Genet Resour Crop Evol (2015) 62:525–538
DOI 10.1007/s10722-014-0178-2

RESEARCH ARTICLE

Screening the primary gene pool of field pea (*Pisum sativum* L. subsp. *sativum*) in Ethiopia for resistance against pea weevil (*Bruchus pisorum* L.)

Abel Teshome · Esayas Mendesil · Mulatu Geleta · Derege Andargie · Peter Anderson · Birgitta Rämert · Emiru Seyoum · Ylva Hillbur · Kifle Dagne · Tomas Bryngelsson

Received: 14 April 2014 / Accepted: 8 September 2014 / Published online: 27 September 2014

Abstract Field pea (*Pisum sativum* L. subsp. *sativum*) is an important agricultural crop worldwide, as a main source of protein in human diet and as animal fodder. In Ethiopia, it is the second most important legume crop next to faba bean (*Vicia faba* L.). However, the production is threatened by pea weevil (*Bruchus pisorum* L.), which is a rapidly spreading insect pest throughout the country. During June–October 2011, a total of 602 pea accessions from Ethiopia were screened for pea weevil resistance at three field sites in Ethiopia. From this trial, accessions with relatively low mean percent seed damage (PSD) were selected and evaluated during June-October 2012 in replicated trials. Some genotypes from the selected accessions were also studied under greenhouse conditions for up to three generations. Both in the field and greenhouse trials, a significant level of variation in PSD were observed among accessions/genotypes. However, a few of them showed relatively consistent results across sites and years. The gene bank accessions *32454* and *235002* had consistently <40 % PSD. These accessions had 17 and 33 % PSD, respectively, at a site where the highest and overall mean PSD were 92 and 75 %, respectively. Also, promising genotypes with consistently low levels of seed damage were identified in accessions *226037* and *32410*. The incorporation of such promising accessions/genotypes into pea breeding programs may lead to the development of field pea varieties with enhanced resistance against pea weevil and consequently contribute to sustainable field pea production in Ethiopia and beyond.

Keywords *Bruchus pisorum* · Field pea · Host-plant resistance · Pea weevil · *Pisum sativum*

Abel Teshome and Esayas Mendesil have contributed equally to this work.

A. Teshome (✉) · M. Geleta · T. Bryngelsson
Department of Plant Breeding, Swedish University of Agricultural Sciences, Box 101, 23053 Alnarp, Sweden
e-mail: Abel.Teshome@slu.se

E. Mendesil · P. Anderson · B. Rämert · Y. Hillbur
Department of Plant Protection Biology, Swedish University of Agricultural Sciences, Box 102, 23053 Alnarp, Sweden

D. Andargie
Adet Agricultural Research Centre, P.O. Box 8, Bahir Dar, Ethiopia

E. Seyoum
Department of Zoological Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

Y. Hillbur
International Institute of Tropical Agriculture, Oyo Road, Ibadan, Nigeria

K. Dagne
Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

Introduction

Field pea (*Pisum sativum* L. subsp. *sativum*) is a cool-season legume crop which is an important source of protein for humans in the developing world and a major fodder crop in developed countries. It also plays a key role in soil fertility due to its nitrogen fixing ability (Stenvovic et al. 2005). The main field pea producing countries include Canada, Russia, China, India and France. Ethiopia ranks first in Africa and number six in the world in field pea production (FAOSTAT 2012). Field pea is the second major pulse cultivated in Ethiopia next to faba bean (*Vicia faba* L.). It serves as staple food for millions of people in the country (CSA 2011) and has through export become a source of foreign currency. The production of field pea is, however, hampered by various biotic stresses like diseases and pests (Fikere et al. 2010). In recent years, pest pressure has been the main reason for the sharp decline in field pea production in Ethiopia (Tesfaye et al. 2002). Among the field pea pests, pea weevil (*Bruchus pisorum* L.) is the main threat that can cause up to 60 % reduction in yield annually (Assayehegne 2002; Teka 2002).

Pea weevil is a major pest in many pea growing countries (Clement et al. 2009; Aryamanesh et al. 2012). Usually, pea weevils invade pea fields at flowering stage and feed on pea pollen and flowers before they start mating. A few weeks after mating, the adult female oviposits on young green pods. The larvae hatch on the surface of the pods, burrow through the pod wall and seed coat and embed themselves inside the seeds. Multiple larval infestations per seed are possible but only one will survive and complete its life cycle (Smith et al. 1982). The larvae have four instars and feed on the cotyledons of field pea. The infested pods are prone to shattering during harvesting which contributes to the loss in yield (McDonald 1995). Two to three months after harvest, with more than 50 % of the cotyledons consumed, infested seeds become unfit for human consumption and have low germination rates (McDonald 1995; Clement et al. 2002).

Pea weevil was inadvertently introduced to Ethiopia around the mid-1970s, probably with infested seeds imported for research purpose and/or as food aid (Abate 2006). Since then, it has spread throughout the country through seed exchange and trading (Teka 2002). At present, farmers in the northern and central parts of the country are giving up field pea production due to this pest. Unfortunately, the decline in field pea production has caused a sharp rise in field pea prices in the local market, which makes it unaffordable for resource-poor farmers. Therefore, a quick intervention is needed to circumvent the spread of the pest and reduce the economic loss both at the national and international level.

At present, the most efficient pea weevil control method is the use of chemical pesticides which is environmentally unfriendly and costly, especially for subsistence farmers in developing countries (Clement et al. 1996; Byrne 2005; Aryamanesh et al. 2012). In addition, transgenic field pea lines with α-amylase inhibitor gene from *Phaseolus vulgaris* L. have been developed to tackle this pest (Schroeder et al. 1995). The inhibitor prevents pea weevil larvae from digesting seed starch, which retards their development and leads to death. Despite the fact that these lines have less seed damage as compared to those without the a-amylase inhibitor gene, the seeds still have the sting mark from the larval entry which makes them less marketable. These transgenic lines are yet to be adopted in breeding programs though. Furthermore, limited public awareness and acceptance of transgenic cultivars would further delay the availability of these pea weevil resistant transgenic lines to farmers and consumers.

