Resistance of Arcelin Incorporated Bean (*Phaseolus vulgaris* L.) Hybrids and their Parental Cultivars against the Bean Bruchid *Zabrotes subfasciatus* (Boh.)

Misangu, R. N.*, Susan Nchimbi-Msolla and S.O.W.M. Reuben

Departiment of crop science and Production, P.O.Box 3005, Morogoro, Tanzania.

Abstract

A hybridization bean breeding programme aimed at breeding beans resistant against the bean bruchid species Z. subfasciatus, which destroys beans in storage was carried out at Sokoine University of Agriculture (SUA) Morogoro, Tanzania between 1994 - 1997. Five potential local bean varieties/lines were crossed to a bruchid resistant bean line RAZ 24-2 which was developed at SUA by selection from CIAT segregating RAZ bean populations. Seeds of RAZ lines contain arcelin a protein which confers resistance against Z. subfasciatus and can be transferred into other varieties by hybridization. The backcross breeding procedure was adopted and five arcelin containing progenies were developed from this breeding programme. Seeds of the developed progenies and those of the parents were then tested for resistance against Z. subfasciatus in a Randomized Complete Block Design with 5 replications. There were significant differences ($P < 0.05$) in resistance against Z. subfasciatus among the genotypes. Results indicated that arcelin incorporated genotypes were superior over the arcelin deficient parents for resistance against Z. subfasciatus. Generally, the presence of arcelin in bean seeds delayed bruchid development, reduced the number of emerged bruchids and bruchid damage on bean seeds. However, bruchids managed to lay many eggs on seeds of all cultivars tested suggesting that bruchids are not inhibited from laying eggs on arcelin containing seeds.

Key words: Bean Bruchids, Arcelin, Resistance.

Introduction

The bean bruchid species *Zabrotes subfasciatus* (Boh.) and *Acanthoscelides obtectus* (Say) which destroy beans in storage are of economic importance in Tanzania, (Masolwa and Nchimbi, 1991 and Misangu, 1997). The damage on bean seeds due to bean bruchids reduces weight, quality and viability. Bean weight losses of up to 30% have been reported (De Lima, 1973). Various methods for controlling these pests including cultural and chemicals have been recommended (Schoonhoven and Cardona, 1986). Unfortunately, bruchid damage has not been fully controlled by these methods and therefore breeding bruchid – resistant varieties has been thought to be a more reliable control method than others.

Bean bruchid resistance has been attribute to a number of factors. Physical factors such as pod pubescence and seed coat hardness have been cited (Thiery, 1984). However, the presence of arcelin in wild beans as identified by Schoonhoven *et al.*, (1983) confers resistance against *Z. subfasciatus* in *P. vulgaris*. Osborn *et al.*, (1986) identified for variants of arcelin and noted that the transfer of arcelin into white seeded cultivated beans via back crossing resulted in large seeded breeding lines with high resistance against *Z. subfasciatus*. This form of resistance is due to antibiosis mechanisms and is controlled by a single dominant gene (Cardona and Posso, 1987).

Essentially, the resistance resulting from the presence of arcelin in bean seeds is manifested

*Corresponding author

by prolongation of the life cycle and reduced adult emergence. The discovery of arcelin in wild bean seeds has offered possibilities of developing bean varieties which are resistant against *Z. subfasciatus* in locally adapted potential bean varieties. This can be achieved by hybridization between the potential local bean varieties and the RAZ lines and hereby substantially reducing losses of beans caused by this pest. The objective of this study was therefore to incorporate arcelin in superior and locally adapted bean varieties, and to identify the most resistant progenies against *Z. subfasciatus* either for use as parents in future bean breeding programmes aimed at breeding beans resistant to this pest or for release to farmers.

Materials and Methods.

Alleles for arcelin from the donor parent RAZ 24-2 were transferred to five agronomically superior bean cultivars. The line RAZ 24- carries the gene for resistance against *Z. subfasciatus*. The locally adapted superior bean cultivars into which arcelin was incorporated were selected based on their known superior characteristics as shown in Table 1. The standard backcross method was adopted in this breeding programme as illustrated in Figure 1 where SUA 90 and RAZ 24-2 bean parents are used as examples.

