

## Effect of Gamma Radiation on Arcelin Protein and its Influence on *Zabrotes Subfasciatus* (Boheman)

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(Received July 10, 1999)

### ABSTRACT

The research aimed to evaluate the effect of different doses of gamma radiation on the arcelin protein related to *Zabrotes subfasciatus* (Boheman) resistance. Seeds of *Phaseolus vulgaris* L. carrier of arcelin protein and a cv. without this protein, used as control, were irradiated with doses of 0, 0.25, 0.5, 1.0 and 2.0 kGy. After irradiation, the seeds were subjected to confinement test to evaluate different parameters viz. total egg, viable eggs, emerged insects, insect weights, oviposition period and sex ratio. The results showed no influence of radiation doses on the evaluated parameters, and the Arcelin-1 and 2 strains have maintained thus antibiosis grade. The Arcelin-3 and 4, also kept their less expressive behaviour of resistance by antibiosis and only prolonged the oviposition period of the female.

**Key words :** Stored grain insects, *Zabrotes subfasciatus*, Gamma radiation, resistance, arcelin

The common bean (*Phaseolus vulgaris*) is a cultivated species and largely consumed, representing a crop of considerable economic importance<sup>1</sup>. Among the main problems of the *P. vulgaris* stock, caused by *Zabrotes subfasciatus* (Boheman) *Coleoptera:Bruchidae Jare* decrease in the weight, reduction of the germinate power, reduction in the nutritive value and commercial detriment<sup>2,3</sup>. In Brazil, losses of beans caused by *Z. subfasciatus* are estimated at about 20 per cent<sup>4</sup>.

As an alternative to the use of chemical insecticides, *Z. subfasciatus* can be controlled by radiation<sup>5</sup>. Therefore, experiments have been conducted to investigate the nutritional quality of treated food with ionizing radiation<sup>6</sup>.

Discovery of defense mechanisms of *P. vulgaris* to *Z. subfasciatus*, in the presence of an additional protein called arcelin, indicated as the main

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resistance factor or *Z. subfasciatus* on beans from Colombia<sup>7-9</sup> has initiated work related to the genetic improvement aiming the resistance to *Z. subfasciatus*. In Brazil, work was recently initiated with the aim of transferring this protein to cultivated beans<sup>10</sup>. Therefore, this work has the objective of evaluating the effect of different doses of gamma radiation (<sup>60</sup>Co) on the arcelin protein and its influence on the resistance to *Z. subfasciatus*, through confinement test.

## MATERIALS AND METHODS

Four *P. vulgaris* strains carrier of arcelin protein (Arcelin-1, Arcelin-2, Arcelin-3 and Arcelin-4) and a cultivar without the protein (LAC-Carioca Akyta), used as control, were radiated by a gamma radiation source of <sup>60</sup>Co (type panoramic) of the Instituto de Pesquisas Energeticas e Nucleares (IPEN), at a dose rate of 0.678 kGy/h. For each radiation dose i.e., 0, 0.25, 0.5, 1.0 and 2.0 kGy, 250 g of seeds of each strain and cultivar were used. All seeds were provided by the Instituto Agronomico de Campinas (IAC).

The confinement test was performed in the laboratory of the Secao de Entomologia at Instituto Biologico, in Sao Paulo, at 25°C and 70 per cent R.H. Fifty grams of each treatment, with their respective radiation doses, were settled in glasses, able to support 150 g of seeds, closed with cotton cloth and infested with seven pairs of *Z. subfasciatus* of 0-24 h age. The insects were confined inside recipients for egg-laying for 4 days. Insects were then taken off and infested seeds were maintained at the incubator until F<sub>1</sub> emergence. Biological parameters used on the resistance evaluation were; total number of eggs, viable eggs, emerged adults, adult weight, period from egg to adult and sex ratio. Further the adult weight, period from egg to adult and sex ratio were evaluated by a factorial arrangement (5x5x5) and the parameters like total number of eggs, viable eggs and the experimental delineation were completely randomized.