Cultural practices, such as early sowing and early harvest to bypass the weevil's life cycle and after harvest grazing by cattle to minimize re-infestation the following year, could decrease the infestation rate (Baker 1998). However, such practices need to be integrated with other pest management methods such as host-plant resistance. Since the 1970s, efforts have been made to identify resistance against the pea weevil in the primary and secondary gene pools of field pea (Pesho et al. 1977; Hardie et al. 1995; Clement et al. 2002; Byrne 2005), but with limited success. The most successful resistance against pea weevil recorded to date was found in *Pisum fulvum* Sibth. et Sm. lines (Clement et al. 2002; Byrne 2005). *P. fulvum* is a wild *Pisum* species with limited hybridization success with *P. sativum* and a probable linkage drag disadvantage (Hardie et al. 1995). Previous field pea screening efforts for resistance against pea weevil have only considered few accessions from Ethiopia, regardless of the high genetic diversity in field pea grown in the

country (Keneni et al. 2003). Hence, the objectives of the present study was to screen gene bank accessions, newly collected populations and released varieties of field pea in Ethiopia for resistance against the pea weevil and channel resistant germplasm into the national breeding program.

Materials and methods

Plant material

A total of 542 gene bank accessions, 11 released varieties and 49 newly collected populations of field pea were included in this study, all of which were *P. sativum* subsp. *sativum* except two accessions of *Pisum abyssinicum* A. Braun. The two *P. abyssinicum* accessions were included for comparison purpose. The botanical nomenclature of the *Pisum* species mentioned in this paper is according to Lehmann (1954). Gene bank accessions were obtained from the Ethiopian Institute of Biodiversity (EIB), Addis Ababa, Ethiopia, while the released varieties were kindly provided by Holeta Agricultural Research Centre (HARC), Holeta, Ethiopia, and the rest were collected directly from farmers' fields and local markets in areas where the pea weevil is prevalent. For sake of simplicity, the gene bank accessions, populations and varieties used in the field trials will be referred to as "accessions". However, individual plants selected based on the results of field trials and used in greenhouse experiments will be referred to as "genotypes". Names for gene bank accessions are five or six digit numbers (e.g. *32397*, *226037*) whereas that of released varieties are letters (e.g. *Adet*). Newly collected populations were represented by "*NC-nn*" where *nn* is two digit numbers (e.g. *NC-03*). Codes for genotypes combine source accession names and additional identifiers (e.g. *226037-3-VOK-par*).

Field trials

Two consecutive field trials were conducted under rainfed conditions in areas where pea weevil is a major problem. Planting, weeding and harvesting were done manually. During planting, diammonium phosphate (DAP) fertilizer was applied at 100 kg/ha rate but no pesticides were applied. Peas were infested naturally in both trials. Harvesting and threshing were done at pod maturity and seeds were kept in labeled paper bags at room temperature.

First field trial (FT1)

The FT1 was conducted from June to October 2011 in three districts in north and north-western Ethiopia: Ebinat (12°10′N 38°05′E), Liben (11°50′N 37°10′E) and Sekota (13°00′N 38°50′E) (Fig. 1). Specific sites within the districts were selected based on records of high and consistent pea weevil presence during previous planting seasons. The 602 accessions were planted without replication at each site, which allowed screening of a large number of accessions. The plots in each site were arranged into 20 columns with 30 plots each except the last two that had 31 plots. The accessions were randomly assigned to the 602 plots, and randomization was done independently for each site. At each site, all accessions were represented by twenty seeds and planted on a 1×0.2 m (0.2 m^2) plot in two rows. The distance between plants in a row was 10 cm and between rows 20 cm, whereas the distance between plots was 1 m.

Second field trial (FT2)

The FT2 was conducted from June to October 2012 at two sites in Liben district. The first site (site-I) was the same site that was used in FT1 at Liben and the new site (site-II) was situated 15 km north of site-I. The crop fields around each site were similar in terms of types of crops grown.

The accessions used in FT2 were selected based on the results of FT1. At site-I, 100 accessions were planted, of which 77 had <40 % seed damage during FT1. The remaining 23 accessions had more than 60 % seed damage. At site II, only the 77 accessions with <40 % seed damage and one accession known to be highly susceptible to pea weevil were planted. A simple lattice design with two replications was used at each site. Each block was five meters apart from each other. Plot size, distance between plots, and distance between rows and plants within a plot were similar to that of FT1. At pod maturity, five to ten plants were randomly selected from each plot and seeds from each individual plant were separately harvested and kept in labeled paper bags at room temperature.

