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Principal Component Analysis of Chickpea (*Cicer Arietinum* L.) Genotypes Grown Under Different Soil Fertility Levels for the Response of Adzuki Bean Beetle (*Callosobruchus Chinensis* L.) Infestation.

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Abstract: *For the choice of diverse parents in any hybridization programme, multivariate analysis like Principal component analysis has been extensively used. It involves a mathematical procedure that transforms a number of possibly correlated variables in to a smaller number of uncorrelated variables. Quantifying principal component analysis in chickpea varieties grown under different soil fertility levels would lead to improvement in chickpea breeding program. Hundred chickpea genotypes that grown under different soil fertility conditions for the response of Adzuki bean beetle infestesion were screened under laboratory condition at Holetta and Debre Zeit. The principal component analysis showed the first three principal components explained more than 76.7%, 82.1% and 81.0% of the total variation among genotypes managed with neither rhizobium nor phosphorus, only with rhizobium, and with rhizobium and phosphorus condition, respectively. Generally, characters with relatively greater positive weights of Eigen vectors in a given PC those, breeding efforts may need to simultaneously focus on genetic manipulation of these characters in order to reduce infestation and seed damage levels by adzuki bean beetle.*

Keywords: *Adzuki bean beetle, Chickpea, Phosphorus, Principal component analysis Rhizobium*

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a self-pollinating crop believed to be first domesticated in the Middle East. It is a diploid (2n = 2x = 16) crop which belongs to the family leguminoseae, subfamily papilionacea and genus cicer (Van der Maesen, 1987).

Chickpea is among the most important cool season food legumes grown worldwide (FAO, 2008; Gaur *et al.,* 2010). Among the pulse crops, chickpea has consistently maintained a much more significant status, ranking second in area and production after common bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) (Gaur *et al*., 2010).

Chickpea seeds are a major source of human food and animal feed because of their high content of lysine-rich protein (Jukanti et al., 2012). In addition, chickpea cultivation plays a significant role in farming systems as a substitute for fallow in cereal rotations, where it contributes to the sustainability of production and reduces the need for N fertilization through fixing atmospheric nitrogen. Those features make chickpea cultivation of particular importance to food security in the developing world.

Because of its susceptibility to several abiotic (drought, poor soil fertility, and poor cultural practices) and biotic (diseases, insect pests and weeds) factors, the production of chickpea in has remained constantly low. Among the major problems to increase chickpea production include the damage inflicted by storage insects. The most important pests of stored grain legume seeds are *C. chinensis* L., *Callosobruchus maculates* Fabricius, *Callosobruchus analis* Fabricius, *Acanthoscelides obtectus* Say, and *Bruchus incarnates* (Desroches, *et al*., 1995).

Reports indicate that from 25 to 40% of the grain crops are lost in stores annually due to infestations by insect pests in the sub-Saharan Africa (Mulungu *et al*., 2007; Kimatu, *et al*., 2012; Ahmad *et al*.,

2015). Even low initial infestation rates can cause tremendous damage because of the polycyclic nature, high fertility and short generation times of bruchid beetles (Southgate, 1979).

Among the bruchid beetles, adzuki bean beetle is one of the most devastating storage pest throughout the world causing substantial loss during storage (Gowda *et al*., 1982; Sing *et al*., 1994; Desroches *et al*., 1995; Gemechu *et al*., 2012). Reports indicate that adzuki bean beetle in chickpea may cause losses of up to 50% in Ethiopia and 28% in Eritrea (Kemal and Tibebu, 1994; Haile, 2006). It is widely agreed that food losses after harvest can be substantial and are important in terms of quantity, quality, and nutritional and economic values (Homan and Yubak , 2011).

Adzuki bean beetle render quality loss, which is more frequently based on subjective judgment and locally accepted quality standards. It may include the presence of contaminants, such as uric acid and other nitrogenous wastes, the presence of adult beetle inside the seed, exit holes, glued eggs to the seeds, coastal larval skin, species of insect chitin and changes in appearance, and texture and taste, making it unfit for human consumption. Commercial grain buyers usually reject or refuse to accept delivery of insect contaminated grain or may pay very low price for it (Hill, 1990; Espinal, 1993; Nchimbi-Mosolla and Miswangu, 2001).

