Evaluation of Advanced Breeding Lines of Common Bean (*Phaseolus vulgaris* L.) for Resistance to the Mexican Bean Weevil (*Zabrotes subfasciatus*) and Yield Potential

Teshale Assefa[1], Andrea Battisti[2], Cesar Cardona[3], Matthew Blair[3], Steve Beebe[3] Margherita Lucchin[2]
[1]*Melkassa Agricultural Research Center, Bean breeding. P.O.Box 436, Nazreth. Ethiopia*
[2]*Department of Agronomia Ambientale-Entomologia. Agripolis-Via Romea, 16. 350 20 Legnaro (Padova), Italia*
[3]*International Center for Tropical Agriculture, CIAT, Apartado Aereo 6713 Cali, Colombia*

Abstract

The study was carried out to select bruchid-resistant advanced lines of common bean with the presence of arceline determination and to evaluate their yield performance under field conditions in Ethiopia. Forty advanced lines and commercial varieties were tested for resistance to Mexican bean weevil (*Z. subfasciatus)* arranged in randomized complete block design in four replicates of 30 seeds infested with 6 pairs of newly emerged *Z. subfasciatus*. Data were taken on number of eggs, number of emerged adults, days to adult emergence (DAE) and adult dry weight. The insect bioassay and protein analysis were done at CIAT (Colombia) and the field evaluation was made in Ethiopia. The resistant lines containing Arcelin 1 showed for resistance to the Mexican bean weevil. Among these lines RAZ 4, RAZ 101, RAZ 173, RAZ 44 and RAZ 174 revealed consistently high resistance under all the parameters measured. However, the average yield of susceptible varieties (2.11t/ha, SE = 0.05) was moderately higher than that of the resistant lines (1.8 t/ha, SE = 0.02). The number of days to maturity varied between 78 and 96 without significance difference between susceptible varieties and RAZ lines. Using arcelin protein analysis with 12 highly resistant advanced lines and 10 susceptible varieties plus controls also showed a high level of accuracy. Resistance was associated entirely with the presence of the heavy 35 KDa band representing Arcelin 1.

Introduction

Common bean (*Phaseolus vulgaris* L.) is an important crop for food, cash and agro-ecosystem improvement in the central, southern, eastern and western Ethiopia. According to the 2005 Central Statistic Authority of Ethiopia (CSA) data, this crop is grown by nearly 2.5 million small scale farmers on about 250,000-300,000 hectares with annual production near to 234,900 metric tonnes. It is often cultivated by smallholder farmers in small plots of land in association with other crops or as sole crop with low external input. It is rapidly progressing as an important export earning of the country in recent years. The crop is also playing significant role in smallholder farming system serving as an important source of protein and income generation.

Economical losses due to insect attacks on stored beans are known to be substantial all over the world. Losses are higher in third world countries where good storage facilities are not usually available (Schoonhoven and Cardona 1986). Economically important bruchid species affecting bean are Mexican bean weevil, *Zabrotes subfasciatus* (Boheman) and the bean bruchid, *Acanthoscelides obtectus* (Say) (Schoonhoven and Cardona 1982). *Zabrotes subfasciatus* is a pest confined to warmer areas and is a storage pest, while *Acanthoscelides obtectus* is found in colder areas (higher altitude or latitude) infesting beans in the field and storage. Together these insect pests are estimated to cause an average of 13% grain loss to bean crops grown in developing countries (Cardona and Kornegay 1999). *Zabrotes subfasciatus* is the dominant species of storage insect in Eastern and Central Africa (Nchimbi and Misangu 2002). The damage caused by *Z. subfasciatus* in farm storage in Ethiopia was reported to be 38.1%, varying between 3.2% up to 80% at some locations, causing serious problem to Ethiopian bean grower (Ferede 1994).

Insecticides applied as dusts for admixture of seeds or sprays were been reported for the control of bruchids (Tsedeke 1995). Insecticide uses are not applicable at farm level because the farmers' storage structures are not air tight for fumigation and because of problems related to food safety. Hence, alternative methods like use of resistant varieties that could be easily utilized by farmers need to be considered.

Arcelin, a lectin-like protein, present in wild bean accessions is the factor responsible for resistance to the Mexican bean weevil (Osborn et al. 1988). To date, seven variants of Arcelin have been discovered and these variants are all highly similar but provide different levels of resistance (Osborne et al. 1988, Lioi and Bollini 1989 and Acosta-Gallegos et al. 1998). Within the allelic series the level of resistance is higher in the following variants Arc5, Arc4, Arc1, Arc2, Arc6, and Arc3 when the background is the wild progenitor. However, in the cultivated background the alleles that provide the highest resistance are Arc1 followed by Arc2, Arc5, Arc3 and Arc4 (Cardona and Kornegay 1999) with differences in resistance level thought to be due to protein differences or carbohydrate content (Harmsen et al. 1987).

