MUTATION BREEDING FOR RESISTANCE TO MILDEW AND ERGOT IN PENNISETUM AND ASCOCHYTA IN CHICKPEA

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1. The introduction and spread of high yielding varieties and the new technology associated with them have changed the micro-environment in the crops and thrown up new problems of diseases not important previously. The change in pearl millet from an yield level of 290 kg/ha. to a potential of 5000 kg/ha has resulted in such a problem with an unprecedented damage of the crop by downy mildew and ergot in India. Chemical control has not been very effective due to the nature of infection. These diseases are serious in West Africa, India and U.S.A. Similar is the case with Ascochyta rabiei on chickpea in the Near East, Mediterranean and South Asia (Tables 1 and 2). The very few resistant lines are poor in plant type, productivity and grain type and restricted in their adaptation (Tables 3 and 4). Hence, induced variation for resistance is imperative to supplement the conventional breeding methods which have not been successful in these two cases due to linkage and adverse correlated response. This Project is proposed for the above purpose.

2. The work carried out in India and to a

limited extent in Nigeria on pearl millet has shown that the available male steriles are highly susceptible. Some of the races of downy mildew are found to infect Sorghum, millet and maize which requires immediate attention. Evidence on seed-borne nature of this pathogen is also available. Studies on bi-parental progenies have shown that tight linkage between resistance and poor plant type is a major obstacle (Table 5). The screening methods in the field also need improved repeatability for both ergot and downy mildew. The race position is also not clear. Preliminary studies on the use of crude extracts of active principle in leaves infected with mildew indicate that rapid screening may be possible using detached leaves. This method is still to be standardized.

Resistance to ergot is associated with short stigma and shorter period of its receptivity and closer synchronization with anthesis. It may be useful to select for reduction in the receptive period from nearly two weeks to four or five days.

In chickpea, two races of Ascochyta rabiei are already identified. An effective method of screening in the field has been developed (Table 1). Increase in phenols, reducing sugars and peroxidase activity is found in resistant varieties when artifically inoculated with the pathogen, as compared to the susceptible types. The seeds of resistant types are black, wrinkled and/or small and not linked

by consumers. The spectrum of variation of some black-seeded types has shown that limited increase in seed size without detriment to disease resistance is possible. None of the high-yielding yellow-seeded types is resistant.

Genetic studies on the nature of resistance revealed resistance to be partially dominant over susceptibility for downy mildew and Ascochyta with an apparent monogenic control. The situation does not appear to be so simple due to adverse correlated response and the lack of similarity in the results of laboratory inoculation of seedlings and field tests. Field inoculation methods with ergot are not having the desirable extent of repeatability and need improvement.

3. Plot studies on the response to gamma irradiation (Co^{60}- source) @ 25 Kr and 35 Kr to the seeds for resistance to downy mildew are encouraging (Table 6). These results on 8 inbreds and a hybrid show that the rm frequency of resistant mutants is reasonable for selection in M_3 generation. Similar work on Tift 23 A & B a male-sterile line has resulted in the identification of two resistant M_3 families in 84 families from bulks of 70 M_2 progenies with field resistance. In Bil 3B, pollen parent of a hybrid, 16 M_3 families out of bulks of 84 were resistant under artificial epiphytotics in the field. These lines are to be screened in the seedling stage in the laboratory. Thus, the scope for inducing resistance in the male steriles is also good.

4. The objectives in the proposed project are as follows, using mutation breeding:

Pearl millet:

(a) Development of new cytoplasmic male steriles with resistance to ergot and downy mildew in the resistant West African material.

(b) Alteration of the tall photo-sensitive West African types to photo-insensitive and dwarf types.

(c) Improve the frequency of recombination by treating crosses between resistant donors and inbreds with dwarf plant type and good yield potential.

(d) Improvement of screening methods already developed for laboratory testing of seedling resistance to downy mildew and adult resistance to ergot to suit mass screening in the field particularly during off-season November - March.

Chickpea :

(a) Mutational rectification of the resistant but defective black-seeded cultivars.

(b) Induction of variation for resistance to Ascochyta in the yellow-seeded high yielding types.