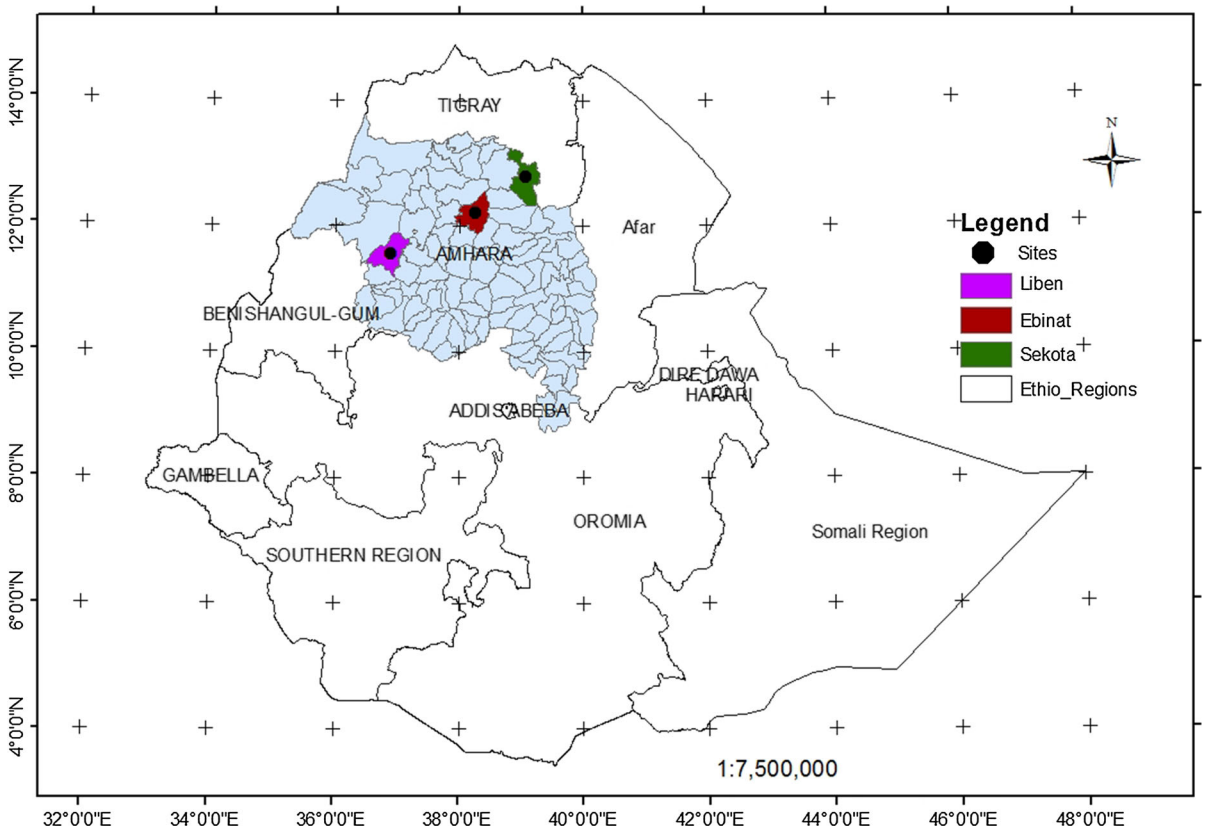

Fig. 1 Geographical positions of the three experimental sites used during first field trail in Ethiopia

Greenhouse experiments

During FT1 and FT2, some least infested and highly infested genotypes were tagged and their seeds were collected separately for greenhouse experiments with the aim of investigating the heritability of pea weevil resistance and developing genotypes resistant to pea weevil. Three separate greenhouse experiments were carried out at the Swedish University of Agricultural Science (SLU), Alnarp, between February 2012 and June 2013. Genotypes selected during FT1 were from accessions *226037*, *32018*, *32397*, *32063* and *230846*, whereas those selected during FT2 were from accessions *32410*, *32487*, *236413* and *Adet*. The first group of genotypes was studied for three generations while genotypes from FT2 were studied only during the third greenhouse experiment.

All plants were grown in the greenhouse in 2 l plastic pots at 22 °C and a minimum of 12 h light. Before flowering, plants were transferred into insect rearing cages (60 cm × 60 cm × 120 cm) made of polyester netting and plastic (MegaView Science Co Ltd, Taiwan). A total of 17 cages were used and five to six plants were placed in each cage. In all greenhouse experiments, progenies of least infested and highly infested genotypes, selected based on the results of the field trials or preceding greenhouse experiments, were randomly distributed across the cages so that each cage contains both groups of genotypes. When the plants started flowering, 25 pairs of naive male and female weevils were released into each cage. These weevils were newly emerged from infested seeds collected from our field trials and farmers' fields in Ethiopia. The sex of the weevils was determined based on the small spine present on the tibia of the middle leg of male insects but absent in female insects (Bousquet 1990). Pods were harvested at maturity from each pot and kept in labeled paper bags and stored at room temperature (20–24 °C).

Data scoring and analysis

Damage assessment of seeds in both field and greenhouse trials was conducted 3 months after

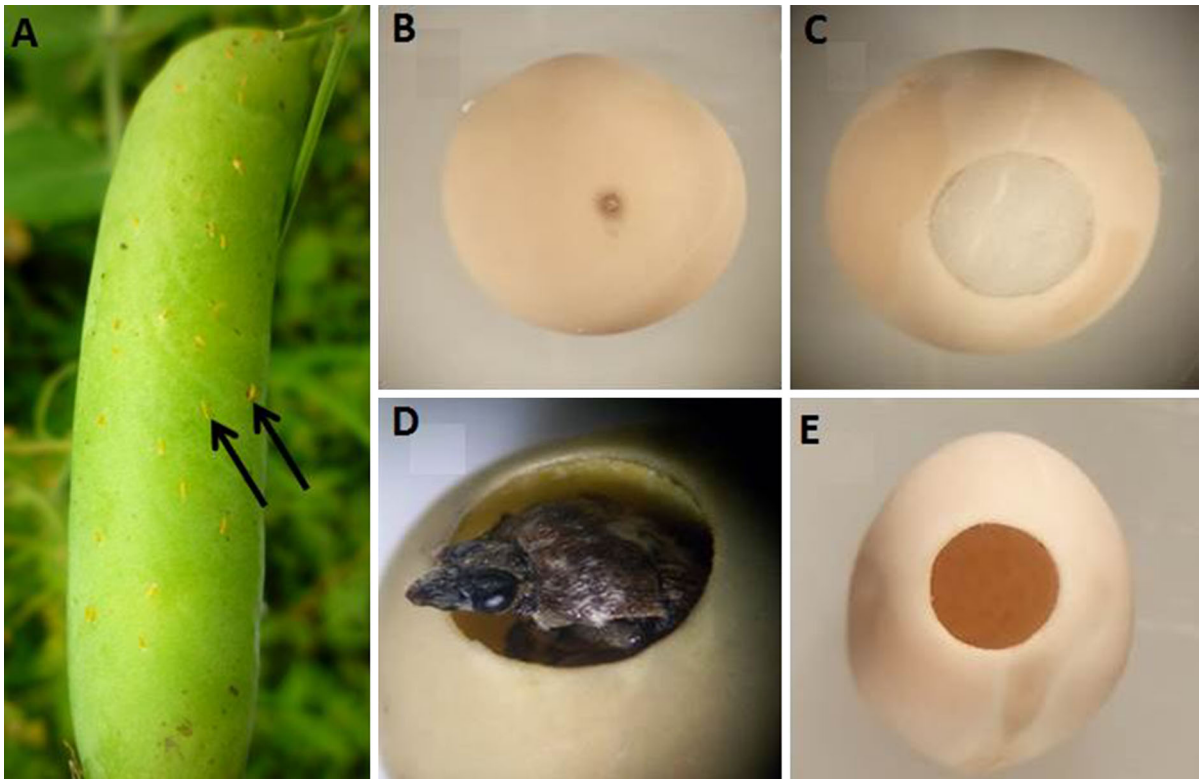

Fig. 2 Pea weevil eggs on pea pod (**A**) and seed damage symptoms at different stages of pea weevil development **B** sting, **C** window, **D** adult pea weevil emerging, **E** adult pea weevil exit hole

