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Genetic Gain for Adzuki Bean Beetle (*Callosobruchus chinensis* L.) Resistance in Ethiopian Chickpea (*Cicer arietinum* L.) Genotypes

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Abstract: Chickpea (*Cicer arietinum* L.) is attacked by a number of insect pests, including the adzuki bean beetle (*Callosobruchus chinensis* L.). Genetic gains from selection to infestation by adzuki bean beetle were studied using 130 chickpea genotypes in 2009/2010. The test was conducted under ambient condition in the Entomology Laboratory of Holetta, Ambo and Debre Zeit, using RCBD in 3 replications. Data on infestation and seed damage levels were recorded and subjected to statistical analysis. Differences among the genotypes were mostly significant. Seed related traits generally exhibited larger heritable variations than insect related traits. The t-test revealed effective selection for almost all traits. Broad sense heritability varied from 43-76% and 0.20-11.00% for seed and insect related traits, respectively. The corresponding expected genetic gain ranged from 28-42% and 0.01-6.00% in the same order. Significant positive correlations were found among seed weight loss and three component characters, i.e. number of eggs, adults emerged and seed size. Number of uninfested seeds (%) and percent seed coat weight showed strong negative correlations with seed weight loss. Selection of genotypes could improve resistance to the beetle in this gene pool, but with a compromise for seed quality. A search for more suitable sources of variability and selection criteria would be advisable with the adoption of molecular plant breeding tools for better selection efficiency and effectiveness.

Keywords: Broad Sense Heritability; Chickpea; Correlation Coefficient; Genetic Gain

1. Introduction

Chickpea (*Cicer arietinum* L.) is widely grown in tropical Africa particularly in Ethiopia, Sudan, Eritrea, Kenya, Tanzania and Malawi (Bejiga and van der Maesen, 2006). Ethiopia, with a total area of 194981 ha and a productivity of 1.19 tons per hectare, contributes 46% of the total production of chickpea in Africa (Kassie *et al.*, 2009). Chickpea ranks second in both area coverage and volume of production from among the highland pulses grown in Ethiopia preceded only by faba bean (*Vicia faba* L.) (CSA, 2013). It is grown in several regions of the country on black Vertisol soils with residual moisture (Bejiga and van der Maesen, 2006; Kassie *et al.*, 2009). Chickpea is produced for different purposes including food and feed, cash and foreign currency earnings. Despite its importance, however, the production and productivity of chickpea is constrained at least in part by field and storage insect pests.

Chickpea is attacked both by pre- and post-harvest insect pests in Ethiopia (Ali and Habtewold, 1994). Storage insect pests, particularly the adzuki bean beetle (*Callosobruchus chinensis* L.), are important constraints in chickpea seed and grain storage. Some reports indicate that the adzuki bean beetle in chickpea may cause a loss of up to 50% in Ethiopia (Ali and Habtewold, 1994; Damte and Dawd, 2006) and 28% in neighboring Eritrea (Haile, 2006). Why more loss was observed in Ethiopia as compared to Eritrea may remain the subject of future investigation but in addition to the actual loss, the chickpea grain, once damaged by bruchid, fits neither for planting (due to poor germination) nor for food (due to spoilage and bad smell) (Aslam, 2004; Haile, 2006).

Chickpea breeding in Ethiopia was initiated in the 1960's and a number of locally collected and newly introduced germplasm have been evaluated to develop varieties having better combination of characters, with major emphasis on high-yielding potential and wide adaptability. The generation of information related to genetics of resistance to storage insects has received little attention as compared to resistance to even field insects. Ethiopia, as the secondary center of genetic diversity, owns an immense wealth of variability for many legumes (Hagedorn, 1984; Mekibeb *et al.*, 1991) including chickpea (van der Maesen, 1987). A large number of Ethiopian chickpea germplasm accessions have been collected and conserved in the Ethiopian Gene Bank (Tanto and Tefera, 2006). Despite the large number of collections, however, their proper utilization in breeding programs is limited by the lack of baseline genetic information.

The two basic requirements for selection to be effective are the presence of adequate variation in the breeding material and the variation must be heritable (Singh, 2002). Selection efficiency also largely depends on the level of selection intensity posed by the breeder (Falconer, 1989) and on the existence of effective selection criteria (Wricke and Weber, 1986). Most studies examining genotypic variation for resistance of storage insect pests in food legumes elsewhere demonstrated existence of genetic variation in landraces, cultivated varieties and their wild relatives

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(Clement *et al.*, 2002; Ku-Hwan *et al.*, 2002; Shaheen *et al.*, 2006; Chen *et al.*, 2007; Somta *et al.*, 2006, 2007, 2008). However, the magnitude of traits heritability and the extent and pattern of their cause-effect relationship vary with the changes in the environment (Rosielle and Hamblin 1981; Simmonds 1991; Banziger and Edmeades 1997; Singh 2002) and nature of the genetic material under consideration (Ceccarelli and Grando, 1996).