## RESULTS AND DISCUSSION

### Total number of eggs/viable eggs

No significant differences were observed for the means of the total eggs and number of viable eggs laid in cultivar and strains, submitted to 0, 0.25, 0.5, 1.0 and 2.0 kGy doses (Table 1). The large number of laid eggs by *Z. subfasciatus* in carrier arcelin protein bean strains was according to previous work<sup>9, 11</sup>, where similar results were reported during the investigation of sources of resistance to *Z. subfasciatus*, in such strains compared to susceptible cultivars.

Present findings in agreement with previous research reported that *P. vulgaris* seed irradiated with 50 Gy did not influence the oviposition preference<sup>12</sup>. The percentage of viable eggs was over 60 per cent for the tested cultivar and strains (Fig 1).

Table 1 : Means of the total number of eggs/viable eggs of *Z. subfasciatus*, in 50 g of *P. vulgaris* cultivar and strain seeds irradiated with different doses of gamma radiation.

Cultivar and Strains	Mean of number of eggs				
	0 (kGy)	0.25 (kGy)	0.5 (kGy)	1.0 (kGy)	2.0 (kGy)
<b>Total No. of eggs</b>					
TAC-Carioca Akyta	169,00a	193,20a	152,20a	140,40a	176,40a
Arcelin-1	195,80a	184,40a	187,00a	160,60a	207,00a
Arcelin-2	218,00a	197,60a	146,80a	157,00a	177,00a
Arcelin-3	214,00a	157,80a	141,60a	152,40a	195,00a
Arcelin-4	198,20a	175,40a	172,40a	183,60a	178,40a
C.V(%)	13,85	14,86	18,06	22,88	12,24
<b>Viable eggs</b>					
TAC-Carioca Akyta	141,40a	155,80a	132,80a	119,20a	145,20a
Arcelin-1	150,60a	152,00a	170,00a	128,40a	159,60a
Arcelin-2	161,40a	165,60a	122,60a	129,00a	140,60a
Arcelin-3	149,40a	139,00a	128,40a	122,40a	154,80a
Arcelin-4	149,60a	141,80a	136,20a	147,80a	130,00a
C.V(%)	14,29	15,80	18,95	21,84	11,82

Means followed by the same letter did not differ statistically among them by the Test of Tukey ( $p=0.05$ ) Means obtained from 5 repetitions of 50g of cultivar and strains seeds.

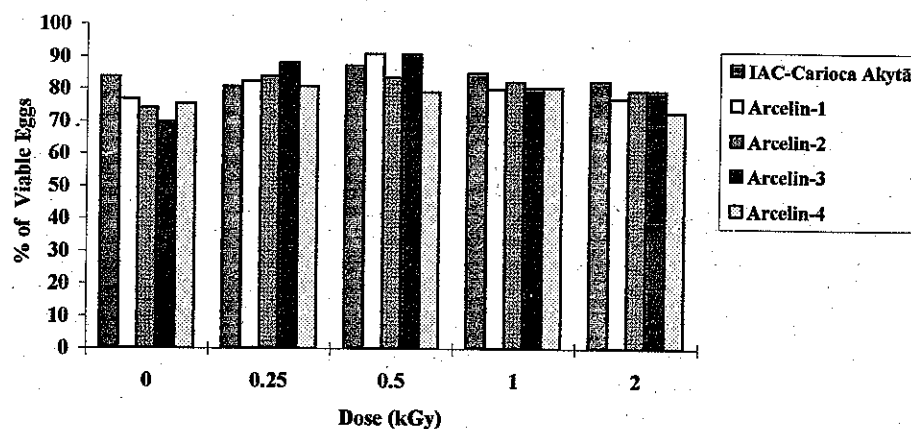


Fig 1. Mean percentage of *Z. subfasciatus* viable eggs in 50 g of seeds of cultivar and strains irradiated with different doses of gamma radiation.

### Number of emerged insects

There was no influence of radiation doses on the number of adults emerged but there were significant differences among the cultivar and the strains (Table 2). The lowest number of emergence was obtained for Arcelin-1 (29.88) and Arcelin-2 (37.36) strains corresponding to an emergence of 19.64 and 25.97 per cent respectively. The cultivar showed 93 per cent emergence, while Arcelin-3 showed 82.30 and 88.03 per cent, respectively.

The lowest means of Arcelin-1 and Arcelin-2 strains were also evident when the genotype behaviour inside each radiation dose was analyzed.