harvest to allow easy identification of infested seeds. Percent seed damage (PSD) was calculated as the number of infested seeds; i.e. number of seeds with sting, window or hollow marks (Fig. 2) caused by the weevil divided by the total number of seeds analyzed. In FT1, the damage in each accession at each site was determined based on pooled seeds of all plants in the accession. Seed color (green, variegated green, brown, variegated brown, cream and violet) and seed shape (round and wrinkled) were also recorded for each accession. Based on the availability of seeds, and the diversity of seed color and shape, 30–180 seeds were used for damage assessment in each accession. In FT2, data was collected separately at least from five individual plants for each accession per replicate and site. Then, mean PSD was calculated for each accession. In the greenhouse experiments, PSD was calculated for each individual plant in each cage based on a minimum of 30 seeds. Furthermore, mean PSD in each cage was calculated as the average of individual plant's PSD. The percentage of plants with infested seeds in each cage was also calculated as the ratio of the number of plants with infested seeds divided by the total number of plants in the cage.

All statistical analyses were carried out using R version 2.15.3 (R Core Team 2013). Before the actual analysis, the PSD scores were first arcsine transformed to make the distribution normal and variances homogenous. For FT1 data, analysis of variance (ANOVA) was carried out by considering data from the three sites as replicates. In the case of FT2, a mixed-effects model with Bonferroni adjustment method was used to compare the mean performance of the accessions with each other and with the susceptible accession *Adet*. In this model, accessions were considered fixed effect while sites and blocks were considered as random effects and a 5 % significance level was used. The Q–Q plot of the residuals was used to check if the mixed model could explain the variation in a linear manner, which was found to be the case with only a few outliers. Re-scoring of the outliers confirmed the first results and hence they were kept for further analysis. In both FT1 and FT2 accessions with incomplete or missing data were excluded from the analyses.

Results

First field trial (FT1)

Out of the 602 accessions planted during FT1, only 487 accessions had complete data from the three sites. ANOVA on the 487 accessions revealed no significant differences in terms of mean PSD between the accessions ($P = 0.99$; Table 1). However, when accessions with a mean PSD between 30 and 60 % were excluded from the analysis, a significant variation was observed between the remaining accessions ($P < 0.001$). Furthermore, the mean PSD for the three sites differed significantly with Ebinat having the highest (52 %) and Sekota the lowest (30 %; Table 2). The Pearson correlation coefficients between PSD values of Ebinat and Liben, Liben and Sekota, and Ebinat and Sekota were 0.11 ($P = 0.02$), 0.11 ($P = 0.02$) and 0.07 ($P = 12$), respectively. In all the three sites, the mean PSD of gene bank accessions and newly collected populations was lower than that of released varieties (Table 2). The mean PSD of the accessions across the three sites ranged from 12 % (*32819*) to 98 % (*Wolmera*; Fig. 3). Overall, there were nine accessions with <20 % and four accessions with more than 80 % PSD (Fig. 3). Interestingly, all accessions with more than 80 % mean PSD were accessions with cream seed coat color. These accessions were *Wolmera* (98 %), *227143* (97 %), *Milky* (93 %) and *227141* (83 %; Fig. 3). The two *P. abyssinicum* accessions had more than 50 % PSD and hence were not considered in the subsequent field trial.

Table 1 Analysis of variance (ANOVA) calculated by considering the three sites as replicates for PSD of 487 accessions that have complete data from the three sites in FT1; and for PSD of 65 accessions that have <30 % or >60 % seed damage at the three sites

	df	Sum Sq	Mean	F value	*P* value
Accessions[a]	486	129199	265.84	0.78	0.99
Residuals	974	333426	342.33		
Accessions[b]	64	64844	1013	2.26	0.000
Residuals	130	58324	449		

[a] ANOVA for the 487 accessions; [b] ANOVA for the 65 accessions

Second field trial (FT2)

Out of the 100 accessions included in FT2, only 53 accessions with complete data from both sites were considered for data analysis, as the remaining accessions did not have enough number of individuals for analysis due to poor germination rates. The mean PSD of block-I and block-II of site-I and block-III and

Table 2 Mean percent seed damage (PSD) at Ebinat, Liben and Sekota for 487 accessions and for their subsets grouped based on sources of the accessions, in FT1

Sites	Number of accessions	Min PSD	Max PSD	Mean PSD	SD
Sekota[a]	487	0.00	90.00	29.71	11.74
EIB accessions	442	0.00	55.56	29.67	11.41
Newly collected populations	38	4.17	68.00	28.90	12.09
Varieties	7	13.33	90.00	36.27	25.47
Liben[b]	487	0.00	100.00	46.48	12.76
EIB accessions	442	0.00	100.00	46.42	12.50
Newly collected populations	38	27.50	72.73	42.98	8.74
Varieties	7	49.17	100.00	69.20	23.51
Ebinat[c]	487	0.00	100.00	52.20	19.42
EIB accessions	442	0.00	100.00	51.63	19.37
Newly collected populations	38	19.18	100.00	54.23	16.59
Varieties	7	45.00	100.00	77.19	22.23

Only accessions with complete data from the three sites were included

Sites with different superscripts are significantly different from each other in mean PSD (ANOVA; $P < 0.05$)