Table 1: Parental bean cultivars used in breeding beans resistant against *z.subfasciatus*

Variety/line	Superior characteristics	Source
SUA 90	Released commercial variety, high yielding, resistant to angular leaf spot, rust, bean common mosaic virus and drough tolerant	SUA
PR 12	High yielding with high protein content, red seededand resistant to angular leaf spot, rust and bean common mosaic virus	SUA
PR 13	High protein content with a dark brown seed colour. Resistant to angular leaf spot, rust and bean common mosaic virus	SUA
EP 1-4	High protein content with a dark brown seed colour. Resistant to angular leaf spot, rust and bean common mosaic virus	SUA
EP 3-2	High yielding, fast cooking and resistant to angular leaf spot, rust and bean common mosaic virus	SUA
RAZ 24-2	*Resistant against Z.subfasciatus*	CIAT

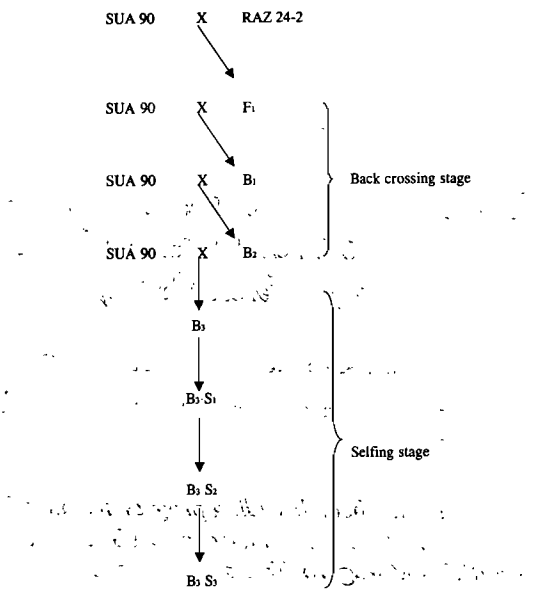

Figure 1: The breeding scheme for resistance against *Z.subfasciatus* using SUA 90 and RAZ 24-2 as an example

Prior to the commencement of the breeding programme, soil was collected from the SUA horticultural unit and filled in 5 liter size plastic pots. Nitrogen in the form of ammonium sulphate and phosporus (P_2O_5) in the form of triple super phosphate were well mixed in the soil contained in plastic pots at rates of 20kg N/ha and 50 kg P_2O_5/ha, respectively. Each cultivar was planted in 10 pots at a rate of one plant per pot. RAZ 24-2 was planted in twenty pots in order to provide adequate flowers from which pollen for making crosses was collected. All pots were arranged in rows on benches in a glass house such that pots planted with one entry were arranged in a single row and there were two rows for the line RAZ 24-2. The pots were placed at a spacing of 50cm within and between rows followed by adequate watering until germination and subsequently throughout the growing period to maturity.

At flowering stage, RAZ 24-2 was crossed to all other parents during which reciprocal crosses were also made. The F_1 seeds were backcrossed to their respective recurrent parents three times. After each backcross generation, seeds of backcross lines were grown, harvested separately as single plants and tested for resistance against *Z. subfasciatus*. After harvest, seeds of single plants were packed and sealed in polythene bags. The seeds were deep frozen in a deep freezer for one week in order to eliminate

Table 2: Levels of resistance to *Z. Subfasciatus* in parental bean lines crosses containing *arcelin* (means)

Entries	Number of eggs laid	Number of days to 50% F1 adults emerged	Number of adults emerged	Number of damaged seeds	(IS)	Resistance level
RAZ 24 -2 x SUA 90	111.4^{cd}	50.6^{bc}	1.4^{e}	2.4^{e}	0.23^{fg}	R
RAZ 24-EP 4-4	120.6^{bcd}	53.2^{d}	1.0^{e}	1.0^{e}	0.18^{g}	R
RAZ 24-2 x EP 3-2	127.2^{bc}	48.8^{cd}	1.4^{e}	3.0^{e}	0.23^{fg}	R
RAZ 24-2 x PR 12	120.4^{bcd}	50.4^{bc}	1.6^{e}	2.4^{e}	0.26^{f}	R
RAZ 24-2 x PR 113	129.6^{ab}	48.2^{d}	2.2^{e}	3.4^{e}	0.43^{d}	R
RAZ 24-2	132.2^{ab}	52.2^{ab}	2.6^{e}	2.8^{e}	0.35^{e}	R
SUA 90	145.8^{a}	30.6^{e}	134.0^{a}	38.0^{a}	2.38^{a}	S
EP 4-4	127.6^{bc}	31.0^{e}	110.0^{bc}	31.4^{b}	2.29^{b}	S
EP 3-2	125.b^{cd}	31.4^{e}	109.6^{bc}	33.4^{b}	2.28^{b}	S
PR 12	108. 8^{d}	30.4^{e}	94.6^{d}	30.6^{b}	2.21^{c}	S
PR 113	124.8^{bcd}	31.2^{e}	102.2^{c}	39.4^{a}	2.24^{bc}	S
Kijivu - susceptible Control	133.0^{ab}	29.4^{e}	114.2^{b}	41.0^{a}	2.30^{b}	S
Mean	125.5	40.6	55.3	19.1	1.3	
SE	5.3	0.7	6.9	1.3	0.02	
CV%	9.3	3.8	27.6	15.4	4.6	

Means in the same column followed by the same letter(s) are not significantly different (P>0.05) following separation by Duncan's Multiple Range Test.

