Inheritance of resistance to *Bacillus sphaericus* toxins in a laboratory selected strain of *Anopheles stephensi* (Diptera Culicidae) and its response to *Bacillus thuringiensis* var. *israelensis*

Anopheles stephensi is recognized as a major vector of urban malaria in India. This species prefers to breed in small man-made water collections and is responsible for frequent outbreaks of malaria, particularly at constructions sites in urban areas. In such circumstances, the strategy to control *An. stephensi* relies mainly on the use of chemical insecticides, which results in the development of resistance and also contaminates the environment. Target-specific microbial preparations based on endotoxins produced by *Bacillus thuringiensis* (*Bt*) H-14 and *Bacillus sphaericus*, have been evaluated extensively to control mosquito breeding in a variety of habitats[1–6]. Among the two bio-larvicidal agents, use of *B. sphaericus* has resulted in the development of resistance in field populations of *Culex quinquefasciatus*[7]. Though *B. sphaericus* resistance in *An. stephensi* has not been reported from field, laboratory selection of a strain of *An. stephensi*, which had shown some tolerance to *B. sphaericus*, resulted in a high level of resistance to *B. sphaericus*[8]. The present study was carried out to see the inheritance pattern of *B. sphaericus* resistance in *An. stephensi*.

Two strains of *An. stephensi*, viz. S – susceptible to *B. sphaericus* (LC_{50} = 0.088 mg/l) and R – resistant to *B. sphaericus* (LC_{50} > 1600 mg/l)[8], were used. 'Spherix', a Russian powder formulation of *B. sphaericus* B-101 serotype H5a,5b, supplied by Biotech Internationals Pvt Ltd, was used to study the inheritance of *B. sphaericus* resistance. 'Bacticide', also a Russian powder formulation of *Bt* H-14 was used to determine cross-resistance. The two strains of mosquitoes were colonized in cages of 30 × 30 × 30 cm in an insectory maintained at 28 ± 1°C and 70–80% humidity. To establish the inheritance and linkage, *B. sphaericus* susceptible and resistant strains were reciprocally crossed, and the F_1 progenies of both the reciprocal crosses were allowed to grow into adult. They were inbred and back-crossed with the parental strains to obtain F_2 progenies. Single female progenies from these crosses were collected and the mutant phenotypes were scored at 2nd larval instars. Susceptibility of the F1 and F2 larvae from individual mosquitoes against *B. sphaericus* was checked by exposing the 3rd instar larvae at a discriminatory dose of 40 mg/l of Spherix, which was determined by carrying out preliminary bioassays against *B. sphaericus* susceptible and resistant strains. To study cross-resistance to *Bt* H-14, dose mortality responses of *B. sphaericus* susceptible and resistant strains of *An. stephensi* were determined against Bacticide, as described earlier[5].

Table 1 shows the response of larvae of two strains of *An. stephensi* used in this study and their reciprocal crosses, to the discriminatory dose of *B. sphaericus* formulation. Exposure of 3rd instar larvae of *B. sphaericus*-resistant strain of *An. stephensi* to a dose of 40 mg/l Spherix, did not induce any mortality among the larvae within 48 h, whereas all the larvae of susceptible strain were killed at this concentration. When *B. sphaericus*-susceptible strain was reciprocally crossed with *B. sphaericus*-resistant strain (crosses 3 and 4), larvae of F1 progeny from both these crosses were found to be completely susceptible to *B. sphaericus*. Since both female and male larvae of the F1 progeny of both these reciprocal crosses were susceptible to *B. sphaericus*, it was concluded that *B. sphaericus* resistance may not be linked to a sex chromosome and was thus autosomal. It may be pointed out that sex in *An. stephensi* is determined by XX and XY mechanism[9], where the female is homogametic (X/X) and the male is heterogametic (X/Y). In an earlier study, resistance to *B. sphaericus* in *Cx. quinquefasciatus* was also reported to be autosomal and recessive.

Table 2 shows the response to *B. sphaericus* by larvae obtained from the single progenies of inbreeding from F1 adult and backcross of F1 adults with parental strain. The F2 progeny obtained from F1 inbreeding (crosses 1 and 2) had approximately 3 : 1 ratio of susceptible and resistant larvae, as expected. Assuming the hypothesis that resistance is monofactorial, no significant difference between observed and expected number for *B. sphaericus* resistant and susceptible larvae was obtained ($P > 0.5$). In backcrosses 3 and 4 (between F1 adults and *B. sphaericus* resistant parents), larvae of F2 progeny consisted of both *B. sphaericus* susceptible and *B. sphaericus* resistant larvae in the approximate ratio of 1 : 1 (Table 2) (cross 3–680 : 658, cross 4–716 : 704) which also confirms the hypothesis for monofactorial resistance in *An. stephensi*. In backcrosses 5 and 6 (between F1 adults and *B. sphaericus* susceptible strain), the entire F2 progeny was susceptible to *B. sphaericus*, as expected. Resistance to *B. sphaericus* toxin, which consists of 51 and 42 kDa protein, was reported to be due to a change in the