The above studies will be integrated with the breeding program and studies on epidemeology and physiological mechanisms of resistance to these pathogens already in progress at the Indian Agricultural Research Institute, New Delhi. Collaboration with other institutions engaged in similar activities will be established.

Detailed Work Plan :

The work will be oriented based on the results of proposed work during the first year as follows:

Pennisetum

(i) Screening of the M_2 generation of the three West African resistant lines and six high yielding inbreds for new sources of cytoplasmic male sterility combined with resistance to mildew and ergot. (Laboratory screening for downy mildew will be in the seedling stage by artificial inoculation with Conidia/Sporangia).

(ii) Evaluation of promising mutants from the West African resistant lines for photo-insensitivity and desirable plant frame.

(iii) Modification of laboratory screening techniques for mass inoculation on a field scale for downy mildew and ergot.

(iv) Irradiation of new lines and crosses with better productivity for increasing the frequency and recovery of desirable recombinants.

Chickpea

(i) Mutagenic treatment of large and medium seeded resistant lines with black seed coat.

(ii) Treatment of yellow seeded types susceptible to Ascochyta to get resistant mutants.

These studies on chickpea will be limited to find effective radiation dose and suitable sample size.

(b) The yellow-seeded but susceptible types C 104, L 44 and NP 58 in the first year, five others in the new collections in the second year will be treated for improving their resistance and maintain their yield level, seed colour and size.

(c) Six locally adapted brown seeded types C 235, NP 53, RS 10, Type 3, Pb 7 and NP 58 will be treated in the second year to get resistant mutants.

(d) Improvement of recombination in crosses between the resistant line P 1528 with susceptible but adapted types NP 58, Pb 7 and RS 10 may have to be taken up at a later stage.

Mutagenic Agents:

(a) It is proposed to treat seed using Co^{60} source (gamma cell) already available with a delivery rate of 1.20 Kr/minute. Seeds equilibrated for uniform level of moisture content will be used. A range of 35 and 45 Kr will be attempted for millet and a dose of 10 Kr and 15 Kr yet to be evolved for chickpea. This is suggested due to the encouraging results already obtained with seed treatment.

(b) Chemical mutagens may be used at a later stage if necessary. One of the mutagens EMS, DES, NMU will be used based on data available on similar small grains and grain legumes.

(c) Controlled environment of temperature ($20^{\circ}C$, $30^{\circ}C$) orygen and seed water content will be provided for seed treatment.

(d) All procedures for treatment and raising of crop selection, and sample size will be done as described broadly in the "Manual on Mutation Breeding". A minimum availability of at least 10,000 surviving plants and or 1000 spikes of 500,000 plants ~~on~~ as possible after treatment is envisaged.

(e) The facility of utilizing off-season nurseries to raise two generations per year in different centres of IARI will be taken advantage.

Creation of Artificial Epiphytotics:

A. Field

	Downy mildew	Ergot	Ascochyta
i) Inoculum	Conidial spore suspension in water; 10-15 spores per field of 200 x ; repeat 6-7 times	Conidial suspension 200 - 300 conidia per field of 200 x ; repeat three times.	Pycnidial spore suspension @ 1000 spores/ml or 3-4 spores per field of 200 x ; One spray in field tents is enough.
ii) Stage of crop	Boot leaf stage (30-45 day old)	Pre-anthesis	6 - week old crop
iii) Humidity and temperature	80% humidity before 6 AM 25 - 28°C	90 - 100% humidity before 8 AM 20 - 25°C	100% humidity Before 8 AM in the field conditions Around 20°C.
IV) Other facilities	Disease sick plot is also used, mixing 00 spores 3" below soil and allow for 3 months before sowing.	-	-
v) Time expected for appearance of disease	5 days	10-12 days	5-6 days

M_3 generation: Laboratory and field screening with a minimum basic productivity. A total of half a million plants in chickpea will be screened in each variety. Male sterility in millet will be rested using normal counter parts in the variety.

M_4 & M_5 generations: As above and also for yield.