For the choice of diverse parents in any hybridization programme, multivariate analysis like Principal component analysis has been extensively used. It involves a mathematical procedure that transforms a number of possibly correlated variables in to a smaller number of uncorrelated variables. estimating principal component analysis in chickpea varieties under different soil fertility levels would lead to improvement in chickpea breeding program. Hence, this research was conducted with the objective of quantifying principal component analysis in chickpea varieties grown under different soil fertility levels for the response of Adzuki bean beetle infestation.

2. Materials And Methods

Description of Experimental Sites

Both the experiments were conducted at Holetta and Debre Zeit Agricultural Research Centers, Ethiopia. Debre Zeit Agricultural Research Center (DZARC) is located in East Shewa Zone of Oromia Regional State in Central Ethiopia, at 08°44'N, 38°58'E and an altitude of 1900 m.a.s.l.. It is characterized by long-term mean annual rainfall of 851 mm and mean maximum and minimum temperatures of 28.3° C and 8.9° C, respectively. Holetta Agricultural Research Center (HARC) is located in West Shewa Zone of Oromia Regional State in Central Ethiopia, at 09°04'12"'N, 38o29'45"E and an altitude of 2400 m.a.s.l. It is characterized by long term mean annual rainfall of 1064 mm and mean maximum and minimum temperatures of 22.5°C and 6.4°C, respectively.

3. Genetic Materials

A total of 100 genotypes were used in the study: 54 chickpea germplasm accessions collected from the major chickpea production areas all over the country (Arsi, East Gojam, West Gojam, North Gonder, South Gonder, West Harerge, East Shewa, North Shewa, West Shewa, Tigray, and Wello), 29 pipe line materials and 17 improved varieties. The pipe line materials and released varieties were originally from the Ethiopian Institute of Agricultural Research (EIAR), the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The test genotypes are described in Table 1.

Table 1. *Description of the test chickpea genotypes included in the experiment*

Origin/region	Zone	No. of genotypes/accessions	Genotypes/accessions
Amahara	E. Gojam	5	41026, 41074, 41021, 41027, 41029
	W. Gojam	5	207745, 41277, 207743, 41015, 41273
	N. Gonder	6	41280, 41308, 41312, 41304, 41303, 41311
	S. Gonder	5	41289, 41290, 41291, 41048, 41053
	N. Shewa	2	41207, 41215
	S. Wello	5	41114, 212589, 207660, 207646, 225874

Oromia	Arsi	5	231327, 231328, 209094, 209098, 41002
	W. Harargie	4	209090, 209091, 209087, 209088
	E. Shewa	5	207661, 207667, 41134, 41168, 41130
	N. Shewa	2	41066, 41008
	W. Shewa	5	209035, 41176, 41174, 41170, 41185
Tigray	Tigray	5	207151, 207563, 207564, 219800, 219803
*ICARDA, *ICRISAT Indian	_____	46	DZ-2012-CK-0029, DZ-2012-CK-0030, DZ-2012-CK-0034,
			DZ-2012-CK-0035, DZ-2012-CK-0037, Akaki, Worku, DZ-
			2012-CK-0001, DZ-2012-CK-0002, DZ-2012-CK-0003, DZ-
			2012-CK-0005, DZ-2012-CK-0007, DZ-2012-CK-0010, DZ-
			2012-CK-0012, DZ-2012-CK-0013, DZ-2012-CK-0032, DZ-
			2012-CK-0033, DZ-2012-CK-0039, DZ-2012-CK-004, DZ-
			2012-CK-0061, DZ-2012-CK-0062, DZ-2012-CK-0065, DZ-
			2012-CK-009, DZ-2012-CK-0170, DZ-2012-CK-0220, DZ-
			2012-CK-0246, DZ2012-CK-0248, DZ-2012-CK-0251, Cheffe,
			Ejere, Fetenech, Habru, Kasech, Teji, DZ-2012-CK-0031, DZ-
			Ejere, Fetenech, Habru, Kasech, Teji, DZ-2012-CK-0031, DZ-
			2012-CK-0038, DZ-2012-CK-006, Akuri, Dalota, Kobo,
			Kutaye, Mastewal, Minjar, Natoli, Shasho, Teketaye

*ICARDA, International Center for Agricultural Reserch in the Dry Areas

*ICRISAT, International Crops Research Institutes for the Semi-Ardi Tropics

Experimental Field Layout and Management of Treatments

All genotypes were grown in 2016 main cropping season in a randomized complete block design with 3 replications at Holetta and Debre Zeit under three different soil fertility levels, i.e., neither rhizobium nor with phosphorus, only with rhizobium and with phosphorus and rhizobium. Phosphorus was applied at the rate of 20 g per plot (1.2 m2) in the form of triple supper phosphate (TSP) as recommended (Eshete, 1994). An effective isolate of Rhizobium, CP EAL 004, was inoculated at the rate of approximately 1 g of inoculum for 40 seeds using 40% gum arabic as an adhesive (Somasegaran and Hoben, 1985).