Direct selections for storage insects' resistance in breeding nurseries may be difficult as artificial bio-assaying is not practically feasible given the limitation in technical requirements. Even though a number of secondary traits associated with seed resistance to storage insects have been suggested for indirect selection without the need for seed bio-assaying (Ahmed *et al.*, 1989; Shaheen *et al.*, 2006), their application was limited as they were found to be inconsistent with changes in genetic materials (Lale and Kolo, 1998; Somta *et al.*, 2007; Srinivasan and Durairaj, 2007). Therefore, specific tests for specific genetic material should be given a due attention. Identification of secondary traits positively associated with bruchid resistance, genetically variable, highly heritable and easily observable among the Ethiopian landraces, therefore, helps in formulating efficient scheme of multiple trait selection, as it provides means of both direct and indirect selections of primary and secondary characters (Lawes *et al.*, 1983; Edmeades *et al.*, 1997; Edmeades *et al.*, 1998). The objective of this study was, therefore, to determine the magnitude of expected genetic gains from selection for response characters to infestation by adzuki bean beetle in Ethiopian chickpea genotypes.

2. Materials and Methods

2.1. Plant Materials

One hundred-thirty chickpea genotypes were considered for the study. These include 117 Ethiopian chickpea germplasm accessions representing over 10% of the germplasm held by the Ethiopian Institute of Biodiversity Conservation and 13 improved varieties or breeding lines from the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). All genotypes were rejuvenated during 2008/2009 under the same condition at Ginchi to minimize initial variation due to difference in seed age. Description of the genotypes tested in this study is given in Table 1.

2.2. Testing Environment and Experimental Design

Freshly harvested seeds with moisture contents of 9.0-9.5% were disinfested in a deep freeze at about -20 ^{o}C for a month prior to the study. Seeds with no insect damage were hand-sorted and exposed to infestation from April to June, 2009. The experiment was conducted in entomology laboratories under ambient temperature and relative humidity (RH) at Holetta, Ambo and Debre Zeit Agricultural Research Centers, Ethiopia. The temperatures and relative humidity of the test period are summarized and presented in Figure 1. Adzuki bean beetles were collected from the Holetta Agricultural Research Center (Entomology Research Section) and mass reared in the laboratory at the same Center on a bulk of chickpea seeds of one of the susceptible Kabuli cultivars, *Shasho*. Two hundred seeds of each genotype were put in a 250 ml (6 cm x 7 cm) plastic jar with openings at one end for free air circulation. Fourteen 1-2 day old unsexed adults were randomly taken and placed in each jar. The male to female ratio in this insect being nearly 1:1 (Lemma, 1990), it was assumed that each jar received 7 males and 7 females. The ovipositing adults were kept in the jars for 10 days after introduction and then were removed from the jars. Records on the first progeny were taken until complete adult emergence. The first progeny was removed from the jars in the same way as the initial parents for further evaluation of the level of attack and loss incurred by the second progeny. The experiment was conducted in a randomized complete block design (RCBD) with 3 replications. The chickpea genotypes were assigned to jars at random within each block.

2.3. Data Collection and Statistical Analysis

Data were collected on number of eggs female^{-1} (first progeny), proportion of uninfested seeds (%) (first progeny), days to adult emergence (first and second progenies), number of adults emerged (first and second progenies), adult recovery (%) (first progeny) and seed weight loss (g) (first and second progenies). Days to adult emergence were recorded only at Holetta and Ambo. Seed size as 1000 seed weight (g) and the proportion of seed coat by weight (%) were taken from a 4 times replicated field trial conducted using RCBD (plot size = 1 row 4 m long) on the same genotypes grown under the same conditions. The field experiment was conducted in 2009/2010 (September to January) on Vertisol soil with residual moisture at two locations, namely Ginchi (09° 00′ N latitude; 38° 10′ E longitude; altitude = 2200 meters above sea level; rainfall = 10-80 mm or on the average 35 mm month^{-1}; RH = 30-65%; temperature = 10-26 ^{o}C) and Ambo (09° 00′ N latitude; 37° 22′ E longitude; altitude = 2225 meters above sea level; rainfall = 5-80 mm or on average 38 mm month^{-1}; RH = 45-70%; temperature = 10-25 ^{o}C). The locations are considered to somehow represent the major chickpea production areas in the country.

Weight loss adjusted to 10% moisture was calculated for each genotype at the end of the experiment by separating healthy seeds (without holes) from each jar as suggested by Shaheen *et al.* (2006):

$$\text{Weight loss} = \text{Initial weight} - (\text{Weights of healthy} + \text{damaged seeds}) \quad (1)$$

Data based on count and percentage values were log and ARCSINE transformed, respectively, for statistical analysis when necessary (Little and Hills, 1978; Gomez and Gomez, 1984). Untransformed means were used for all the analyses except for analyses of variance (ANOVA) which was computed to quantify the total variation among the genotypes using the following model:

$$P_{ijk} = \mu + (b/l)_{ik} + g_j + l_k + (gl)_{jk} + e_{ijk} \qquad (2)$$

where P_{ijk} = phenotypic observation on genotypes j in block i (at location) k (i = 1…B, j = 1…G and k = 1…L) and G, L and B = number of genotypes, location and block, respectively, μ = grand mean, $(b/l)_{ik}$ = the effect of block i (within location k), g_j = the effect of genotype j, l_k = the effect of location k, $(gl)_{jk}$ = the interaction effect between genotype j and location k and e_{ijk} = the residual or effects of random error.