The Pearson correlation coefficients between PSD values of Ebinat and Liben, Liben and Sekota, and Ebinat and Sekota were 0.11 ($P = 0.02$), 0.11 ($P = 0.02$) and 0.07 ($P = 12$)

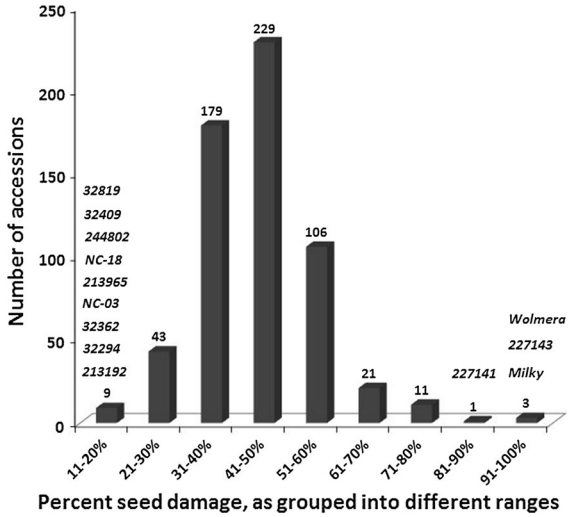

Fig. 3 Number of accessions with different levels of PSD in FT1. Accessions with <20 % and >80 % PSD are listed on top of the corresponding bars. *NC-3* and *NC-18* are newly collected populations; *Milky* and *Wolmera* are released varieties; whereas the remaining accessions listed are from the gene bank (EIB)

block-IV of site-II were 74, 77, 11 and 18 %, respectively (Table 3). A pair-wise comparison of the mean PSD at site-I and site-II for individual accessions revealed a significantly lower PSD at site-II than at site-I ($P < 0.0001$; Fig. 4). Among the 53 accessions, only three (*32471*, *230844* and *203084*) had a mean PSD of <30 % (Fig. 4). The most infested accession was *Adet*, which has cream-colored seeds, with a mean PSD of 92 and 32 % at site-I and site-II, respectively. When the data from the two sites were pooled and compared with the most susceptible accession, *Adet*, 37 out of the 52 accessions (71 %) had lower PSD at a highly significant level ($P < 0.01$) whereas 10 accessions (19 %) were not significantly different (Table 4).

Comparison of the PSD of least infested and highly infested accessions at site-I during FT2 with the performance of the same accessions grown at the same site during FT1 revealed a significantly higher infestation during FT2 (mean PSD = 76 %) than in FT1 (mean PSD = 43 %; Fig. 5). However, six accessions with the lowest PSD during FT2 had similar or lower levels of infestation during FT2 than during FT1 (Fig. 5).

Greenhouse experiments

Among the genotypes selected from FT1 for greenhouse experiments, some genotypes of accessions *226037* and *32397* showed relatively consistent results across the three generations in their resistance against pea weevil. The overall mean PSD of moderately resistant genotypes selected from accession *226037* (4 %) was much lower than that of susceptible genotypes selected from accession *32397* (58 %; Table 5). Similarly, some moderately resistant genotypes selected from accessions *236413* and *32410* were less infested as compared to susceptible genotypes selected from accessions *32487* and *Adet* (Table 5).

Almost all genotypes under group-I (Table 5) had significantly lower PSD values as compared to other plants in the same cage, unlike the genotypes under group-II. For example, genotype *226037-3-VOK-par* had no seed damage while the mean PSD of plants in the cage where this genotype was placed (MPSDC; see Table 5) was above 50 % and all other plants in the

Table 3 Mean percent seed damage (PSD) for blocks I and II at site-I and blocks III and IV at site-II, mean PSD for each site, and their corresponding *t* values, in FT2

Sites	Blocks	Mean % seed damage^{arc}	Mean % seed damage^{ori}	Standard error	*t* value
I	I	0.84	0.74	0.03	29.86
	II	0.88	0.77	0.04	23.34
	I and II combined	0.85[a]	0.75	0.02	39.7
II	III	0.11	0.11	0.01	8.58
	IV	0.18	0.18	0.01	13.46
	III and IV combined	0.14[b]	0.14	0.020	6.75

Values with different superscripts are different at highly significant level ($P < 0.0001$)

Mean % seed damage^{arc} = Mean percent seed damage for arcsine transformed data; Mean % seed damage^{ori} = Mean percent seed damage for original data