Arcelin is inherited as a single gene, its presence being dominant to its absence (Kornegay et.al. 1993, Romero-Andreas et al. 1986). The precise mode of action of arcelin is not known, but the arcelin negatively affects the digestion process in the larval gut, resulting in lower emergence rate and lighter weight of surviving adults (Minney et al. 1990). Researchers at CIAT (International Centre for Tropical Agriculture, Bean Programme based at Cali, Colombia) have used the Arc1 variant widely in their breeding program to create resistant breeding lines known as RAZ (Resistant against *Z. subfasciatus*) through back crossing (Cardona et al. 1990). The objective of this study was to select bruchid-resistant advanced lines of common bean crossed at CIAT , to determine the presence of arcelin 1 and to evaluate their yield performance under field condition in Ethiopia.

Materials and Methods

Resistance evaluation

Techniques to maintain insect cultures and to test lines and commercial varieties were identical to those described by Schoonhoven and Cardona (1982). A set of 40 advanced lines of RAZ and susceptible commercial varieties were used in the trial from September to January, 2007. Of these, 30 were RAZ Mesoamerican and Andeans genotypes crossed at CIAT; 10 were Mesoamerican and Andean susceptible commercial varieties of different colour (Table 1).

The bean variety Calima was used to rear *Z. Subfasciatus* and obtained from a mass culture maintained at CIAT in a controlled environment chamber (27 ^{0}C and 70% RH).

Four replications were tested in randomized complete block design using 6 pairs of newly emerged Mexican bean weevil (*Z. subfasciatus)* to infest 30 seeds per replicate at 10 % seed moisture. Beans and weevils were put into small mesh covered clear plastic vials (2 x 1 inch) whose walls were covered with sandpaper (No. 150), the rough side facing inwards, to avoid egg laying on the surface of the plastic vials. Vials were incubated at 27 °C and 70 % relative humidity in a dark room. Data were collected on number of eggs at 15 days after infestation at which point initial insect parents were removed, number of emerged adults, percentage emergence and adult dry weight. In addition, to facilitate comparisons of resistance levels among genotypes, data on progeny per female and days to adult emergence were used to calculate Dobie's (1974) Index of susceptibility (IS).

$$IS = \frac{\text{Log progeny/female}}{\text{Days to adult emergence}} X100$$

Table 1: Resistant lines and susceptible varieties used in the experiment and their market classes.

Genotype	Reaction to bruchids	Source	Seed color	Seed size
Awash melka	S	Ethiopia	White	Small
Bat 41	S	Africa	Colored	Medium
Cal 96	S	Africa	Colored	Large
Cal 143	S	Africa	Colored	Large
Carioca	S	CIAT	Colored	Small
Emp 250	S	CIAT	Colored	Medium
Ex-Rico 23	S	Ethiopia	White	Small
ICA- Bunsi	S	CIAT	White	Small
ICA-Pijao	S	CIAT	Black	Small
PC 50	S	CIAT	Colored	Large
Raz 11	R	CIAT	White	Small
Raz 17	R	CIAT	Colored	Small
Raz 19	R	CIAT	White	Small
Raz 2	R	CIAT	White	Small
Raz 20	R	CIAT	Colored	Small
Raz 22	R	CIAT	White	Small
Raz 26	R	CIAT	White	Medium
Raz 34	R	CIAT	White	Small
Raz 36	R	CIAT	White	Small
Raz 37	R	CIAT	White	Small
Raz 4	R	CIAT	Cream	Small
Raz 40	R	CIAT	White	Small
Raz 42	R	CIAT	White	Small
Raz 44	R	CIAT	White	Small
Raz 49	R	CIAT	Colored	Small
Raz 54	R	CIAT	Colored	Small
Raz 7	R	CIAT	White	Small
Raz 101	R	CIAT	Colored	Small
Raz 105	R	CIAT	Colored	Large
Raz 11-1	R	CIAT	White	Small
Raz 111	R	CIAT	White	Small
Raz 114	R	CIAT	White	Small
Raz 119	R	CIAT	White	Small
Raz 120	R	CIAT	White	Small
Raz 136	R	CIAT	White	Small
Raz 138	R	CIAT	White	Small
Raz 151	R	CIAT	Colored	Small
Raz 173	R	CIAT	White	Medium
Raz 174	R	CIAT	White	Medium
Raz 24-2	R	CIAT	Colored	Large

CIAT: International Center for Tropical Agriculture. R: Resistant. S: Susceptible. Seed size is expressed as weight in grams of 100 randomly chosen seeds. Small: <25gm, medium: 25-40gm and large: >40gm.