Table 1. Origin and name of genotypes.

Origin	No. of genotypes	Name of genotypes
Arsi	11	Acc. No. 231327, Acc. No. 231328, Acc. No. 209093, Acc. No. 208829, Acc. No. 209094, Acc. No. 209096, Acc. No. 209097, Acc. No. 209098, Acc. No. 41002, Acc. No. 207761, Acc. No. 207763
East Gojam	10	Acc. No. 41026, Acc. No. 41074, Acc. No. 41075, Acc. No. 41076, Acc. No. 41021, Acc. No. 41027, Acc. No. 207734, Acc. No. 41103, Acc. No. 41320, Acc. No. 41029
West Gojam	12	Acc. No. 41015, Acc. No. 41271, Acc. No. 41276, Acc. No. 207745, Acc. No. 41275, Acc. No. 41277, Acc. No. 207743, Acc. No. 207744, Acc. No. 41273, Acc. No. 41274, Acc. No. 207741, Acc. No. 207742
North Gonder	13	Acc. No. 41316, Acc. No. 41298, Acc. No. 41311, Acc. No. 41313, Acc. No. 41280, Acc. No. 41312, Acc. No. 41315, Acc. No. 41308, Acc. No. 41299, Acc. No. 41046, Acc. No. 41047, Acc. No. 41304, Acc. No. 41303
South Gonder	9	Acc. No. 41295, Acc. No. 41296, Acc. No. 41289, Acc. No. 41290, Acc. No. 41291, Acc. No. 41297, Acc. No. 41293, Acc. No. 41048, Acc. No. 41053
West Harargie	8	Acc. No. 41054, Acc. No. 41052, Acc. No. 209084, Acc. No. 209091, Acc. No. 209087, Acc. No. 209088, Acc. No. 209089, Acc. No. 209090
East Shewa	13	Acc. No. 41159, Acc. No. 41160, Acc. No. 41161, Acc. No. 207661, Acc. No. 207667, Acc. No. 207666, Acc. No. 41141, Acc. No. 207665, Acc. No. 41134, Acc. No. 41128, Acc. No. 41168, Acc. No. 41129, Acc. No. 41130
North Shewa	10	Acc. No. 41110, Acc. No. 41111, Acc. No. 207658, Acc. No. 41142, Acc. No. 41207, Acc. No. 41215, Acc. No. 41066, Acc. No. 41011, Acc. No. 41007, Acc. No. 41008
West Shewa	10	Acc. No. 209035, Acc. No. 41176, Acc. No. 41175, Acc. No. 41174, Acc. No. 41170, Acc. No. 41171, Acc. No. 41185, Acc. No. 209036, Acc. No. 41190, Acc. No. 41195
Tigray	9	Acc. No. 207151, Acc. No. 207563, Acc. No. 207564, Acc. No. 207895, Acc. No. 219797, Acc. No. 219799, Acc. No. 219800, Acc. No. 219803, Acc. No. 221696
South Wello	12	Acc. No. 41114, Acc. No. 212589, Acc. No. 41113, Acc. No. 207659, Acc. No. 207660, Acc. No. 225878, Acc. No. 225873, Acc. No. 225874, Acc. No. 225877, Acc. No. 207645, Acc. No. 207646, Acc. No. 225876
Improved genotypes (ICRISAT, ICARDA)	13	ICC 5003, ICC 4918, ICC 4948, ICC 4973, ICC 15996, Shasho (ICCV 93512), Arerti (FLIP 89-84C), Worku (DZ-10-16-2), Akaki (DZ-10-9-2), Ejere (FLIP-97-263c), Teji (FLIP-97-266c), Habru (FLIP 88-42c), Natoli (ICCX-910112-6)