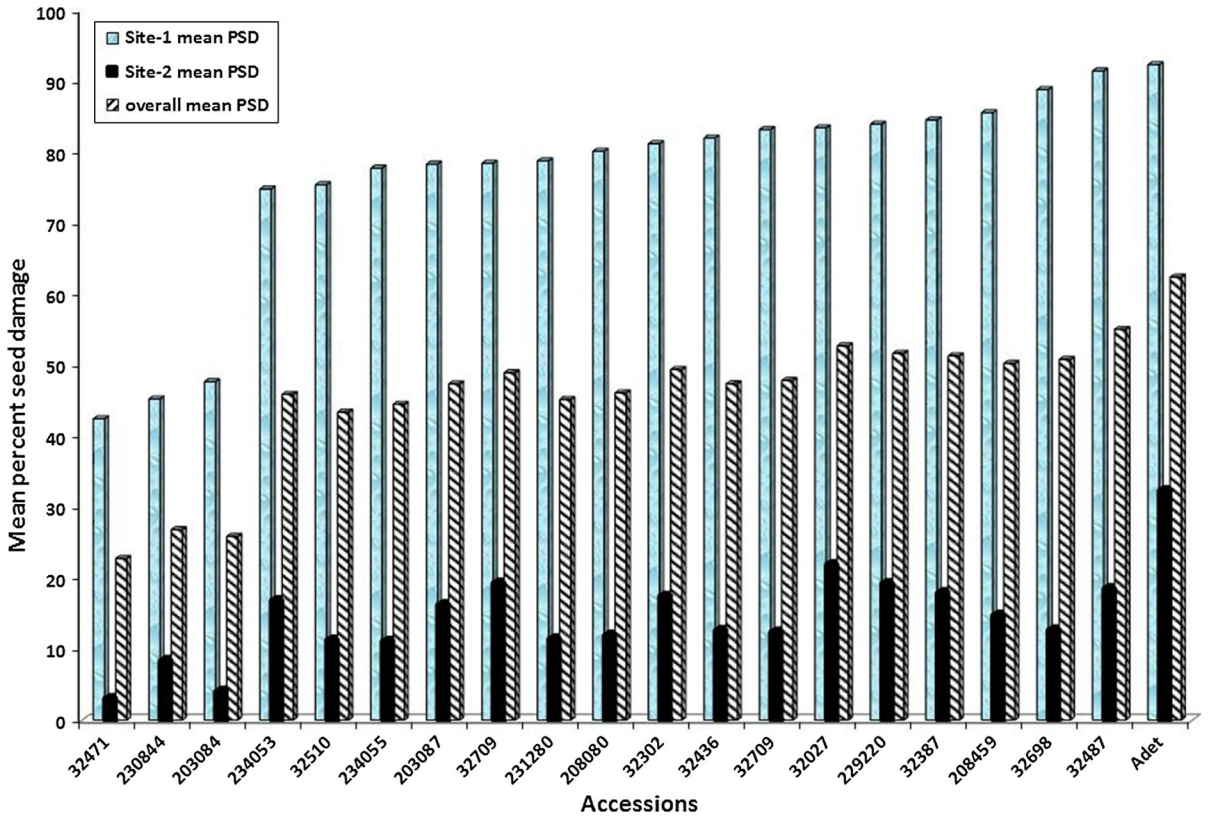

Fig. 4 Comparison of each accession's PSD in FT2 at site-I and site-II, and their mean PSD (only three accessions with lowest mean PSD and 17 accessions with highest mean PSD were included)

cage were attacked (PPISC = 83 %; see Table 5). The progenies of this genotype, *226037-3-VOK-pro-1* and *226037-3-VOK-pro-2* also had comparatively low infestation. In contrast, individuals like *32397-2-GV-par* and its progenies had equal or higher PSD as compared to the mean PSD of plants in their corresponding cages. A similar trend is noted for all genotypes under group-II (Table 5).

Discussion

In FT1, the level of damage caused by pea weevil in most accessions varied significantly between the three sites, which appeared to be partly due to differences in pest pressure. Furthermore, there was poor correlation between the PSD values of the three sites suggesting inconsistency in the overall performance of most accessions against pea weevil under different environmental conditions. Such inconsistency resulted in insignificant variation among accessions when all accessions were included, as revealed by ANOVA. Nonetheless, a number of accessions showed relatively high or low levels of infestation across the three sites. Most of these accessions had overall mean PSD of <40 % or >60 %. Some of the accessions with <40 % mean PSD during FT1 also had a relatively low level of infestation during FT2 (e.g. *32454* and *235002*). The incorporation of the accessions with a relatively low infestation across the different sites during the two field trials into a pea breeding program may lead to the development of field pea varieties with enhanced resistance to pea weevil and consequently contribute to sustainable field pea production. Such approach will be more effective if it is combined with crop rotation in which field pea and non-host plants of pea weevil such as cereals are involved to break the life-cycle of the pest and thereby minimize pest pressure.

Similar to this study, previous field screening efforts reported variation among field pea accessions in the level of seed damage caused by the pea weevil

Table 4 Comparison of mean percent seed damage (PSD) of 52 accessions with the most infested accession (*Adet*) during FT2

Accession	Mean PSD[a]	Δ-mean PSD[b]	SE	*P* value	Accession	Mean PSD[a]	Δ-mean PSD[a]	SE	*P* value
Adet	0.78	0.00	–	–	*206988*	0.39	−0.38	0.08	<0.01[c]
32019	0.59	−0.19	0.07	0.23[e]	*208080*	0.55	−0.22	0.07	0.06[e]
32027	0.62	−0.15	0.06	0.29[e]	*208459*	0.63	−0.15	0.07	0.41[e]
32065	0.44	−0.33	0.07	<0.01[c]	*215517*	0.48	−0.30	0.07	<0.01[c]
32229	0.62	−0.16	0.06	0.17[e]	*216905*	0.48	−0.29	0.07	<0.01[c]
32302	0.55	−0.23	0.06	0.01[c]	*223331*	0.52	−0.26	0.07	<0.01[c]
32387	0.59	−0.19	0.07	0.13[e]	*226037*	0.50	−0.28	0.07	<0.01[c]
32426	0.47	−0.31	0.07	<0.01[c]	*229220*	0.64	−0.14	0.08	0.92[e]
32433	0.54	−0.24	0.07	0.03[d]	*229222*	0.36	−0.42	0.08	<0.01[c]
32436	0.54	−0.24	0.07	0.02[d]	*230049*	0.42	−0.35	0.06	<0.01[c]
32471	0.19	−0.59	0.07	<0.01[c]	*230054*	0.51	−0.26	0.08	0.02[d]
32476	0.55	−0.23	0.07	0.01[c]	*230844*	0.28	−0.49	0.07	<0.01[c]
32487	0.50	−0.28	0.06	<0.01[c]	*231266*	0.36	−0.42	0.07	<0.01[c]
32496	0.49	−0.29	0.07	<0.01[c]	*231277*	0.50	−0.23	0.07	0.02[d]
32510	0.53	−0.25	0.07	0.01[c]	*231279*	0.54	−0.24	0.07	<0.01[c]
32527	0.69	−0.09	0.07	0.99[e]	*231280*	0.51	−0.26	0.06	<0.01[c]
32588	0.45	−0.33	0.06	<0.01[c]	*234053*	0.54	−0.24	0.06	<0.01[c]
32670	0.40	−0.38	0.08	<0.01[c]	*234055*	0.51	−0.27	0.07	<0.01[c]
32698	0.61	−0.16	0.07	0.25[e]	*235000*	0.42	−0.36	0.07	<0.01[c]
32703	0.45	−0.33	0.06	<0.01[c]	*235004*	0.37	−0.40	0.07	<0.01[c]
32709	0.58	−0.20	0.06	0.02[d]	*236413*	0.49	−0.29	0.07	<0.01[c]
32779	0.41	−0.37	0.07	<0.01[c]	*237065*	0.50	−0.28	0.07	<0.01[c]
203083	0.38	−0.39	0.07	<0.01[c]	*237945*	0.48	−0.30	0.07	<0.01[c]
203084	0.34	−0.44	0.05	<0.01[c]	*NC-05*	0.44	−0.34	0.06	<0.01[c]
203087	0.58	−0.19	0.08	0.25[e]	*NC-20*	0.49	−0.28	0.07	<0.01[c]
203088	0.45	−0.33	0.06	<0.01[c]	*NC-26*	0.35	−0.43	0.07	<0.01[c]
203108	0.34	−0.44	0.07	<0.01[c]					