Seed yield assessment

The same advanced lines and commercial varieties used for resistance evaluation were planted for seed yield evaluation in Ethiopia during 2008 cropping season at Melkassa Research Center (39° 12'N and 8° 24'E and 1550 meter above sea level). A randomized complete block design with four replications was used. The plot size was two rows of 3m long each with 40 and 10cm between and within row spacing, respectively. Data on days to physiological maturity and seed yield (t/ha) were recorded.

Arcelin determination

The presence of the arcelin protein and the arcelin alleles were determined according to the methods described by Ma & Bliss (1978) where a 0.75 g of bean flour was dissolved in 250 µl of extraction buffer, vortexed and centrifuged at 14,000 for 15 minutes. The supernatant was transferred and mixed with 50 µl cracking buffer, which was vortexed, boiled for 5 minutes, allowed to cool and centrifuged before loading on to a 5 µl stacking polyacralymide gel. Samples were run at a constant 150 volts until the samples passed in to running gel where a constant 25 mA was maintained. Protein gels were stained for 4 to 5 hours in 120 ml of 0.025 % Coomassie Blue R-250, 45.4 % methanol, 9.2 % acetic acid, and 45.4 % distilled water and then transfer to distaining solutions (I: 10 % acetic acid, 50 % methanol and II: 7 % acetic acid, 50 % methanol) for approximately 4 to 5 hours.

Data analysis

Percentage data were arcsin transformed wherever necessary. Analysis of variance (ANOVA) was computed using SAS 2002, Statistical Analysis Software.

Results

Resistance evaluation

The number of eggs laid on beans of each line differed significantly among genotypes ($F_{(39,120)} = 6.5$, $p < 0.01$), varying from 62.2 (SE 7.9) for RAZ 42 to 221.5 (SE 26.7) for RAZ 174 (Figure 1). Days to adult emergence (DAE) also differed significantly among lines ($F_{(39,120)} = 23.5$ $p<0.01$), where the susceptible commercial varieties resulted in lower number of days, ranging from 31 to 34, whereas the resistant lines did prolong DAE (Figure 1). The percentage of adult emerged showed significant difference among genotypes tested ($F_{(39,120)} = 229.0$). Percentage of emerged adults was lower on RAZ 4, RAZ 120, RAZ 42, RAZ 101, RAZ 173, RAZ 119, RAZ 44, RAZ 174, and RAZ 151 and a considerable variation in emergence rate was also observed among resistant lines, with values ranging from 33.4% to 0.7% (Figure 2). A significantly higher percentage adult emergence was recorded from varieties EX-Rico 23, PC-50, ICA-Pijao, ICA-Bunsi and Carioca (Figure 2). The dry weight of the adults obtained from the bioassay varied significantly among genotypes ($F_{(39,120)} = 6.4$ $p<0.01$). The heaviest adults were found in susceptible varieties while the lightest in resistant lines (Figure 2). The index of susceptibility of RAZ 4, RAZ 101, RAZ 173, RAZ 44, RAZ 174, RAZ 36, RAZ 2 and RAZ 20 was lower ranging from 1 to 3 than commercial varieties which showed a value ranging from 8.6 to 9.7. The remaining lines had intermediate resistance with values ranging from 3.1 to 5.0 (Figure 3).

Seed yield assessment

The seed yield had also significant difference among genotypes ($F_{(39,120)} = 6.3$ $p<0.01$). The average yield of susceptible varieties (2.11t/ha, SE = 0.05) was significantly higher than that of the resistant lines (1.8 t/ha, SE = 0.02) (Figure 3 and Table 2). The number of days to physiological maturity varied between 78 and 96, without any difference between susceptible commercial varieties and RAZ lines ($F_{(36,120)} = 0.74$, $p = 0.78$) (Table 2).

Table 2. Mean values of 30 RAZ lines and 10 susceptible commercial varieties for seed yield (t/ha) and physiological maturity with their market classes under rainfed condition in 2008 at Melkassa, Ethiopia.