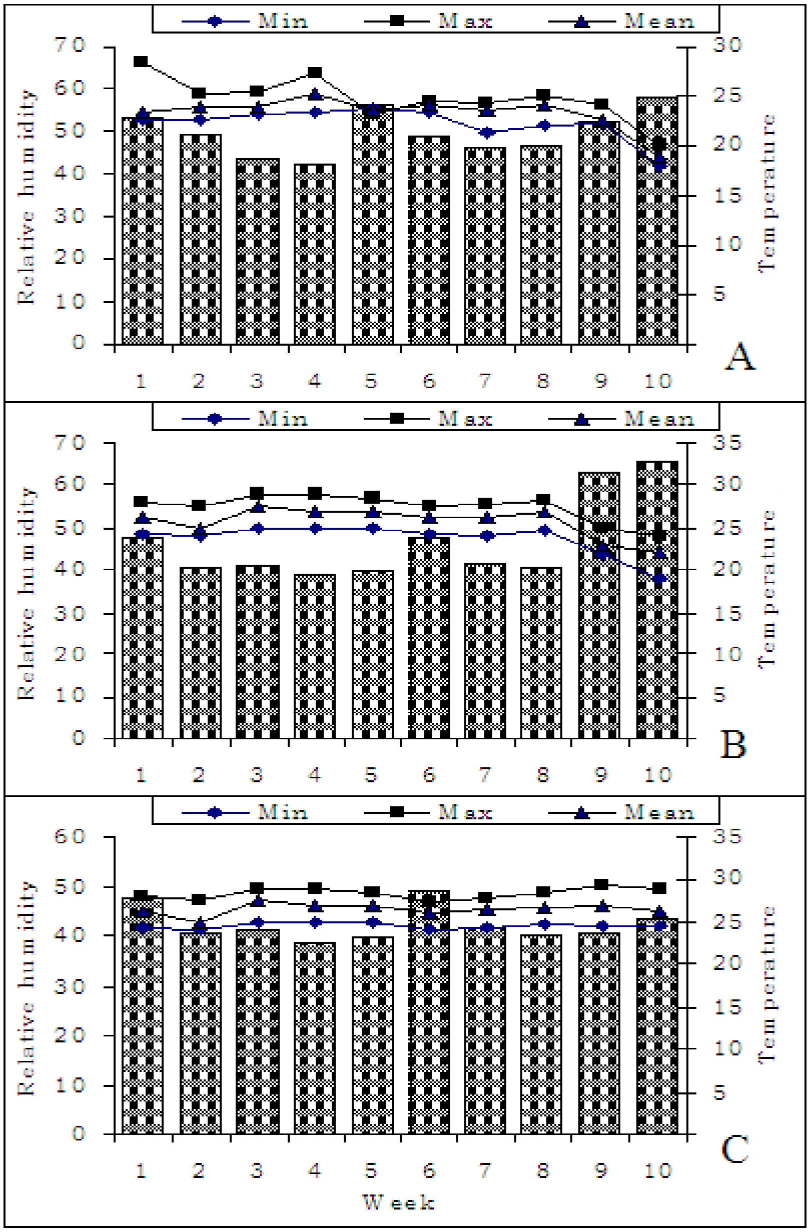

Figure 1. Relative humidity (%, bar graphs) and temperature (°C, line graphs) in laboratories at (A) Holetta, (B) Ambo and (C) Debre Zeit during the study period.

Some means were adjusted for initial seed weight as a covariate whenever significant covariance was observed. Existence of significant difference among the genotypes for the attributes was determined using the F-test. Mean separation was done using Tukey's honestly significant difference test as suggested by Sokal and Rohlf (1997).

To compare selected subsets of the 5% best genotypes within the whole population, they were sorted and means were independently computed for each character. The absolute value of Student's t test was calculated to compare genotypic values of the 5% best selected genotypes with the base population as:

$$t = \left| \frac{\overline{X} - \mu}{\sigma / \sqrt{n}} \right| \qquad (3)$$

where $\overline{X}$ = mean of selected genotypes, μ is mean of the base population, σ = the standard deviation calculated for the base population and n = the number of genotypes selected from the base population for better resistance.

Partitioning of the total variance into components due to genotype (σ_g^2), environment (σ_e^2) and genotype by environment interaction (σ_{ge}^2) variances was performed from the analyses of variance by assuming various observed mean squares equal to their expected mean squares (Table 2) as suggested by Singh and Chaudhary (1985):

$$\sigma_g^2 = [(\sigma_e^2 + R\sigma_{ge}^2 + RL\sigma_g^2) - (\sigma_e^2 + R\sigma_{ge}^2)]/RL = (MS3\text{-}MS4)/RL \qquad (4)$$

$$\sigma_e^2 = MS5 \qquad (5)$$

$$\sigma_{ge}^2 = [(\sigma_e^2 + R\sigma_{ge}^2) - (\sigma_e^2)]/R = (MS4\text{-}MS5)/R \qquad (6)$$

where σ_g^2 = genotype variance, σ_e^2 = environmental variance and σ_{ge}^2 = genotype by environment interaction variances. Broad-sense heritability (h^2) was calculated as:

$$h^2 = \sigma_g^2 \ / \ [\sigma_g^2 + \sigma_{ge}^2/L + \sigma_e^2/RL] \times 100 \qquad (7)$$

where the components of the equation are as described in equations (4) and (6) above.