Table 2 : Mean number of *Z. subfasciatus*, emerged in 50 g of cultivar seeds of *P. vulgaris* irradiated with different doses of gamma radiation

Cultivar and Strains	Doses in kGy					Mean
	0	0.25	0.5	1.0	2.0	
IAC-Carioca Akyta	131,20aA	144,80aA	125,00aA	113,40aA	131,40aA	129,16a
Arcelin-1	12,40bA	23,60cA	33,00bA	34,00bA	46,40bA	29,88b
Arcelin-2	22,60bA	39,00cA	35,80bA	42,00bA	47,40bA	37,36b
Arcelin-3	120,80aA	100,60bA	100,00aA	115,20aA	134,60aA	114,24a
Arcelin-4	126,20aA	123,40abA	122,40aA	133,60aA	115,40aA	124,20a
C.V(%)			23,30			
CV(%)			23,30			

Means of the rows (cultivar and strains) followed by the same letters and on the lines (dose in kGy) followed by the same capital letter did not differ among themselves by the Test of Tukey (P=0.05).

Means obtained from 5 repetitions of 50g of seeds/cultivar and strains

Arcelins-1 and 2 maintained their adverse effects against *Z. subfasciatus* showing lower means. These strains exhibited resistance to antibiosis type. Arcelins-3 and 4 also maintained their behaviour expressing their lower effect over this parameter. The results obtained were considered coherent with the early reports<sup>9, 10, 13</sup>.

A gradual increase on the per cent emergence of *Z. subfasciatus* for the strains Arcelin-1 and 2 was observed with increase in radiation doses (Fig 2). The per cent emergence varied from 8.23 (0 kGy) to 29.07 (2.0 kGy) for Arcelin-1, representing an increase of more than 3 times of the emergence. On Arcelin-2, the percentage was 14 (0 kGy) and 33.71 (2.0 kGy) duplicating the pest emergence. It indicates a possible interference of radiation dose on the Arcelin. Thus, the resistance of these strains could be broken with doses over 2.0 kGy.

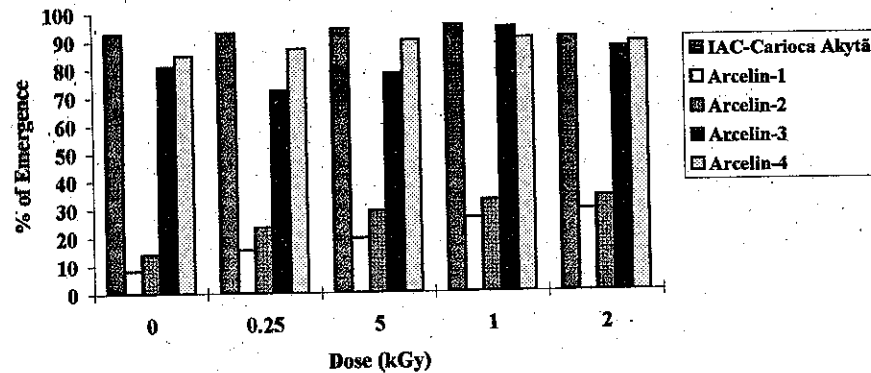


Fig 2. Mean percentage of emerged insects of *Z. subfasciatus* in 50 g of seeds of cultivar and strains of *P. vulgaris*, irradiated with different doses of radiation.

#### Weight of *Z. subfasciatus* adults

According to the weight of *Z. subfasciatus* male and female (Table 3) it can be observed that the cultivar differed from strains because it showed the highest weight even to males or to females, independently of the radiation dose. There was no difference on the insect weights in arcelin carrier beans. These results showed that there is no interference of the radiation doses on the insect weights.

#### Period of egg to adult and sexual rate of *Z. subfasciatus*

The parameter of the period of *Z. subfasciatus* egg to adult confirmed the manifestation of the resistance or the antibiosis type for the strains. According to the results of Table 4, there was no interaction between the cultivar and strains with the radiation doses. Statistical differences always occurred in relation to the cultivar and strains. The lower period of egg to adult was observed on the cultivar even on the general means as on each radiation dose analyzed alone. This result was similar to those obtained by several authors, which found low levels of resistance to *Z. subfasciatus* in cultivar of *P. vulgaris* with the absence of arcelin<sup>14, 15</sup>.