Genotype	Yield (t/ha)	Maturity	Seed color	Seed size
Awashmelka	2.57	96	White	Small
Bat 41	2.12	79	Colored	Medium
Cal 96	1.92	80	Colored	Large
Cal 143	1.93	81	Colored	Large
Carica	2.22	90	Colored	Small
Emp 250	2.02	81	Colored	Medium
EX-Rico23	2.45	91	White	Small
ICA-Bunsi	2.10	86	White	Small
ICA-Pijao	1.78	84	Black	Small
PC 50	2.02	78	Colored	Large
RAZ 11	1.71	89	White	Small
RAZ 17	1.64	86	Colored	Small
RAZ 19	1.49	79	White	Small
RAZ 2	1.82	82	White	Small
RAZ 20	1.42	81	Colored	Small
RAZ 22	1.67	83	White	Small
RAZ 26	1.95	78	White	Medium
RAZ 34	1.84	90	White	Small
RAZ 36	1.91	91	White	Small
RAZ 37	1.51	80	White	Small
RAZ 4	1.23	83	Cream	Small
RAZ 40	1.60	87	White	Small
RAZ 42	1.63	82	White	Small
RAZ 44	2.12	89	White	Small
RAZ 49	1.88	81	Colored	Small
RAZ 54	1.48	84	Colored	Small
RAZ 7	1.89	85	White	Small
RAZ 101	2.01	83	Colored	Small
RAZ 105	2.00	89	Colored	Large
RAZ 11-1	1.51	91	White	Small
RAZ 111	1.88	80	White	Small
RAZ 114	1.36	86	White	Small
RAZ 119	1.92	93	White	Small
RAZ 120	1.96	80	White	Small
RAZ 136	1.43	91	White	Small
RAZ 138	1.68	90	White	Small
RAZ 151	1.92	82	Colored	Small
RAZ 173	1.90	94	White	Medium
RAZ 174	2.06	86	White	Medium
RAZ 24-2	1.76	78	Colored	Large
Mean	1.83	85		

