Indian J. Genet., 49(1): 85–89 (1989)

INDUCTION, ISOLATION AND CHARACTERIZATION OF LEAF RUST RESISTANT MUTANTS IN WHEAT CULTIVAR KALYAN SONA

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(Received: May 9, 1988; accepted: June 18, 1988)

ABSTRACT

Mutations for resistance to culture IL011 (race 77A) of *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* were induced in wheat cultivar Kalyan Sona using ethyl methanesulphonate (EMS) treatment and 40 kR gamma irradiation. Two mutants identified after EMS treatment were designated as KSM 4233-US and KSM 4234. One mutant, designated as KSM 4238, was identified after gamma irradiation. Mutants 4234 and 4238 were resistant to leaf rust in field tests but KSM 4233-US segregated for susceptibility up to M_5 generation. The resistance of KSM 4234 to race 77A was due to one dominant and one recessive genes. The multipathotype tests using cultures IL007 (race 108), IL011 and IL017 (race 106) and the allelic tests suggest the origin of three new alleles.

Key words: Rust resistance, induced mutations, wheat.

Over 30 genes for resistance to leaf rust of wheat (*Triticum aestivum* L.) caused by *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* have been identified and designated [1]. None of the genes documented so far from *T. aestivum* give satisfactory resistance to the prevalent races, particularly to race 77 and its biotypes. The Lr genes derived from alien sources are effective, but they are associated with undesirable characters and are difficult to use in breeding programmes. Therefore, search for new variability for resistance to leaf rust is necessary to ensure continuing support to the breeding programmes. Induced mutations have been used to create new variability for rust resistance in wheat [2–5]. However, induced mutations have been rarely systematically characterised to establish any new variation.

Cv. Kalyan Sona is good combiner for many agronomic traits and was grown over a large area in India up to 1975 till it became susceptible to leaf rust race 77 and its biotypes. The present paper reports induction, isolation and genetic characterization of mutations for leaf rust resistance in cv. Kalyan Sona against race 77A.

MATERIALS AND METHODS

Cultivars and mutagenic treatments. Cv. Kalyan Sona (Penjamo 62 "S" × Gabo 55), Agra Local (a land race susceptible to all the known leaf rust races from India)

were used in the study. Two mutagenic treatments (ethyl methanesulphonate, 0.6%–12 h, and 40 kR gamma rays) were given to 1000 healthy dry seeds of cv. Kalyan Sona. After EMS treatment, the seeds were thoroughly washed in running water, surface dried and sown 3 cm apart in 2 m long rows. The gamma-ray treated seeds were also planted similarly. The M_1 plants were bagged and harvested as bulk for each treatment.

Pathogen cultures. Three highly virulent pathogen cultures, viz. IL007 (race 108), IL011 (race 77A), and IL017 (race 106) were used. The avirulence/virulence formulae of these cultures are given in Table 1. Culture IL011, which is virulent on all the known Lr genes from *T. aestivum* at seedling stage [6] was used for isolation of mutants. In addition, cultures IL007 and IL011 were used for characterization of the mutants.

Table 1. Avirulence/virulence formulae of the leaf rust cultures used for isolation and characterization of mutants

Culture	Avirulence/virulence formulae
IL007	PLr3, Lr9, Lr15, Lr19, Lr24, Lr25, Lr26, / pLr1, Lr2, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr16, Lr17, Lr18, Lr20, Lr21, Lr22a, Lr22b, Lr23.
IL011	PLr9, Lr18, Lr19, Lr21, Lr24, Lr25, Lr26,/ pLr1, Lr2, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr15, Lr16, Lr17, Lr22a, Lr22b, Lr23.
IL017	PLr9, Lr19, Lr23, Lr24, Lr25, Lr26,/ pLr1, Lr2, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr15, Lr16, Lr17, Lr18, Lr20, Lr21, Lr22a, Lr22b.

Isolation of mutants. The two M_2 populations were evaluated for leaf rust reaction to culture IL011 at seedling stage in a growth chamber maintained at 20±1°C and relative humidity above 80%. Seven-day-old seedlings were inoculated with a homogeneous mixture of uredospores and neutral talcum powder at the density of 5-6 spores per microscopic field at 100× magnification. The inoculated seedlings were incubated for 48 h under 100% relative humidity and subsequently transferred to the benches in growth chamber. Observations on infection types were recorded 14 days after inoculation according to the scale given by Stakman et al. [7]. The resistant seedlings were transferred to the field and raised to maturity. The mutation frequency was expressed as mutants per 1000 M_2 seedlings.

Characterization of the mutants. The M_3 seeds from each mutant were harvested separately and tested at seedling stage to identify the homozygotes. The resistant seedlings from homozygous M_3 progenies were transplanted to the field and progeny tested. True breeding families thus identified were crossed in the M_4 generation with the susceptible cultivar, Agra Local. They were also crossed with near-isogenic leaf rust resistant lines carrying genes Lr9, Lr18, Lr19, Lr21, Lr24, Lr25 and Lr26 to check the allelic relationship of these genes in the mutants and also to rule out the possibility of outcrossing. Multistrain tests on seedlings using cultures IL011, IL007 and IL017 were done to establish genetic differences in the mutants and the

parent cv. Kalyan Sona. Adult plants of cv. Kalyan Sona and the mutants were repeatedly inoculated with culture IL011 under field conditions. Leaf rust intensity on these plants was recorded according to the modified Cobb's scale as described by Peterson et al. [8].