The predicted response of seed weight loss and the components to selection or the expected genetic advance (GA) were calculated, assuming the selection intensity of 5%, as:

$$GA = K. \sqrt{\sigma_P^2}. (\sigma_g^2/\sigma_p^2) = K. \sigma_P. h^2 \qquad (8)$$

$$\text{GA as \% of mean} = \frac{GA}{\bar{X}} \times 100 \qquad (9)$$

where, GA = expected genetic advance from selection and K = the selection differential (K = 2.06 at 5% selection intensity), σ_P = square root of phenotypic variance and h^2 = heritability (Singh and Chaudhary, 1985)

Correlation coefficients between characters were estimated based on the standard procedure as:

$$r = Cov_{xy} / \text{sqrt} [\sigma_x^2 + \sigma_y^2] \qquad (10)$$

where $Cov_{(xy)}$= co-variance of traits x and y, σ_x^2 = variance of x and σ_y^2 = variance of y.

Table 2. Model used to compute combined analysis of variance (ANOVA) that was used to calculate variation among chickpea genotypes.

Source of variation	Degree of freedom	Mean square (MS)	Expected mean square (EMS) [1]
Locations	L –1	MS1	$\sigma_e^2 + G\sigma_r^2 + GRL\sigma_l^2$
Replications/location	L(R -1)	MS2	$\sigma_e^2 + G\sigma_r^2$
Genotypes	G –1	MS3	$\sigma_e^2 + R\sigma_{gl}^2 + RL\sigma_g^2$
Genotype x Location	(G –1)(L -1)	MS4	$\sigma_e^2 + R\sigma_{gl}^2$
Error	L (G -1)(R –1)	MS5	σ_e^2

[1] *For explanation of abbreviations used refer to equations (4) and (5)*

3. Results and Discussion

3.1. Performance of the Genotypes

Pooled analyses of variance across the three locations showed statistically significant differences for all the characters recorded except for non-significant differences among the genotypes for the proportion of un-infested seeds, i.e. seeds with no signs of eggs or emergence holes. Different ranges of variability were found in different characters with the least differences observed between minimum and maximum values for days to adult emergence (Table 3). On average, the damage and loss by the second progeny were higher than that incurred by the first progeny in almost all the cases (data not shown).

Average performances of selected 5% best genotypes for attributes of both infestation levels and seed weight loss are presented in Table 4. Comparison of the selected 5% best genotypes showed lesser number of eggs and adults, lower adult recovery and seed weight loss, longer emergence period, and higher number of uninfested seeds and thicker seed coat cover as compared to the original population. This revealed the efficiency of selection in recovering genotypes with better resistance levels in just one cycle of selection. Selected genotypes scoring least number of eggs and adults, adult recovery, seed weight loss and smallest seed size on the one hand and those with delayed adult emergence, highest number of uninfested seeds (%) and largest seed coat weight (%) on the other hand were considered as the most resistant as compared to the population means. The Student's t-test showed significant differences between means of the selected 5% best genotypes ($\bar{X}$) and the population mean (μ) for different traits except seed weight loss recorded for the second progeny which was not significantly changed through selection. This indicates that the selected accessions were not true representatives of the population and that almost all characters effectively responded to phenotypic selection (Singh, 2001). Even though one or two days differences appeared to be statistically significant (Gemechu *et al.*, 2011), it would practically be impossible to delay adult developmental period through selection among the populations studied.

Table 3. Mean square for genotypes, range, mean, standard deviation (Sd) and coefficients of variation (CV) for response characters to infestation by Adzuki bean beetle in the 130 chickpea genotypes.

Character	Progeny	Mean square for genotype	Range	Mean±Sd	CV (%)
Total Number of eggs	First	**	152-359	234±35	26.69
Days to adult emergence	First	*	31-33	32±0.35	2.32
	Second	**	33-37	34±0.55	3.31
Number of adults emerged	First	**	104-222	160±26	27.33
	Second	**	456-1136	590±129	22.31
Number of uninfected seed (%)	First	NS	12-46	31±6	20.87
Adult recovery (%)	First	**	56-85	70±6	16.89
1000 seed weight (g)	---	**	85-276	114±28	19.17
Seed coat weight (%)	---	**	5-21	18±3	8.85
Seed weight loss (g)	First	**	2-13	5±2	30.77
	Second	**	5-15	7±2	19.89

*** = Highly significant ($P \leq 0.01$); * = Significant ($P \leq 0.05$); NS = Non-significant ($P > 0.05$)*

Generally, resistance to storage insects in grain crops is of rare occurrence in nature. For instance, only one out of 6000 cowpea (*Vigna unguiculata*) accessions was reported resistant to *C. maculatus* (Redden and McGuire, 1983). From the present study, complete resistance was not observed and accessions identified as best for one character may not consistently repeat the same performance for another character (data not shown). This may indicate that in different genetic backgrounds, different mechanisms of resistance, starting from deterring oviposition to hindering adult development, may operate. Despite the inconsistencies, however, some accessions like Acc. No. 41320, Acc. No. 41289, Acc. No. 41291, Acc. No. 41134, Acc. No. 41315 and Acc. No. 207658 were found to be among the best 5% as sources of resistance based on relative ranking. Note that these accessions with lesser infestation and seed damage levels were generally characterized by small seed size and thicker seed coat cover as attributes that conferred better protection (Table 4). Therefore, the observed genetic enhancement in this study is encouraging to increase the frequency of resistance genes through further intra-accession selections that would help to select more resistant lines. Thus, it is necessary to evaluate more number of local and exotic collections in order to obtain genotypes with a required level of resistance. Interestingly, none of the improved genotypes stood one among the selected 5% best genotypes for any one of the attributes of resistance.