Maturity: number of days from emergence to physiological maturity

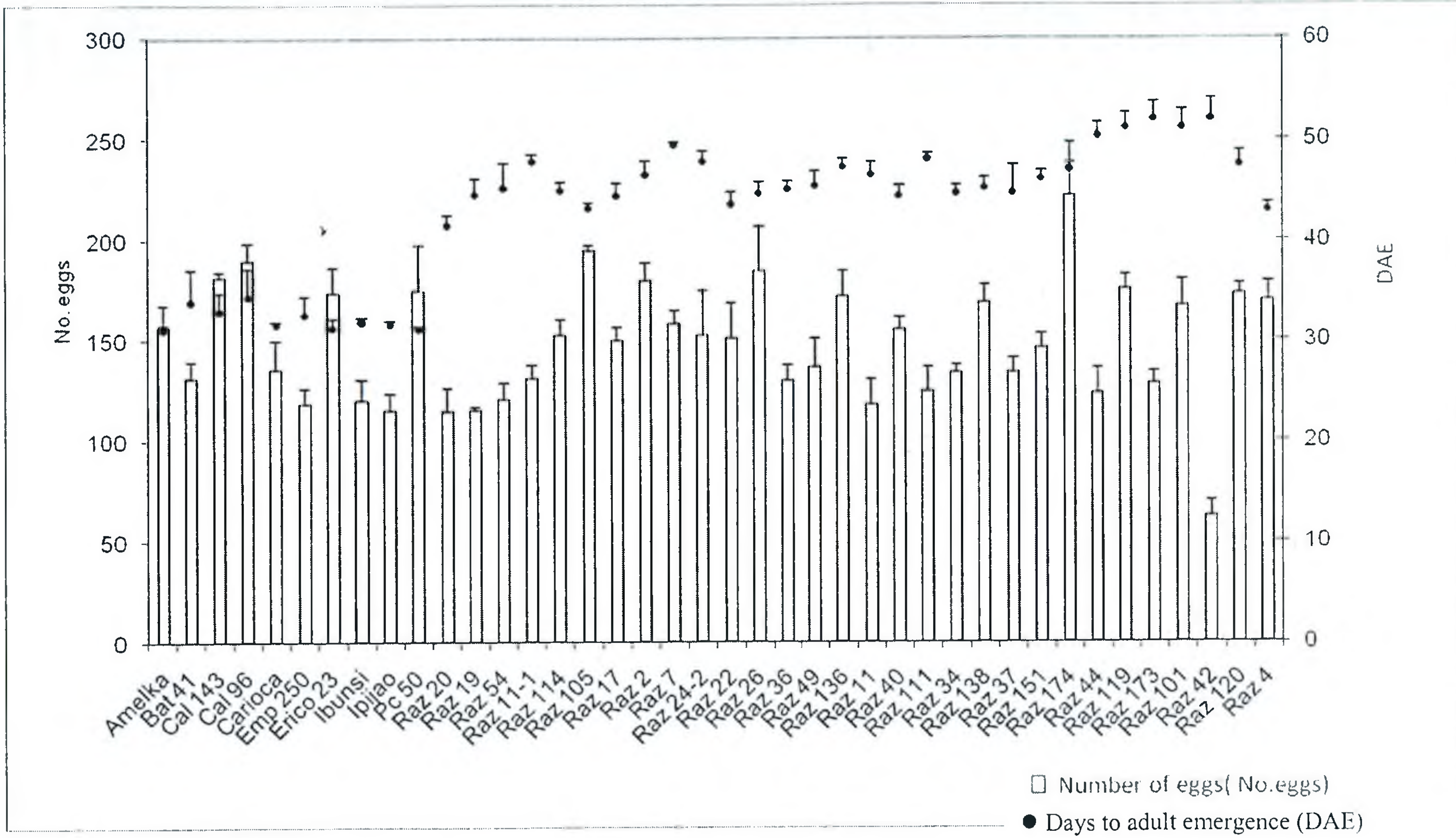


Figure 1. Number of eggs (▯ SE) and days to adult emergence (▯ SE) of Mexican bean weevil in bioassay with 10 susceptible and 30 resistant (RAZ) lines