Table 1. Response to *B. sphaericus* by *An. stephensi* larvae of two parental strains (*B. sphaericus* resistant and susceptible) and progenies of their reciprocal crosses

Cross no.	Parental genotype F	Parental genotype M	F1 progeny	Response to *B. sphaericus* No. larvae exposed to *B. sphaericus* (conc. 40 mg/l) (F + M) exposure	No. of dead larvae after 48 h
1	R/R	R/R	R/R	250 (125 + 125)	0
2	S/S	S/S	S/S	250 (125 + 125)	250
3	R/R	S/S	R/S	250 (125 + 125)	250
4	S/S	R/R	S/R	250 (125 + 125)	250

R, *B. sphaericus* resistant; S, *B. sphaericus* susceptible.

Table 2. Results from F-1 inbreed and backcrosses of F-1 with *B. sphaericus* resistant and susceptible strains of *An. stephensi*

Cross no.			No. of larvae/families exposed to *B. sphaericus*	No. of larvae alive after 48 h (expected*)	No. of larvae dead after 48 h (expected*)	χ^2
1	R/S	R/S	1016/7	243 (254)	773 (762)	0.2034**
2	S/R	S/R	1139/8	287 (285)	852 (855)	0.001**
3	R/S	R/R	1338/9	658 (669)	680 (669)	0.027**
4	R/R	R/S	1420/11	704 (710)	716 (710)	0.0071**
5	R/S	S/S	1551/12	0 (0)	1551 (1551)	0
6	S/S	R/S	1297/11	0 (0)	1297 (1297)	0

R, *B. sphaericus* resistant; S, *B. sphaericus* susceptible.
*, Based on the hypothesis that resistance is due to a single major factor, expected number of larvae that would die at a concentration of 40 mg/l within 48 h.
**, Not significant ($P > 0.05$).

receptors of larval midgut brush border membrane in the resistant strain of *Cx. quinquefasciatus*[10]. Both these toxins require only single class of receptors, one helping in binding and the other performing toxic actions[11].

Bioassay tests with *Bt* H-14 against 3rd instar larvae of *B. sphaericus* resistant and susceptible strains of *An. stephensi* gave LC_{50} values of 0.117 and 0.136 mg/l. These results show that resistance to *B. sphaericus* in *An. stephensi* does not show any cross-resistance to *Bt*, probably because of the different modes of action of two the bacterial toxins[12]. Thus in case of *B. sphaericus* resistance, *Bt* can be used to control the resistant mosquito larvae.

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ACKNOWLEDGEMENT. Assistance provided by Sh. Brij Mohan and Sh. Rajender Singh is acknowledged.

Received 4 April 2005; accepted 28 April 2005

P. K. MITTAL[1,*]
T. ADAK[1]
S. K. SUBBARAO[2,3]

[1]*Malaria Research Centre,*
2, Nanak Enclave,
Radio Colony,
Delhi 110 009, India
[2]*Malaria Research Centre,*
22, Sham Nath Marg,
Delhi 110 054, India
[3]*Present address:*
Indian Council of Medical Research,
New Delhi 110 029, India
**For correspondence.*
e-mail: pk_mittal52@yahoo.co.in

Oryza rufipogon: A possible source of novel resistance specificities against rice blast (*Magnaporthe grisea*)

Blast disease caused by *Magnaporthe grisea* is a serious production constraint for rice world wide. In the past, rice breeders have successfully tapped sources of resistance available in cultivated rice germplasm for developing disease-resistant varieties. To date more than 40 genes conferring resistance to blast have been identified from cultivated rice lines[1]. Besides the cultivated types, the wild and weedy species within the primary gene pool of *Oryza* represent a potential source of easily transferable novel pest and disease resistance[2]. Among the most successful examples of utilization of wild germplasm in rice resistance breeding has been the use of gene(s) of an accession of *O. nivara* from northwestern Himalayas, to provide long-lasting resistance to grassy stunt virus[3]. The hilly state of Himachal Pradesh (HP), representing the northwestern Himalayan region of India, is one of the centres of diversity of cultivated rice[4]. This region abounds in diversity of wild rice varieties identified as types within wild rice *O. rufipogon*, which is believed to be the progenitor of Asian cultivated rice (*O. sativa*)[5].

Since, *O. rufipogon* – *M. grisea* pathosystem has a long co-evolutionary history than the *O. sativa* – *M. grisea* system, we believe considerable diversity for R-genes must have evolved in the wild rice com-