Maintenance & Field testing:

(a) Inter-mating of mutants in pearl millet for creating a gene pool.

(b) Field tests of mutants.

Measuring resistance:

(a) Score on the extent of damage to the vegetative parts, since estimation on the basis of yield reduction will be more time consuming.

(b) Evaluation of progenies in M_4 onward will be on population level.

Maintenance and Utilization of Mutants:

(a) Resistant mutants with an accepted level of productivity will be preferred.

(b) Intermating of the desirable mutants from M_3 stage in a biparental mating system will be attempted. This will increase the recovery of lines with diverse sources of resistant alleles and genetic backgrounds.

(c) Creation of gene pools of mutants will be useful in out breeders like millet to supplement existing

world collection.

(d) Field testing of mutants in a coordinated program will provide information if their resistance is broad-based against different races.

(e) Biochemical analysis of resistant mutants for phenol, and peroxidase activities will be taken up after testing them for stability.

(f) Use of the available trisomics in millet for locating the loci for resistance.

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Table 1. RESISTANCE TO SOME DISEASES IN PEARL MILLET AND CHICKPEA

	PEARL MILLET		CHICKPEA
	Downy mildew	Ergot	Ascochyta
1. Problem areas	Nigeria, Ghana, Senegal, India	All African countries,	India, Near East, Russia and Mediterranean
2. No. of lines screened (3 years)	2290	1600	6500
3. No. of lines with field resistance	741	413	670
4. Lines with resistance under artificial inoculation.	18	3	1 High degree 3 Moderate degree
5. Regions of resistance	Nigeria, Senegal	Nigeria	Morocco
6. Chemical control	Not effective	Not effective	Not effective
7. Possible resistance mechanism	Root exudales	Short stigma receptivity 6 days	Phenols and peroxidases
8. Genetics of resistance	Polygenic	Polygenic	Simple Dominant
9. Method of artificial inoculation (a) Laboratory (b) Field	Standardized	Standardized	Standardized

Table 2 NO OF PEARL MILLET CULTURES RESISTANT TO DOWNY MILDEW AND ERGOT IN THE WORLD COLLECTION SHOWNING FIELD RESISTANCE

Geographical	No. of cultures	Green ear (Delhi-1965)	Ergot (Junagadh,1964)	No. of resistant under artificial inoculation	
				Downy mildew	Ergot
I Africa					
Congo	2	2	-	0	0
Ghana	3	-	-	0	0
Kenya	15	12	4	0	0
Mali	50	50	5	0	0
Nigeria	122	122	38	16	3
N.Rhodesia	3	3	-	0	0
Nysaland	5	5	-	0	0
Senegal	31	24	-	2	0
S.Rhodesia	2	1	2	-	
Sudan	4	4	-	-	
S.Africa	14	13	4	-	
Uganda	8	7	-	-	
II Australia	2	-	-	-	
III Asia					
Pakistan	5	-	-		
India	1078	357	337	0	0
IV America					
USA	99	90	7		
V Exotic unknown	65	51	4		
Exotic	430	384 (89%)	76 (13%)	18	3
India	1078	357 (33%)	337 (31%)	0	0
Total	1508	741 (49%)	413 (27%)	18	3

Table 3. CHARACTERISTICS OF THE ERGOT RESISTANT LINES AND DWARF DERIVATIVES IN MILLET

S.No.	Lines	Origin	Ergot score ( )	Height in cms.	Days to 50% flower	Ear Length (cms)	Tillers per ½ m.	Yield gm./½m
				EXOTIC RESISTANT				
1.	I.P.517	Mali	R(1.5)	175	54	29	11.5	182
2.	I.P.1956	Nigeria	R(1.0)	173	53	35	10.5	133
3.	I.P.1961	Nigeria	R(1.0)	199	54	28	8.5	157
4.	I.P. 326	Senegal	R(2.0)	198	51	36	7.0	107
5.	I.P. 231	Uganda	R(1.5)	222	57	31	8.0	170
6.	I.P. 938	U.S.A.	R(1.5)	206	53	28	10.5	132
				INDIAN - RESISTANT				
7.	I.P.1902	Gujarat	R(1.0)	197	50	24	8.0	163
8.	I.P. 54	M.P.	R(1.0)	170	54	30	9.5	120
				DWARF - SUSCEPTIBLE				
1.	D_1 x I.P.81	F_{10}	S	90	56	19	7.5	60
2.	D_2 x I.P.81	F_9	S	86	60	27	10.0	71
3.	D_2 x I.P.81	F_8	S	79	51	18	9.0	68
4.	S 530	S_7	S	81	53	26	12.0	95

