patterns with disease resistance.

Further studies on biochemical characterisation on the nature of disease resistance would appear to be necessary.

Semn II

~~28~~ 27/3/78

Prof. K. S. Gill

Tift 23 A1 — 1962 released

A_1 — 23A, 101 A, 102 A, 104 A, 105 A, 106 A, 107 A, 110 A, 111 A, 201 A, 202 A, 204 A. Selected from Tift 23 A

A_2 . 66 A, 103 A, 239 D A 301 A

A_3 67 A 108 A, 109 A, 401 A

1) Problem of stability of male sterile [illegible] had to be overcome.

2) Need for restorers also resistant to downy mildew

PIB 155 Highly resistant to good combiner with L111 A

PIB 228

→ parent to PHB-10

→ parent to PHB-14.

PHB 12,

PHB-37

PHB-56 earlier maturing

Cultivation	A_1	A_2	A_3	
				564
1977	339	112	113	

No. of hybrids produced on [illegible]

PB 201 A1
(L102A x PIB 1244)

PB 202 A1
(L402A x PIB 1335)

PB 301 A 2
(239 D A x J104)

PB 401 A3
(L67 A x PIB 1246)

Hutchinson [illegible] [illegible] disease

~~Diversification of cytoplasm~~

Ergot resistance not to [illegible] lower [illegible] of attack

1. Status of disease – Losses caused 8m to 3.5m hag
First little – 1969 – sell [illegible] – 1970-72 [illegible]
Possible
Seed borne nature and debris, soil inoculum
1 a) Maharashtra – an endemic area of DMS [illegible]
2. Genetic vulnerability due to same cytoplasm male sterile widely adopted, but other cytoplasm had been unproductive –

3. Diversification attempts – (a) Diverse source of resistance nucleogenic donors b) ~~Diverse~~ incorporation of superior genotypes into other cytoplasms – as alternatives. c) Mutational rectification. ~~[illegible]~~ (a) and (c) successful.
(b) Partly successful as [illegible]

4. Rectification of both parents – ~~[illegible]~~ –
Fate of only one parent is regulated –
Reconstituted hybrids. – ~~[illegible]~~

5. Wide testing of female parents for possible racial variation – and disease incidence in different environments of crop seasons.
– seed production
– Stability of male sterility –