[a] PSD values are arcsine transformed values

[b] Difference in mean PSD between *Adet* and corresponding accession

[c] Highly significant

[d] Significant

[e] Not significant

(Pesho et al. 1977; Hardie et al. 1995; Gantner et al. 2008). However, to the best of our knowledge, none of these accessions or genotypes has been incorporated into a breeding program. On the contrary, Hardie et al. (1995) and Clement et al. (2002) argued for the absence of such resistance in the primary gene pool of field pea that could be transferred into cultivars. These reports put forward lines of *P. fulvum* as a potential source of resistance through antixenosis and antibiosis mechanisms. In this regard, recent efforts have led to successful hybridization and transfer of resistance from *P. fulvum* to susceptible *P. sativum* varieties (Clement et al. 2009; Aryamanesh et al. 2012). However, despite the fact that the hybrids had the desired resistance against pea weevil, other agronomic traits like maturity, yield, seed size and seed quality was not reported. Although, a direct comparison of the present findings with those of resistant hybrid lines is not possible due to differences in experimental set up, our results have reopened the door to explore the primary gene pool of field peas as a potential source of pea weevil resistance.

In FT2, significantly higher seed damage levels were recorded at site-I than at site-II. Analysis of randomly sampled seeds from neighboring farmers' fields at each site revealed similar levels of pest pressure at both sites. However, the number of weevils emerging within the fields was not measured and it is possible that there were differences in the density of weevils emerging and then staying in the experimental fields between the two sites. There was also a difference between the two sites in the number of

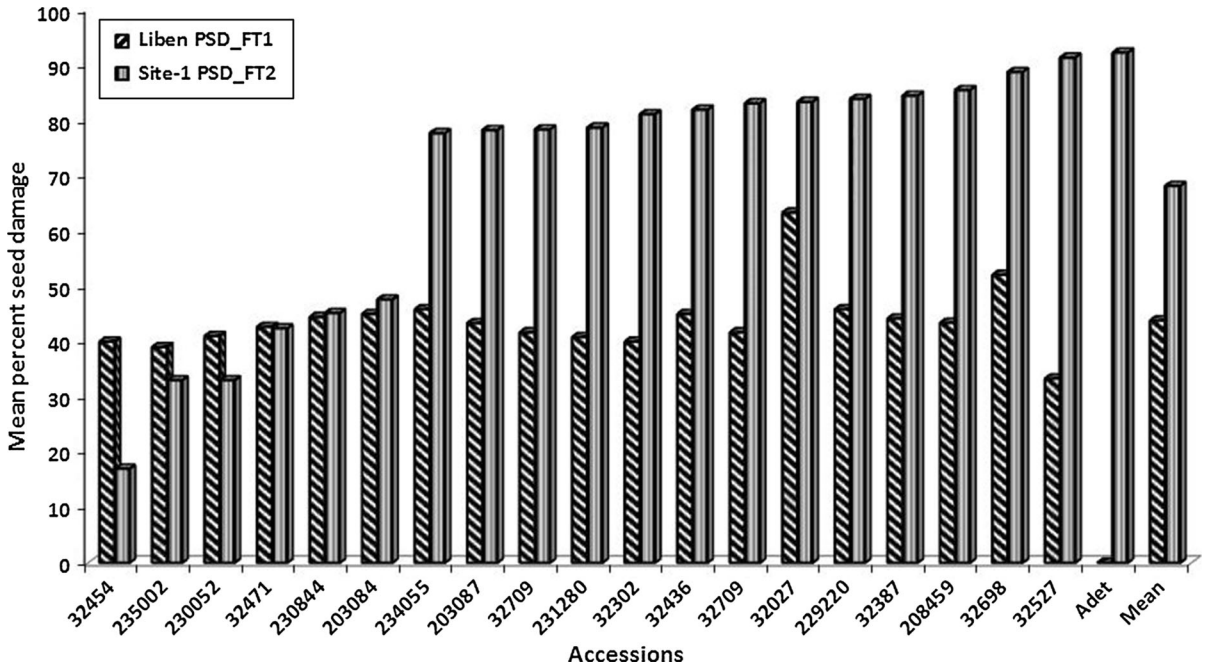

Fig. 5 Comparison of PSD of six least damaged and fourteen highly damaged accessions at site-I in FT2 with PSD of the same accessions grown at the same site in FT1. *Note Adet* was not included in FT1

highly susceptible accessions planted. At site-I, 23 of 100 accessions were highly susceptible while only one highly susceptible accession was included at site-II. It is possible that the large number of highly susceptible plants at site-I could have attracted more weevils, e.g. via release of attractive volatiles, which subsequently resulted in higher seed damage. Clement et al. (1996) reported that flowers from accession *263026* from Ethiopia was significantly less attractive to female pea weevils as compared to susceptible checks (Alaska and Garfield) in greenhouse-based experiments.

A number of recent studies have demonstrated the role of cultivar mixtures in suppression of insect pests in various cropping systems (Ratnadass et al. 2012; Tooker and Frank 2012). A screening of barley (*Hordeum vulgare* L.) genotypes for resistance against the bird cherry-oat aphid (*Rhopalosiphum padi* L.) showed that resistance can be invoked in susceptible genotypes from nearby partially resistant genotypes under greenhouse conditions (Ninkovic and Åhman 2009). Such allelopathic plant interactions were also observed to reduce bird cheery-oat aphid acceptance significantly in certain *Hordeum* genotypes when grown with another genotype at the field level (Ninkovic et al. 2002).