R - Resistant S - Susceptible

Table 4 CHARACTERISTICS OF SOME CHICKPEAS RESISTANT TO ASCOCHYTA

Variety	Area	Resistant	No. of days to mature	Seed appearance	Seed colour	100 seed wt.(gms)	Yield Q/ha.
P. 1528-1	Morocco	High	190	Not desirable	Black	23.6	15 - 20
P. 1137-1	India	Moderate	180	Not desirable	Light pink	33.1	5 - 8
EC. 26414	Morocco	Moderate	180	Desirable	Dark Brown	43.0	12 - 15
EC. 26435	Unknown	Moderate	175	Acceptable but small	Light Brown	11.00	20

Table 5. MATING SYSTEM IN RELATION TO BREEDING FOR DISEASE RESISTANCE IN PEARL MILLET

S.No.	DETAILS	No.	Early vigour 1-Poor 10-Best	Days to 50% Bloom	Leafiness 1-Poor 10-Best	Chlorophyll Depth 1-Poor 10-Best	Leaf Drying 1-Best 10-Poor	Tillers per Metre	Yield per Metre	Disease 1-Best 10-Poor
1	Selections (A+T Series)	40	6.74	50.68	6.78	6.72	4.51	29.25	311.05	2.26
2	A - Series	22	6.73	50.88	7.12	7.04	4.77	29.86	327.15	1.89
3	T - Series	18	6.76	50.44	6.37	6.33	4.18	28.51	291.38	2.73
4	Checks (HB 1 & HB 4)	2	6.00	49.83	7.17	5.33	4.00	30.33	349.25	3.08
5	Selection vs check	-	NS	NS	S=C	S C	S C	S=C	S=C	S=C
6	A vs T series	-	NS	NS	A T	A T	A T	A T	A T	A T
	h^2	-	0.02	24.25	7.35	10.86	19.78	28.24	29.50	73.17

A - Biparental mating, B - Direct selfing

From B.B.Singh (1971)

Table 6. NATURE OF r-ray INDUCED VARIATION FOR RESISTANCE TO DOWNY MILDEW IN SOME LINES OF PEARL MILLET

Variety/ Dose Inbred	M_1			M_2			
	No. of seeds irradiated	Survival %	Propn of disease free plants in field	Total	No. of families Resistant	Segregating	Susceptible
K560 25Kr	3000	85.0	9.7	60	10	29	21
35Kr	"	82.0	10.3				
K559 25Kr	"	90.3	7.3	48	10	36	2
35Kr	"	67.0	8.7				
K230 25Kr	"	81.3	6.3	39	16	15	8
35Kr	"	70.0	6.7				
I2032 25Kr	"	63.3	3.7	39	10	20	9
35Kr	"	79.0	9.3				
I2033 25Kr	"	69.7	5.7	41	7	14	20
35Kr	"	63.3	8.0				
I1318 25Kr	"	58.3	2.3	14	0	7	7
35Kr	"	36.3	2.3				
I2013 25Kr	"	63.3	1.7	12	0	5	7
35Kr	"	36.7	2.3				
I2040 25Kr	"	58.3*	1.7	10	0	5	5
35Kr	"	70.0	1.7				
$23D_2A$ x IP 1967 25kr	"	73.0	6.7	39	8	19	12
35Kr	"	37.3	3.7				
25Kr		71.7	5.0	135	32	62	41
35Kr		60.2	5.9	167	29	88	50

* Only these M_1 plants which ar were 302 apparently healthy in the field were carried forward to the next generation.
The resistant lines will be screened again in the laboratory at the seedling stage.