Table 4. Comparison of the mean performances of the selected 5% best accessions and population for response characters to infestation by Adzuki bean beetle.

Characters	Progeny	Population parameter (μ)	Mean of selected genotypes ($\bar{X}$)	Change through selection (/μ- $\bar{X}$ /)	Change as % of population parameter (μ)	t
Total Number of eggs	First	234	177	57	24.36	3.99**
Days to adult emergence	First	32	33	1	3.13	7.00**
	Second	34	35	1	2.94	4.45**
Number of adults emerged	First	160	112	48	30.00	4.52**
	Second	590	465	125	21.19	2.37*
Number of uninfected seed (%)	First	31	43	12	38.71	4.90**
Adult recovery (%)	First	70	59	11	15.71	4.49**
1000 seed weight (g)	__	114	90	24	21.05	2.10*
Seed coat weight (%)	__	18	21	3	16.67	2.45*
seed weight loss (g)	First	5	3	2	40.00	2.45*
	Second	7	6	1	14.29	1.23NS

** = Highly significant ($P \leq 0.01$); * = Significant ($P \leq 0.05$); NS = Non-significant ($P > 0.05$)

3.2. Broad-sense Heritability and Expected Genetic Gain from Selection

Seed related traits (seed size, percent seed coat weight and seed weight loss) generally exhibited larger heritable variation than insect related traits (total number of eggs, days to adult emergence, number of adults emerged, number of uninfested seed and adult recovery). This was revealed from the higher broad-sense heritability and predicted genetic gains, values for seed related and the extremely lower values for insect related characters (Figure 2). The levels of broad-sense heritability ranged from 43-76% for seed related traits indicating that the observed phenotypic values for these traits could better reflect the genotypic values. On the other hand, heritable genetic variations for insect related traits were extremely lower ranging from 0.20-11.00%.

The corresponding expected genetic gains from selection varied from 28-42% for seed related traits. The present study indicated that resistance could either be improved through direct selection for reduced seed weight loss as a primary trait or through indirect selection for small seed size and increased seed coat weight as component characters or both. Progresses that could be expected from selection for the component traits ranged from 2 g for seed weight loss in first progeny to 38 g for seed size. In other words, it is possible to reduce seed weight loss by 31% through direct selection for first progeny or by 42% at second progeny. Likewise, seed size can also be reduced by 33% while seed coat weight can be increased by 28%. Better expected genetic gain from selection for seed size as a seed quality trait of agronomic importance was also reported earlier (Yücel *et al.*, 2006).

It is obvious that genetic gain from selection depends on the extent of genetic variation and on the magnitude of the heritable portion of this variation. Some authors (Rosielle and Hamblin, 1981; Simmonds, 1991; Singh, 2002) advise that when heritability values are as high as 80% or more, selection for such a trait should fairly be easy. This is because there would be a close correspondence between genotype and phenotype due to a relatively smaller contribution of environment to the phenotype. Considering heritability values between 40-80% as moderate, even if selection for insect-based traits would not be warranting, fortunately, infestation could fairly be reduced using seed-based traits. This study showed that targeting seed weight loss for direct selection and use of seed size and seed coat thickness for indirect selection as secondary traits would result in effective selection to increase resistance to Adzuki bean beetle in this gene pool. However, we necessarily need to compromise seed size and thinness of seed coat as attributes of seed quality for developing Adzuki bean beetle resistant chickpea.

The expected genetic gain from selection for insect related traits ranged from 0.01-6.00%, suggesting that, having such extremely low heritability and genetic gain values from selection, indirect selection for these traits to reduce infestation and seed damage would rather be a difficult proposition whatever the level of correlation of these characters with seed weight loss (Figure 2). Selection could be difficult or virtually impractical for a trait with low heritability, say less than 40%, due to the masking effect of the environment on the genotypic effects (Singh, 2002). As environmental and genotype by environment interaction effects are not heritable and, hence, the higher the environmental and the genotype by environment interaction effects, the lesser will be the level of success from selection. Therefore, it appeared that making genetic progresses from selection in this gene pool based on insect related traits would at least be very difficult. If at all expected, response to selection for insect related traits may only be possible with the use of larger number of germplasm accessions, greater selection intensity and more precise evaluation to capture the very rare trait of this type.