RESULTS

Two leaf rust resistant seedlings were isolated from 6370 M_2 seedlings (0.31 mutants/1000 M_2 seedlings) after EMS treatment. One mutant was true breeding in M_3 generation and designated as Kalyan Sona mutant 4234 (KSM 4234). The second mutant was not stable and segregated for susceptible seedlings in the M_3, M_4 and M_5 generations. It was designated as KSM 4233-US. Only one resistant seedling was isolated from 6430 M_2 seedlings (0.16 mutations/1000 M_2 seedlings) after gamma irradiation. This mutant was also true breeding in M_3 generation and was designated as KSM 4238.

The F_2 generations from the crosses of KSM 4234 and KSM 4238 with Agra Local inoculated with rust culture IL011 segregated in 3:1 and 13:3 ratios of resistant and susceptible plants, respectively (Table 2). The F_2 generations from the crosses of mutants KSM 4234 and KSM 4238 with the near-isogenic leaf rust resistant lines segregated for susceptibility.

Table 2. Segregation for leaf rust reaction against culture IL011 in F_2 generation from the crosses of Kalyan Sona mutants 4234 and 4238 with cv. Agra Local

Cross	No. of seedlings		Total	Probable ratio (R : S)	χ^2
	resistant	susceptible			
KSM-4234 × Agra Local	79	29	108	3:1	0.20
KSM-4238 × Agra Local	750	161	911	13:3	0.69

Cultivar Kalyan Sona was susceptible to cultures IL011 and IL017, but gave infection type (IT); 33+ against culture IL007. Mutant KSM 4234 gave IT=; against cultures IL007, IL011 and IL017. KSM 4238 gave IT=; against cultures IL011 and IL007, and developed IT=12 against culture IL017.

In field tests, 80% of leaf area of Kalyan Sona was covered with rust, whereas KSM 4234 was free from rust and KSM 4238 showed rust reaction up to 5 MR.

DISCUSSION

Both ethyl methanesulphonate and gamma rays have been successfully used to induce mutations for disease resistance in crop plants [4, 9, 10]. Rust resistant mutants were found with a frequency of 0.31 and 0.16 per 1000 M_2 seedlings in EMS and gamma-ray treated populations, respectively. From low to fairly higher

mutation rates have been reported for disease resistance, but these mutation rates cannot be compared because the probability of detecting a mutation is proportional to the number of avirulence genes in the pathogen culture used [11].

The observed segregation for leaf rust reaction to culture IL011 in the crosses of two mutants with Agra Local suggests the presence of one dominant gene in KSM 4234 and one dominant plus one recessive gene in KSM 4238. Race 77A, from which culture IL011 has been derived, is virulent on all the known genes for resistance to leaf rust from *T. aestivum*. Only the alien genes Lr9, Lr18, Lr19, Lr21, Lr24, Lr25 and Lr26 show resistance to this race [6]. Segregation for susceptibility observed in the crosses of the two mutants with near-isogenic lines for these seven alien genes suggests that the changes identified here are not a result of outcrossing with any of the genes and the new variability appears to have been generated following the mutagenic treatment. Origin of such variability through induced mutations in wheat has been reported earlier [5].

True breeding homozygotes were not recovered from the progeny of KSM 4233-US, therefore, this mutant could not be characterized. Instability of induced mutants is well known, but specific reasons for this instability are not known [12].

Table 3. Seedling and field reaction of cultivar Kalyan Sona and its mutants

Stock	Seedling reaction and culture			Field reaction against culture
	IL007	IL011	IL017	IL011
Kalyan Sona	;33+	3	3+	805
KSM-4234	0	0 ;	0	Free
KSM-4238	0	0 ;	12	5MR

Cv. Kalyan Sona is susceptible to cultures IL011 and IL017 at seedling stage and also shows a high IT (IT=; 33+) against culture IL007, however both the mutants have shown high degree of seedling resistance to cultures IL011, IL007 and IL017 (Table 3). The ITs seen on the seedlings of KSM 4238 against culture IL017 (IT=12) is higher than those on the seedlings of KSM 4234. If one of the two genes present in mutants KSM 4234 and KSM 4238 is common, an IT=12 against culture IL017 is unexpected on KSM 4238. The present observations suggest that the two mutants have different resistance genes and at least three new genes controlling resistance to leaf rust have been created by mutagenic treatments. A comparison of field reactions of the two mutants also suggests that they carry different resistance genes. Cultures IL011, IL007 and IL017 are among the most virulent races of wheat leaf rust prevalent in India. The high degree of resistance in mutants of a widely adapted high yielding cv. Kalyan Sona against these cultures may prove useful as a new source of resistance in breeding programmes.

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