Freshly harvested seeds of each genotype were cleaned manually from foreign materials, adjusted to 9.0-10 % moisture contents by sun drying and disinfected in a deep freeze at about -20 oC for a month prior to the study to eliminate any pre-storage infestation (eggs, larvae and adult bruchids).

Mass-rearing of the Insects

Adult beetles were mass-reared using a susceptible chickpea variety Shasho as suggested by Gemechu et al. (2012). The beetles were introduced in to 10` kg of seeds from the susceptible variety and kept at ambient temperature and relative humidity for seven days to allow oviposition. Mass-rearing was made at Holetta and Debre Zeit Agricultural Research Centers, Entomology Laboratories. To standardize the age of the progeny, the parent insects were sieved out after seven days. After parent removal, the progenies that emerged were used for re-culturing, and subsequently, 1-2 day old adult insects that emerged were used for the purpose of infestation.

Laboratory Experimental Design and Infestation

The experiment was conducted under ambient room temperature and relative humidity in a randomized complete block Design (RCBD) with 3 replications. Two hundred seeds of each genotype were allocated per experimental unit (a plastic jar of 250 ml; 6 cm x 7 cm). The chickpea genotypes were assigned to jars at random within each block. Fourteen 1-2 days old unsexed adults of Adzuki bean beetles were collected from the maintained culture and randomly selected and released 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 7 days after introduction and then were removed from the jars. The plastic jars containing seeds were inspected on daily basis for the emergence of first progeny. When emergence of the first progeny was completed, the first progeny was removed from the jars for evaluation of the level of attack and loss incurred by the first progeny.

4. Data Collection

Total number of eggs: Total number of eggs laid on the surface of seeds of each genotype was counted on daily basis starting from the 4th day to the 14th day of infestation.

Days to adult emergence: The number of days required to adult emergence was recorded on daily basis starting from the 25th day of infestation until the first adult emerged from seeds.

Number of adults emerged: Total number of emerged adults from each genotype were counted on a daily basis starting from the 25th day of infestation to the emergence of the last adult of the first progeny from seeds.

Susceptibility index (SI): Susceptibility index was calculated after Howe (1971) as modified by Dobie (1977) using the formula:

$$SI = \frac{\text{Log } Y}{T} X\ 100$$

Where SI = susceptibility index, Log Y= log number of first emerged adults, T = mean developmental periods (days) estimated as the time from the middle of oviposition period to 50% emergence of the first progeny. The values of the susceptibility indices were used to rank genotype susceptibility to the bruchids into five categories according to Mensah (1986) as follows:

i. Genotypes with values ranging from 0.0-2.5 were considered resistant genotypes (R).

ii. Genotypes with values ranging from 2.6-5.0 were considered moderately resistant (MR).

iii. Genotypes with value ranging from 5.1-7.5 were considered moderately susceptible (MS).

iv. Genotypes with values ranging from 7.6-10.0 were considered susceptible (S).

v. Genotypes with values greater than 10.0 were considered highly susceptible (HS).

The percentage of seed damage: The percent damage of each genotype was calculated by separating healthy grains (without holes) from the sieved samples and used for percent damage calculations using the formula described by Khattak et al. (1987) as:

$$\text{Percentage of seed damage} = \frac{Nds}{Tns} \times 100$$

Where Nds = number of damaged seeds, Tns = total number of seeds.

Adult recovery (%): The actual number of adults that emerged compared with the actual number of eggs laid on the surface of seeds. i.e., the ratio of number of adults emerged to number of eggs multiply by one hundred.

Thousand seed weight (g): Cleaned grains sample was taken from each genotype and 1000-grains were weighed in grams after adjusting the moisture content to the standard level (10%).