The explanation for the lower genetic variation for insect-based characters may be related to excessive environmental control of the traits than the genetic control. Other reports also showed that extremely lower heritability in some characters often causes a limited expected success from genetic manipulation of inter-specific parents for resistance to storage insects in legumes (Byrne *et al.*, 2008). There was also a stronger interrelationship between phenotypic and genotypic changes expected from selection in seed-based characters ($R^2 = 0.92$) than it was from insect-based characters ($R^2 = 0.35$) (Figure 3), which is the indication of better response to selection (Singh, 2002).

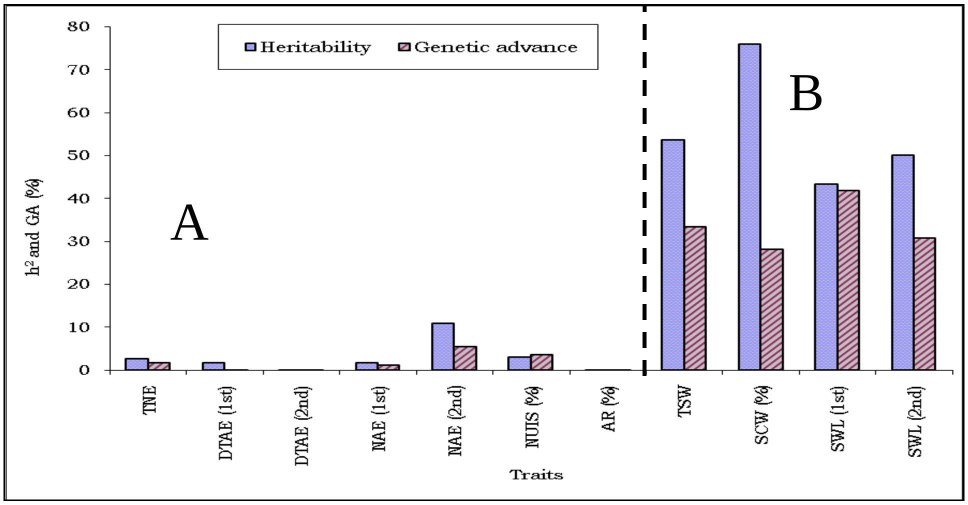

Figure 2. Broad-sense heritability (h^2) and expected genetic advance (GA) for insect-based (A) and seed-based (B) response characters to infestation by Adzuki bean beetle in 130 chickpea genotypes (Table 5 for abbreviations).

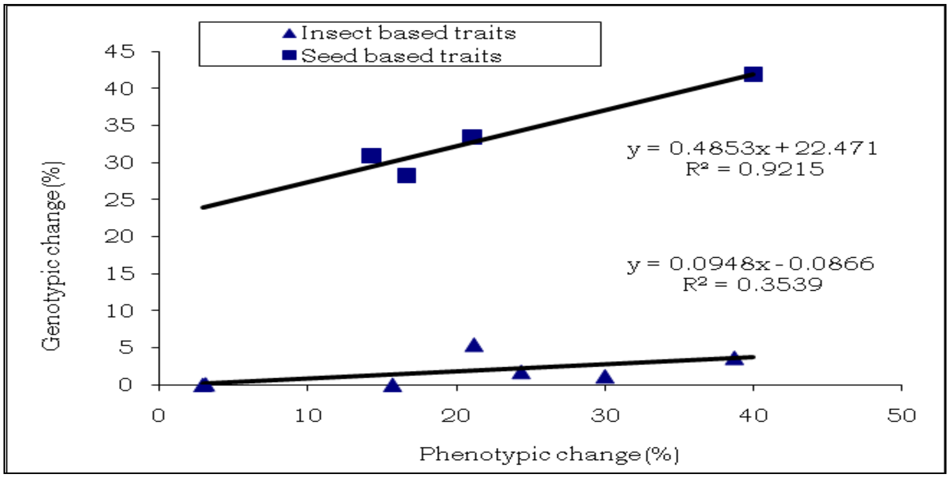

Figure 3. Relationship of phenotypic change from selection of the 5% best genotypes with genotypic change.

3.3. Selection Criteria

Interrelations between the characters revealed strong associations in a number of cases both in positive and negative directions (Table 5). Positively significant associations were observed between seed weight loss and three component characters, namely total number of eggs, number of adults emerged and seed size in both progenies. Conversely, number of uninfested seeds (%) and proportion of seed coat weight (%) had strong negative correlation with seed weight loss. Seed weight loss could, therefore, be reduced by selection for smaller seed size, least number of eggs and adult emergence or from selection for increased percent seed coat weight and number of uninfested seeds. The implication is that in order to reduce seed weight loss, smaller seed size should be combined with least numbers of eggs, adult emergence and proportion of uninfested seeds with thicker seed coat into a single genotype. Smaller seed size and thicker seed coat could be considered as good secondary traits for use as selection criteria for resistance to Adzuki bean beetle as they had also better heritability and expected genetic gain from selection (Figure 2), strongly correlated with seed weight loss and are easily measurable. Other reports have indicated that chickpea varieties with smooth, soft and thin seed coats may be preferred for oviposition than those with rough, hard, wrinkled and somewhat spiny seed coats (Ahmed *et al.*, 1989; Shaheen *et al.*, 2006). Desroches *et al.* (1995) also found that the seed coat in faba bean acted like a physical barrier against penetration by *C. chinensis* and *C. maculatus*.