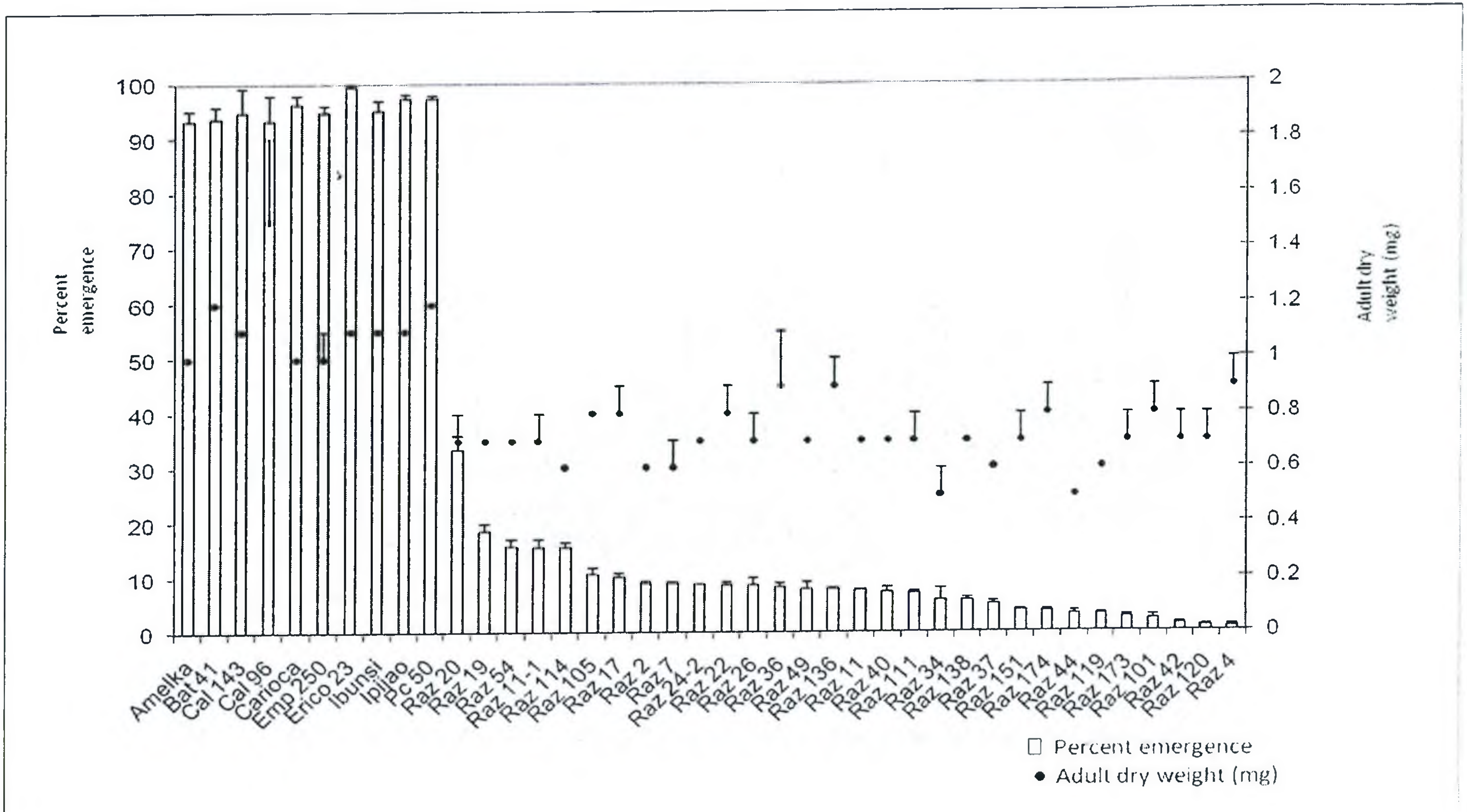


Figure 2. Percentage of emergence (▯ SE) and dry weight of adult Mexican bean weevil (▯ SE) in bioassay with 10 susceptible and 30 resistant (RAZ) lines

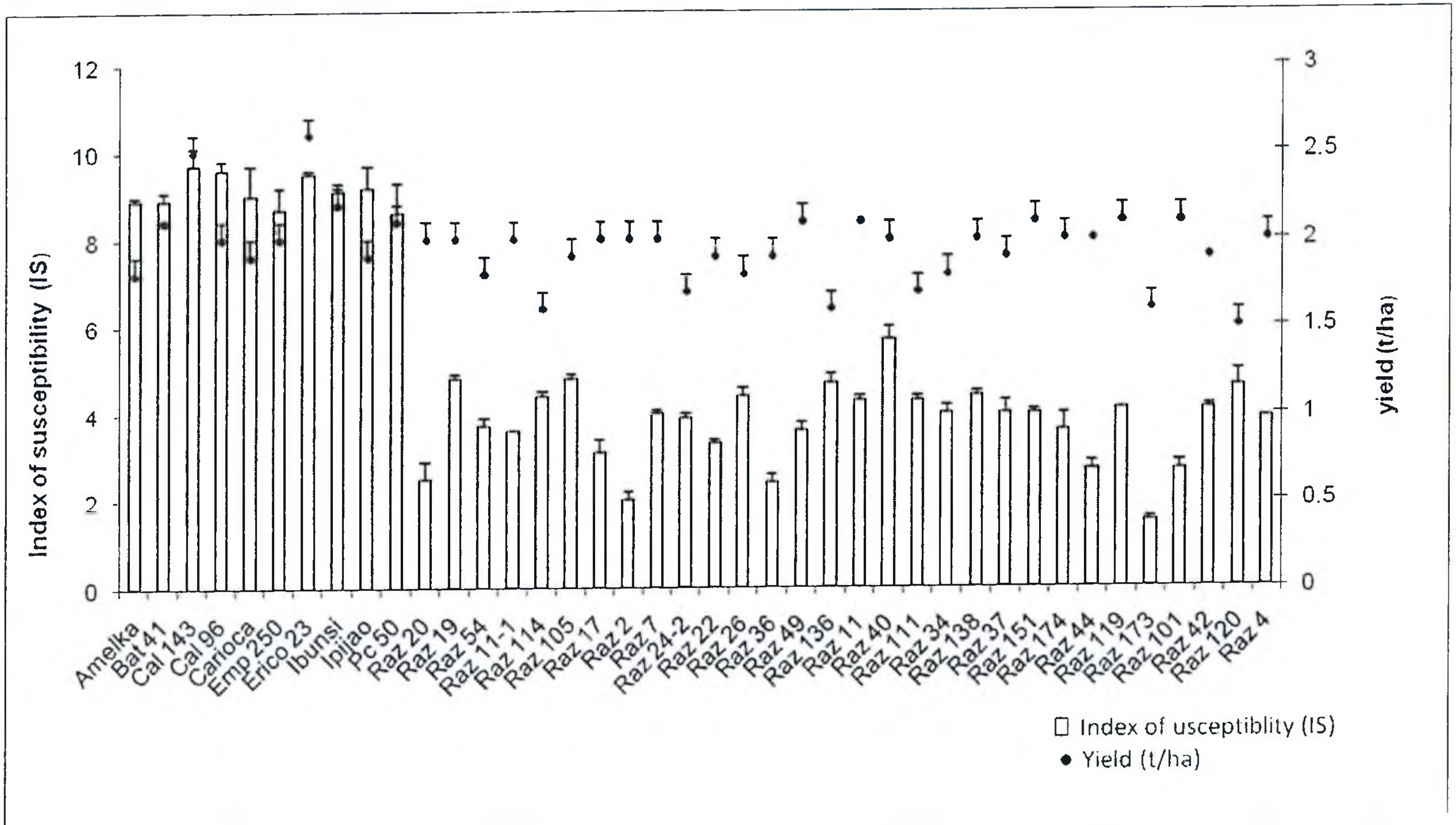
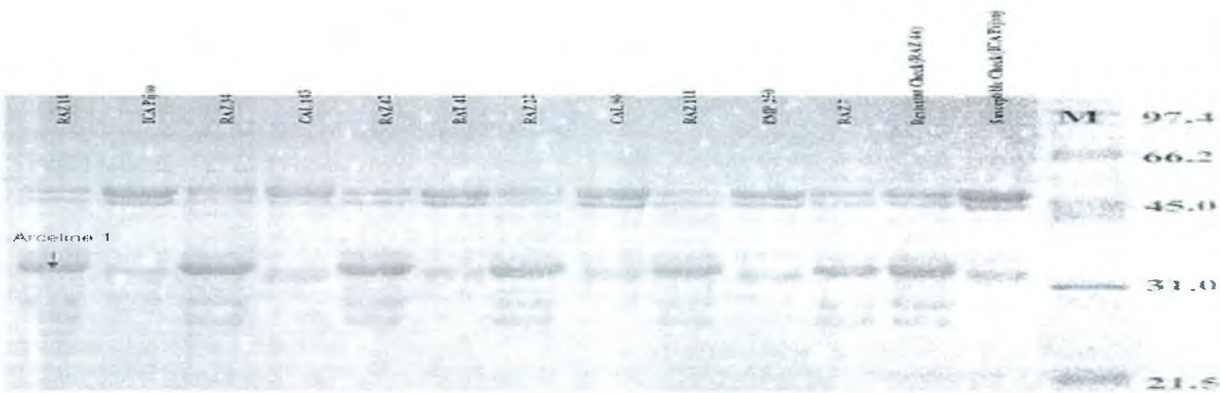