The sex ratio of *Z. subfasciatus* was not influenced by the presence or absence of arcelin as well as by the radiation doses. It verified the proportion of males to females on the tested beans (Table 4).

Table 3 : Weight of *Z. subfasciatus*, adults emerged from 50 g of cultivar and strain seeds of *P. vulgaris* irradiated with different doses of gamma radiation

Cultivar and Strains	Doses in kGy					Mean
	0	0.25	0.5	1.0	2.0	
<b>Male</b>						
IAC-Carioca Akyta	1,72aA	1,74aA	1,73aA	1,71aA	1,75aA	1,73a
Arcelin-1	1,39bA	1,33bA	1,28bA	1,36bA	1,38bA	1,35b
Arcelin-2	1,30bA	1,39bA	1,28bA	1,28bA	1,32bA	1,31b
Arcelin-3	1,35bA	1,37bA	1,30bA	1,32bA	1,36bA	1,34b
Arcelin-4	1,30bA	1,35bA	1,32bA	1,35bA	1,35bA	1,34b
C.V(%)			8,99			
CV(%)			8,99			
<b>Female</b>						
IAC-Carioca Akyta	3,26aA	3,17aA	3,17aA	3,10aA	3,05aA	3,15a
Arcelin-1	2,37bA	2,26bA	2,13bA	2,46bA	2,37bA	2,32b
Arcelin-2	2,32bA	2,36bA	2,32bA	2,39bA	2,42bA	2,36b
Arcelin-3	2,38bA	2,24bA	2,13bA	2,42bA	2,33bA	2,30b
Arcelin-4	2,27bA	2,30bA	2,34bA	2,31bA	2,24bA	2,29b
C.V(%)			9,64			
CV(%)			9,64			

Means of the rows (cultivar and strains) followed by the same letters and on the lines (doses in kGy) followed by the same capital letter did not differ among themselves by the Test of Tukey (P=0.05)  
Means obtained from 5 repetitions of 50g of seeds cultivar and strains

Table 4 : Means of the egg to adult period (days) and sex ratio of *Z. subfasciatus* in 50 g of cultivar and strain seeds of *P. vulgaris* irradiated with different doses of gamma radiation

Cultivar and Strains	Doses in kGy					Mean
	0	0.25	0.5	1.0	2.0	
<b>Egg to adult period (days)</b>						
IAC-Carioca Akyta	34,46bA	34,41bA	34,99bA	35,38bA	35,36bA	34,92c
Arcelin-1	41,53aA	41,93aA	40,57aA	42,24aA	41,92aA	41,64a
Arcelin-2	38,80aA	39,16aA	39,10aA	39,12aA	39,28aA	39,09b
Arcelin-3	41,92aA	41,23aA	41,92aA	40,81aA	42,30aA	41,63a
Arcelin-4	41,99aA	41,99aA	41,15aA	42,00aA	41,52aA	41,73a
C.V(%)			4,69			
CV(%)			4,69			
<b>Sex ratio</b>						
IAC-Carioca Akyta	0,49aA	0,50aA	0,47aA	0,52aA	0,49aA	0,49a
Arcelin-1	0,48aA	0,49aA	0,48aA	0,51aA	0,49aA	0,49a
Arcelin-2	0,45aA	0,54aA	0,46aA	0,49aA	0,55aA	0,50a
Arcelin-3	0,49aA	0,51aA	0,49aA	0,48aA	0,50aA	0,49a
Arcelin-4	0,48aA	0,46aA	0,48aA	0,46aA	0,53aA	0,48a
C.V(%)			13,77			
CV(%)			13,77			

Means of the rows (cultivar and strains) followed by the same letters and on the lines (doses in kGy) followed by the same capital letter did not differ among themselves by the Test of Tukey (P=0.05)  
Means obtained from 5 repetitions of 50g of seeds cultivar and strains

Concluding, the radiation doses did not have influence on the resistance manifestation of antibiosis type on strains of carrier arcelin. Meantime, doses over 2.0 kGy could break such a resistance, because a gradual increase in the percentage of insects emerged from *Z. subfasciatus* was observed when the doses were enlarged.

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