Proportion of seed coat by weight (%): Seed coat weight as percent of total seed weight of the same genotypes grown under the same conditions was taken from the replicated field trial. i.e., the ratio of seed coat weight to total weight of the seed multiplied by one hundred.

Seed weight loss (g): The seeds were separated into damaged and undamaged categories and weight loss was adjusted to 10% moisture content. The damaged and undamaged seeds were counted and weighed. Percent weight loss was calculated using the formula given by Adams (1976) as follows:

$$Percent\ loss\ in\ weight = \frac{UNd - DNu}{U\ (Nd + Nu)}\ X\ 100$$

Where U = weight of undamaged grain; D = weight of damaged grain; Nd = number of damaged grain; Nu = number of undamaged grain.

Data Analysis

Count data including total number of eggs, number of adults emerged and mean number of holes per seed were log-transformed. Likewise, percentage data, adult recovery, proportion of seed coat weight by weight, percentage of damage seeds, percent seed weight loss and index of susceptibility were angular-transformed (arcsine proportion) in order to stabilize the variance (Gomez and Gomez,1984). Data on thousand seed weight and days to adult emergence were untransformed because variance heterogeneity was not observed. The quantitative data from each of the locations were subjected to analysis of variance (ANOVA) using SAS version 9.3 statistical software package (SAS Institute, 2010).

The principal components (PC) were analyzed to identify the traits contributing large part of the total variation among the genotypes. The characters with larger absolute PC values closer to unity within each principal component influence the clustering more than those with lower absolute values closer to zero (Chahal and Gosal, 2002). The principal components with Eigen value greater than one was used as criteria to determine the number of principal components (Kaiser, 1960). General formula to compute scores on the first component extracted in principal component analysis:

$$C1 = bi1(X1) + bi2 + \cdots bip(XP) \quad (1)$$

Where, C1 = the subject's score on principal component 1 (the first component extracted) b1p = the regression coefficient (or weight) for observed variable p, as used in creating principal component 1 Xp = the subject's score on observed variable.

5. Results and Discussion

Principal Component Analysis

The principal component analysis showed that the first three principal components with Eigen values greater than unity altogether explain more than 76.7%, 82.1% and 81.0% of the total variation among 100 genotypes evaluated for ten traits to infestation by adzuki bean beetle managed with neither rhizobium nor phosphorus, with rhizobium and with rhizobium and phosphorus conditions respectively (Table 2).

This indicate that when genotypes grown under neither rhizobium nor phosphorus condition, the first principal component accounted for 43.32 % of the total variation with high positive and negative weight for TNE (0.391), DTAE (-0.213), NAE (0.447), MNHPS (0.447), PDSBN (0.358), PSWL, (0.263) and SI (0.421), but least positive and negative weight for AR (0.108), TSW (0.073), PSCBW (-0.100). Whereas the second principal component (PC2) accounted for 22.27% of the total variation with high positive and negative weight for AR (0.375), TSW (0.623) and PSCBW (-0.914), but least positive and negative weight for TNE (-0.237), DTAE (0.055), NAE (-0.070), MNHPS (-0.075), PDSBN (0.129), PSWL, (-0.090) and SI (-0.002). Similarly the third principal component (PC3) accounted for 11.13% of the total variation with high positive and negative weight for most characters but least negative weight for TNE (-0.081) and TSW (-0.134). A positive and high Eigen vector for a given trait indicate that positive correlation between that trait and the given PC while high and negative eigenvector indicates negative correlation between the trait and a given PC (Mussa, 2017).

When genotypes grown with rhizobium condition, the first principal component accounted for 51.2% of the total variation with high positive and negative weight for TNE (0.383), NAE (0.425), AR (0.234), MNHPS (0.425), PDSBN (0.359), PSWL (0.288) and SI (0.416), but least positive and negative weight for DTAE (-0.119), TSW (0.138) and PSCBW (-0.135). Likewise, the second component (PC2) accounted for 20.82% of the total variation with high positive and negative weight for DTAE (0.376), TSW (0.627) and PSCBW (-0.631), but least positive and negative weight for TNE (-0.128), NAE (-0.057), AR (0.039), MNHPS (-0.041), PDSBN (0.120), PSWL (-0.084) and SI (-0.154). Furthermore, the third principal component (PC3) accounted for 10.11% of the total variation with least positive and negative weight for most characters but high positive and negative weight for AR (0.776) and PSWL (-0.446).