The effectiveness of selection on seed-based traits as a strategy could be useful for reducing infestation. However, seed size in many legumes including chickpea is an economic trait which is recognized on the second priority next to grain yield as a prime objective of the breeding program in Ethiopia. Nevertheless, improvement in seed size and seed coat thinness could result in a negative selection for resistance to the beetle.

Previous studies also showed that the larger the seed size in chickpea, the thinner the seed coat may be (Singh, 1987), and the more susceptible it would be to storage insects (Yadav *et al*., 2006). As increased seed size and decreased seed coat thickness are desirable seed quality traits in chickpea (Singh, 1987), for improving seed size and seed coat thinness through selection, a compromise must be made for resistance to Adzuki bean beetle or the breeder must set minimum standards for one trait while selecting for the other. Or else, it is better to follow separate breeding programs for these seed quality attributes and resistance to Adzuki bean beetle.

Strong positive associations were also observed among total number of eggs with number of adults emerged and seed size, and number of uninfested seeds with the proportional weight of the seed coat. Adult recovery showed strong positive association only in one case with number of adults emerged. Days to adult emergence was weakly associated with a number of other traits. Strong negative associations were observed among total number of eggs with number of uninfested seeds and percent seed coat weight, number of adults emerged with number of uninfested seeds, number of uninfested seeds with seed size and seed size with percent seed coat weight (Table 5). Aslam *et al.* (2006) evaluated varieties of chickpea for response to the beetle and found significant positive correlation between number of eggs and adults, number of adults and percent weight loss, number of adults and number of holes, number of eggs and weight loss, number of eggs and number of holes, percent weight loss and number of holes. More or less similar patterns of association were reported in cowpea infested by *C. maculates* (Redden and McGuire, 1983). Existence of strong association indicates that selection for one trait can indirectly improve the other be it in the positive or negative direction due to either genetic linkage or existence of pleiotropic gene effects. Weak association indicates existence of an independent genetic control between the two traits and improvement in any one of the two would not cause significant change on the other (Singh, 2002).

4. Conclusions

The populations studied were found to contain useful genetic variability for seed related insect resistance traits which can be used for future breeding. We believe that exploring more genetic variability will be among promising alternatives to reduce seed weight loss either through direct selection or indirect selection of component traits. While doing this, the existence of extremely lower heritable variability in insect related traits could hamper genetic progresses to be expected from selection and, therefore, it is necessary to check more collections from both local and exotic sources.

Heritability and expected genetic gains from selection in this study clearly revealed that selection based on seed related traits would be more effective to reduce seed weight loss in Ethiopian chickpea gene pool. The search for more suitable secondary traits particularly biochemical characters associated with resistance to storage insects, genetically variable, highly heritable, and easily observable but with no compromise for seed quality should be continued with the mechanism involved.

The conventional approach has been playing decisive roles in breeding for storage insect pest resistant cultivars of many legume crops so far. However, where it is possible, the conventional approach should be supported by molecular tools for better efficiency and effectiveness.

Table 5. Correlation coefficients (r) between response characters to infestation by Adzuki bean beetle in chickpea genotypes.

Characters [a]	TNE	DTAE (1st)	DTAE (2nd)	NAE (1st)	NAE (2nd)	NUIS	AR	TSW	SCWT	SWL (1st)
DTAE (1st progeny)	-0.34**									
DTAE (2nd progeny)	-0.09NS	-0.11NS								
NAE (1st progeny)	0.83**	-0.28**	-0.05NS							
NAE (2nd progeny)	0.45**	-0.08NS	-0.17NS	0.34**						
NUIS (%)	-0.64**	0.30**	0.20*	-0.59**	-0.54**					
AR (%)	-0.06NS	0.03NS	-0.05NS	0.40**	0.08NS	-0.09NS				
TSW	0.60**	-0.18*	-0.12NS	0.51**	0.84**	-0.45**	0.12NS			
SCW (%)	-0.51**	0.31**	0.13NS	-0.38**	-0.80NS	0.40**	-0.05NS	-0.77**		
SWL (1st progeny)	0.58**	-0.13NS	-0.16NS	0.49**	0.44**	-0.58**	0.06NS	0.51**	-0.48**	
SWL (2nd progeny)	0.60**	-0.10NS	-0.19*	0.47**	0.78**	-0.55**	0.09NS	0.77**	-0.66**	0.80**

[a] *TNA = Total number of eggs; DTAE = Days to adult emergence; NAE = Number of adults emerged; NUIS = Number of uninfected seed, AR = Adult recovery, TSW = 1000 seed weight, SCW = Seed coat weight; SWL = Seed weight loss; ** = Highly significant ($P \leq 0.01$); * = Significant ($P \leq 0.05$); NS = Non-significant ($P > 0.05$).*

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