Resistance level: IS ≤0.5 resistant
IS >0.05 - 1.0 moderately resistant
IS >1.0 susceptible

residual bruchid infestation after which they were stored in a refrigerator at 5 – 8°C. From the refrigerator, 45 seeds of each single plant were drawn and placed in glass vials such that each vial contained 15 seeds replicated three times. To each vial, two pairs (i.e. 2 females and 2 males) of freshly emerged *Z. subfasciatus* were introduced after which the vials were covered with perforated plastic lids. A susceptible local variety Kijivu, served as a control. All vials were kept undisturbed in an incubator adjusted at 26 ± 2°C and 60 – 70% R.H. for 60 days. Seeds of each single plant were then assessed for bruchid damage on the basis of either damaged or undamaged. Seeds of a single plant with a mean number of more than one emerged adult insects were considered damaged while those with less than one insect were considered undamaged. Only seeds of undamaged single plants thus, carrying the gene for resistance were backcrossed to the respective recurrent parents and seeds of susceptible single plants were discarded. After three successive backcrosses seeds of resistant single plants were selfed three times in order to identify homozygous resistant single plants. At each selfing generation single plants were subjected to insect feeding tests as described earlier. The selfing process resulted in producing B_3S_3 generation seeds. Finally seeds of homozygous resistant single plants for each initial cross were mixed and multiplied. Thus, the programme developed five been breeding lines incorporated with arcelin. The five developed progenies and their parents were then evaluated for resistance against *Z. subfasciatus* using the procedure described by Schoonhoven and Cardona (1986). The variety Kijivu was include in the experiment as a susceptible control while the resistant line RAZ 24-2 served as a resistant control.

Freshly harvested seeds of the 12 treatments (Table 2) were conditioned and stored in a re-

frigerator as described earlier. For each treatment, 250 seeds were drawn from the refrigerator, divided into five seed lots and separately placed in 5 vials each containing 50 seeds. To each vial 7 pairs of freshly emerged adults of *Z. subfasciatus* were added and left to oviposit on the seeds. The vials were covered with perforated plastic lids and placed in an incubator as described earlier. The experimental design was a RCBD with five replications. The five vials per treatment represented replications and blocking was done by infesting one vial of each treatment per day for five consecutive days. After 20 days from the date of infestation for each replication, the introduced bruchids were sieved out (by that time all had died) using a 3mm mesh screen and the number of eggs laid per treatment counted. The vials were re-incubated and left undisturbed until the emergence of F_1 adult bruchids commenced. From then all emerged F_1 adults were removed from the vials daily and counted until 60 days from the infestation day after which the experiment was terminated. Data on progeny per female and days to adult emergence were used to calculate the index of susceptibility (IS) by adopting the formula proposed by Schoonhoven and Cardona (1986) as follows:

$$IS = \frac{\text{Natural log (progeny) female}}{\text{Day to 50\% } F_1 \text{ adult emergence}} \times 100$$

Results and Discussion

The relative degrees of resistance against *Z. subfasciatus* displayed in arceliin – containing and arcelin – deficient genotypes presented in a number of variables are summarized in Table 2. There were significant ($P < 0.05$) variations among the entries with respect to the number of eggs laid on bean seeds ranging from 108.8 – 145.8 eggs. Generally, bruchids laid many eggs on all treatments and there was no clear indication that bruchids laid more or few eggs on either arcelin – incorporated or arcelin – deficient entries. However, significant variations were observed in number of days to 50% F_1 adults emergence among the genotypes. This ranged from 29.4 to 53.2 days in the susceptible check (Kijivu) and the arcelin–inocorporated progeny RAZ 24-2 x EP 4-4, respectively. The results indicated that bruchids required more days to emerge from seeds of arcelin – containing than from arcelin – deficient genotypes. Thus, arcelin prolonged the bruchid life cycle. The number of days to 50% F_1 emergence ranged from 48.2 to 53.2 and 29.4 to 31.2 in arcelin – incorporated and arcelin – deficient entries, respectively. Significant differences among the entries in number of emerged adults were observed. However, all progenies and the arcelin – containing parent RAZ 24.2 had relatively low mean numbers of emerged adults, ranging between 1.0 and 2.6 adults. On the contrary, more adults emerged from the arcelin – deficient cultivars which ranged form 94.6 to 134.0 adults for PR 12 and SUA 90, respectively. This indicated that the presence of arcelin in bean seeds significantly reduced the total number of emerged adults. The most striking finding was that more bruchids emerged from the commercial variety (SUA 90) than from the susceptible check (Kijivu suggesting that SUA 90 is very susceptible to *Z. subfasciatus*.