Figure 3. Index of susceptibility (± SE) and yield of (± SE) of bean lines tested in bioassay with 10 susceptible and 30 resistant (Raz) under field conditions in Ethiopia

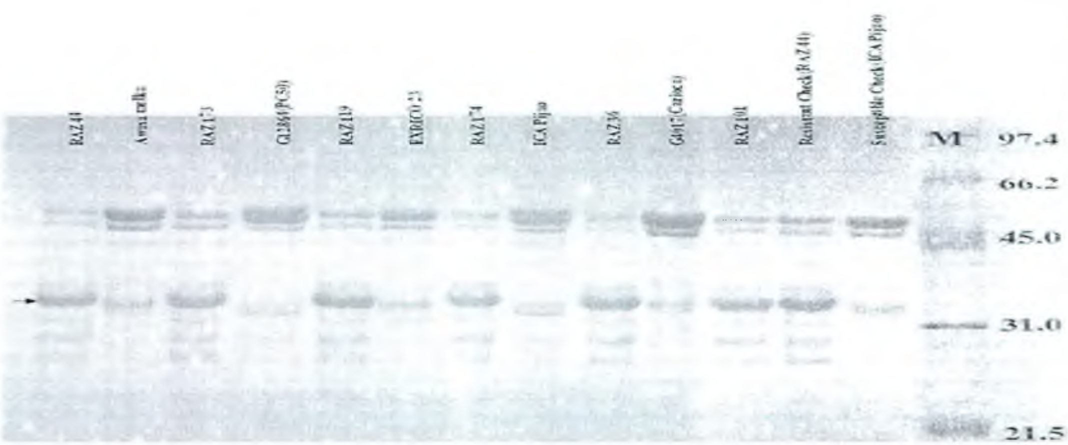
Arcelin determination

Electrophoretic patterns for advanced lines with high reconfirmed levels of resistance to Mexican bean weevil are shown in Fig.4 and 5. Lanes 1, 3,5,7,9 and 11 correspond to advanced RAZ lines whereas 2, 4, 6, 8 and 10 were susceptible varieties. Lanes 12 and 13 were standard checks for resistant and susceptible lines, respectively. The advanced resistance lines (except RAZ 101) were identical in terms of seed size, colour and growth habit. Arcelin protein analysis of 12 resistance advanced lines and 10 susceptible commercial varieties plus controls showed with high. reconfirmed level resistance to Mexican bean weevil (Figure 4 and 5). The electrophoretic analysis resulted in heavy 35 KDa band for the resistant lines that represents the presence of a seed protein Arcelin 1. This protein is not formed for the susceptible varieties. Therefore, resistance could be associated with presence of Arcelin 1 protein.