When the genotypes grown with rhizobium and phosphorus condition, the first principal component accounted for 50.16% the total variation with high positive and negative weight for TNE (0.369), NAE (0.422), AR (0.234), MNHPS (0.422), TSW (0.207), PSCBW (-0.202) PDSBN (0.376), PSWL (0.272) and SI (0.408), but least positive and negative weight for DTAE (-0.097) and AR (0.180). Likewise, the second component (PC2) accounted for 19.61% of the total variation with least positive and weight for most characters, but high negative and positive weight for DTAE (-0.409), TSW (-0.581) and PSCBW (0.588). Similarly the third principal component (PC3) accounted for 11.23% the total variation that with least positive and negative weight for most characters, but high negative and positive weight for TNE (-0.416), AR (0.764), TSW (0.234) and PSWL (-0.359).

In general, the variables with Eigen vector of large absolute magnitude (close to unity) reflects a strong influence while those of small magnitude (near zero) reflect little influence for a particular variable provided that the first principal component accounts for a substantial portion of the variation (Chahal and Gosal, 2002). Similarly Gemechu et al. (2012) noted that characters individually contributed small effects to the variation in a given PC and, hence, the differentiation of the accessions into different clusters was rather dictated by the cumulative effects of a number of characters, however characters with relatively greater positive weights of Eigen vectors in a given PC those, breeding efforts may need to simultaneously focus on genetic manipulation of these characters in order to reduce infestation and seed damage levels by adzuki bean beetle.

Table2. *Principal component analysis of ten traits of 100 genotypes tested with neither rhizobium nor phosphorus, with rhizobium, with rhizobium and phosphorus to infestation by adzuki bean beetle.*

Principal components			
	PC1	PC2	PC3
Neither rhizobium nor phosphorus			
Characters			
Total n number of adults emerged	0.391	-0.237	-0.081
Days to adult emergence	-0.213	0.055	0.370
Number of adults emerged	0.447	-0.070	0.239
Adult recovery	0.108	0.375	0.589
Mean number of holes per seed	0.447	-0.075	0.232
Thousand seed weight	0.073	0.623	-0.134
Proportion of seed coat weight	-0.100	-0.614	0.209
Percentage of damage seeds	0.358	0.129	-0.372
Percentage of seed weight loss	0.263	-0.090	-0.378
Susceptibility index	0.421	-0.002	0.235
Eigen values	4.33	2.23	1.11
Proportion (%)	43.32	22.27	11.13
Cumulative (%)	43.32	65.59	76.71
With rhizobium			
Characters			
Total n number of adults emerged	0.383	-0.128	-0.278
Days to adult emergence	-0.119	0.376	0.171
Number of adults emerged	0.425	-0.057	0.124
Adult recovery	0.234	0.039	0.776
Mean number of holes per seed	0.425	-0.041	0.108
Thousand seed weight	0.138	0.627	-0.075

Proportion of seed coat weight	-0.135	-0.631	0.106
Percentage of damage seeds	0.359	0.120	-0.168
Percentage of seed weight loss	0.288	-0.084	-0.446
Susceptibility index	0.416	-0.154	0.144
Eigen values	5.12	2.08	1.01
Proportion (%)	51.2	20.82	10.11
Cumulative (%)	51.2	72.02	82.14
With rhizobium and phosphorus			
Characters			
Total n number of adults emerged	0.369	0.003	-0.416
Days to adult emergence	-0.097	-0.409	-0.032
Number of adults emerged	0.422	0.123	0.030
Adult recovery	0.180	0.223	0.764
Mean number of holes per seed	0.422	0.119	0.030
Thousand seed weight	0.207	-0.581	0.234
Proportion of seed coat weight	-0.202	0.588	-0.198
Percentage of damage seeds	0.376	-0.030	-0.102
Percentage of seed weight loss	0.272	-0.110	-0.359
Susceptibility index	0.408	0.238	0.084
Eigen values	5.02	1.96	1.12
Proportion (%)	50.16	19.61	11.23
Cumulative (%)	50.16	69.77	81.00

6. SUMMARY AND CONCLUSIONS

The variables with eigenvector of large absolute magnitude (close to unity) reflects a strong influence while those of small magnitude (near zero) reflect little influence for a particular variable in a given PC, breeding efforts may need to simultaneously focus on genetic manipulation of these characters in order to reduce infestation and seed damage levels by adzuki bean beetle.

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