Similarly, the total number of damaged seeds was relative low in all arcelin – containing genotypes. This ranged from 1.0 to 3.4 for RAZ 24-2 x EP 4-4 and RAZ 24-2 x PR 113, respectively. There were no significant differences among arcelin – incorporated cultivars for this variable. High numbers of damaged seeds were observed on arcelin – deficient genotypes, indicating that these were highly susceptible to *Z. subfasciatus*. The highest number of 41.0 damaged seeds was recorded on the susceptible control (Kijivu) but did not differ significantly ($P < 0.05$) from SUA 90 and PR 113 with 38.0 and 39.4 damaged seeds, respectively. This indicated that the agronomically superior cultivars SUA 90 and PR 113 were also very highly damaged by *Z. subfasciatus*. The lack of significant differences among the arcelin – incorporated genotypes in number of emerged adults and number of damaged seeds suggested that the presence of arcelin in bean seeds was the major determining factor for resistance against *Z. subfasciatus* in these treatments. The levels of resistance against *Z. subfasciatus* measured as IS's were significant among the treatments. The progeny RAZ 24-2 x EP 4-4 had the lowest IS of 0.18 and was thus the most resistant line among the genotypes tested. The commercial variety SUA 90 was more susceptible than the control variety Kijivu, which had IS's of 2.38 and 2.30, respectively. As it was indicated in earlier discussed variables,

all arcelin – containing entries were resistant to *Z. subfasciatus* while arcelin – deficient entries were all susceptible to the pest. This was a clear indication that arcelin protects bean seeds from attack by *Z. subfasciatus*. These results are consistent with those of Schoonhoven *et al.*, (1983) who found that the presence of arcelin in bean seeds confirmed resistance against *Z. subfasciatus*.

Conclusion

It is apparent from this study that the presence of arcelin in bean seeds confers resistance against *Z. subfasciatus* and that gene recombinants which are more resistant against *Z. subfasciatus* than RAZ lines from CIAT can be developed by crossing the locally adapted varieties to CIAT genotypes. The line RAZ 24 – 2 x EP 4-4 was more resistant against *Z. subfasciatus* than the arcelin donor parent RAZ 24 –2. It is brown with medium seed size. This line is therefore potential as an arcelin donor parent in future hybridization programmes aimed at incorporating arcelin in locally adapted bean varieties. It can be considered for release after it has been tested for other agronomical characteristics.

Acknowledgements

We are grateful to CIAT for providing the RAZ bean lines as sources of resistance against *Z. subfasciatus* for this study. We are also thankful to the CRSP Bean/Cowpea Project for funding the study.

References

Cardona, C. and Posso, C.E. (1987). Resistance to bean cultivars to the stored grain weevil, source, mechanisms and response factors. Hojas de Frihol. 9: 2, 1-4

De Lima, C.P. (1973). A technical Report on 22 Grain Storage Projects at subsistence farmers level in Kenya. 23 pp.

Masolwa, P.E. and Nchimbi, S. (1991). Distribution Patterns of Bean Bruchids: *Zabrotes subfsciatus* (Boh.) and *Acanthoscelides obtectus* (Say) (Bruchidae: Coleoptera) in some parts of Tanzania. Undergraduate Special Project : Unpublished.

Misangu, R.N. (1997). Distribution of bean bruchid species in ten major bean growing regions of Tanzania and breeding beans for resistance to *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boh.). Ph. D Theses submitted at Sokoine University of Agriculture.

Osborn, T.C.; Alexander, D.C.; Sun, S.M. ; Cardona, C and Bliss, F.A. (1986). Insecticidal activity and lectin homology of arcelin seed protein. Science 240: 207 – 210.

Schoonhoven, A.V. and Cvardona, C. (1986). Main insect pests of stored beans and their control. Study Guide. Central International de Agricultura Tropical.

Schoonhoven, A.V., C. and Valor, J. (1983). Resis tance to the Bean Weevil and the Mexican Bean Weevil (Coleoptera:Bruchidae) in non cultivated common bean accessions. Journals of Economic Entomology. 76 (6): 1255 – 1259.

Thiery, D. (1984). Hardness of some fabaceuos seed coats in relation to larval penetration by *Acanthoscelides obtectus* (Say) Coleoptera: Bruchidae). Journal of Stored Products Research. 20 (4): 177 – 181.