1 2 3 4 5 6 7 8 9 10 11 12 13
M

Figure 4. Electrophoretic patterns of resistant lines and susceptible varieties with reconfirmed level of resistance to MBW. Lane 1: RAZ 11;3: RAZ 34;5:RAZ 42; 7:RAZ 22; 9:RAZ111; 11:RAZ 7;12:Resistance check (RAZ 44) & 13: Susceptible check (ICA-Pijao). 2, 4, 6,8,10 are susceptible varieties (ICA-Ppijao, Cal.143, Bat41, Cal96 & EMP 250). M: standard molecular marker. Arrow points to Arcelin 1 bands.



1 2 3 4 5 6 7 8 9 10 11 12 13
M

Figure 5. Electropheretic patterns of resistance lines and susceptible varieties with reconfirmed level of resistance to MBW. Lane 1: RAZ 44; 3: RAZ 173; 5: RAZ 119; 7: RAZ 174; 9: RAZ 36; 11: RAZ 101; 12: Resistance check (RAZ 44) & 13: Susceptible check (ICA-Pijao). 2,4,6,8 & 10 are susceptible varieties (Awashmelka, PC 50, EX-Rico23, ICA-Pijao & Carioca). M: Standard Molecular markers. Arrow points on Arcelin 1 bands.

Discussion

Higher antibiosis resistance expressed as reduced emergence, prolonged life cycle and reduced progeny weight was observed in all advanced lines (RAZ). These indicators of resistance to bruchids have been studied by several workers. Redden and McGuire (1983) indicated mean emerging day or developmental time from egg to adult, cumulative adult emergence as percentage of number of eggs at 45 and 56 days as best separation of resistant and susceptible lines. Resistance was expressed as reduced oviposition, prolonged larval development period and reduced progeny weight (Schoonhoven et al. 1983). According to Mueke (1984) number of eggs oviposited, days to adult emergence, weight of adult and index of susceptibility are reliable measures of resistance and susceptibility. This experiment measured most of the above mentioned criteria as an indicator of resistance and susceptibility and the result indicated that the advanced lines containing arcelin 1 variant had good levels of resistance to *Z. subfacsiatus*. RAZ 4, RAZ 120, RAZ 42, RAZ 101, RAZ 173, RAZ 119, RAZ 44, RAZ 174, and RAZ 151 resulted in lower rate of emergence, longer days to adult emergence and lighter adult dry weight that exhibits their higher degree of resistance to *Z. subfasciatus*. The back cross lines containing Arcelin 1 variant have been similarly ranked in past studies for resistance to *Z.subfasciatus* (Harmsen, 1989, Cardona et al. 1990).

Ranking of the advanced lines as resistance factors was facilitated by comparison on Index of susceptibility (IS). It is linearly correlated with the intrinsic rate of increase and thus with the logarithm of the numbers of insects that will be produced in a given period of time, providing a reliable estimate of resistance levels. Under our experimental conditions, IS values for susceptible varieties and checks usually range from 8.6 to 9.7. Genotypes with low IS values are rated as highly resistant and those with high values as susceptible ones (Cardona et al. 1989). Consequently, RAZ 4, RAZ 101, RAZ 173, RAZ 44, RAZ 174, RAZ 36, RAZ 2 and RAZ 20 confer higher level of resistance to Mexican bean weevil. These results agree with those reported by Cardona et al. (1990) and Cardona et al. (1989). In general, there was a marked difference in a susceptible index among the 40 varieties tested ranging from 1.5 for RAZ 173 and over 9.5 for the varieties Cal 96 and Cal 143.

Electrophoretic patterns for advanced lines with high reconfirmed levels of resistance to Mexican bean weevil was associated entirely with the presence of the heavy molecular weight 35KDa band that represents arcelin 1 in seed (Romero Andreas et al. 1986).

In summary, most of the advanced lines crossed at CIAT Colombia showed high to intermediate level of resistance in all the parameters tested as compared to commercial varieties. The advanced lines RAZ 4, RAZ 101, RAZ 173, RAZ 44 and RAZ 174 were consistently resistant for all parameters measured and can therefore be used as source of resistance in a breeding program. As varieties resistant to *Z. subfasciatus* are currently unavailable in the country, it is suggested the national bean research program tests the promising lines for their agronomic excellence and commercial values and then releases the best performing ones for production. Efforts should also be made by the national breeding program to incorporate arcelin alleles in to improved commercial varieties

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