The results from the field experiments could indicate an advantage of using admixtures of moderately resistant accessions in tackling the pea weevil problem in field pea production. Due to the benefits of compensation and cooperation, the use of an admixture of genotypes as mechanism of resistance is better suited to farmer‘s fields where there are various biotic and abiotic stresses as compared to using genetically uniform varieties (Döring et al. 2011). However, the effectiveness of this approach as a defense mechanism needs further evaluation to optimize admixtures that perform best against the pest. Such moderate level of resistance in selected genotypes could also be augmented with other pest management methods like intercropping and crop rotation (Bajwa and Kogan 2004). Different combinations of genotypes are now being evaluated in the greenhouse for resistance against pea weevil, which will later be tested at the field level.

In line with the results from the field trials, the greenhouse experiments also led to the identification of individual genotypes with low or no seed damage in cages where there was significant damage to other genotypes. For example, genotypes like *226037-3-VOK-par*, *226037-3-LGRK-par*, *32410-par* and

Table 5 Comparison of the extent of pea weevil damages in different genotypes of field peas based on greenhouse experiments

Group-I				Group-II			
Plant code	PSD	MPSDC	PPISC	Plant code	PSD	MPSDC	PPISC
226037-3-VOK-par[c]	0	52	83	*32397-2-GV-par*[c]	67	52	83
226037-3-VOK-pro-1[d]	12	36	100	*32397-2-GV-pro-1*[d]	95	36	100
226037-3-VOK-pro-2[d]	4	54	100	*32397-2-GV-pro-2*[d]	21	23	83
226037-3-VOK-pro-mean[d]	8	45	100	*32397-2-GV-pro-3*[d]	50	53	100
226037-3-LGRK-par[c]	0	64	83	*32397-2-GV-Pro-mean*[d]	56	37	94
226037-3-LGRK-pro-1[d]	0	29	54	*32397-2-GV-Pro-1-1*[e]	42	31	100
226037-3-LGRK-pro-2[d]	3	35	100	*32397-4-GVOS-par*[c]	85	64	83
226037-3-LGRK-pro-mean[d]	1	32	77	*32397-4-GVOS-pro-1*[d]	80	35	100
226037-3-LGRK-pro-1-1[e]	27	31	100	*32397-4-GVOS-pro-2*[d]	88	35	100
226037-5-GRK-par[c]	0	35	67	*32397-4-GVOS-pro-mean*[d]	84	35	100
226037-5-GRK-pro-1[d]	0	13	80	*32397-4-GVOS-pro-1-1*[e]	39	26	100
226037-5-GRK-pro-2[d]	0	17	83	*32397-4-GVOS-pro-1-2*[e]	16	9	40
226037-5-GRK-pro-mean[d]	0	15	82	*32397-4-GVOS-pro-1-mean*[e]	28	18	70
226037-5-GRK-pro-1-1[e]	10	15	100	*32397-6-GR-par*[c]	58	35	67
226037-7-GRK-par[c]	0	35	67	*32397-6-GR-pro-1*[d]	46	17	83
226037-7-GRK-pro-1[d]	0	29	54	*32397-6-GR-pro-2*[d]	72	52	100
226037-7-GRK-pro-2[d]	9	52	100	*32397-6-GR-pro-mean*[d]	59	35	92
226037-7-GRK-pro-3[d]	5	41	77	*32397-6-GR-pro-1-1*[e]	28	9	40
226037-mean[a]	4	35	84	*32397-6-GR-pro-1-2*[e]	59	52	100
32410-1-par[c]	0	na	na	*32397-6-GR-pro-1-3*[e]	95	54	83
32410-1-pro-1[d]	18	26	100	*32397-6-GR-pro-1-mean*[e]	61	38	74
32410-1-pro-2[d]	0	9	43	*32397-mean*[b]	58	36	85
32410-1-pro-3[d]	10	9	40	*Adet-1-par*[c]	92	na	na
32410-1-pro-4[d]	0	54	83	*Adet-1-pro-1*[d]	26	9	43
32410-1-pro-mean[d]	7	25	67	*Adet-1-pro-2*[d]	56	51	100
236413-1-par[c]	0	na	na	*Adet-1-pro-mean*[d]	41	30	72
236413-1-pro-1[d]	0	12	60	*32487-1-par*[c]	77	na	na
236413-1-pro-2[d]	24	15	100	*32487-1-pro-1*[d]	18	15	100
236413-1-pro-3[d]	0	9	71	*32487-1-pro-2*[d]	12	9	71
236413-1-pro-mean[d]	8	12	77	*32487-1-pro-3*[d]	16	26	100
				32487-1-pro-mean[d]	15	17	90

The genotypes were placed under two groups based on their levels of resistance across generations (moderately resistant = group-I and susceptible = group-II)

Genotypes under group-1 were from accessions *226037*, *32410* and *236413* whereas genotypes under group-II were from accessions *32397*, *32487* and *Adet*; PSD values for *32410-1-par*, *236413-1-par*, *Adet-1-par* and *32487-1-par* were from FT2

na Not applicable, *par* parent, *pro* progeny, *pro-mean* mean for progeny plants, *pro-1-mean* mean for the second progeny generation, *PSD* percent seed damage, *MPSDC* mean percent seed damage per cage, *PPISC* percent of plants with infested seeds per cage

[a] Overall mean for genotypes originating from accession *226037*. [b] Overall mean for genotypes originating from accession *32397*. [c] Parental generation. [d] First progeny generation. [e] Second progeny generation

236413-par were not infested although other genotypes in the same cages had significant seed damage. The progenies of these genotypes also performed well in comparison with other genotypes. Interestingly, some of these genotypes produced neoplastic pods under greenhouse conditions (Fig. 6). Neoplasm is an outgrowth of callus tissue from the stomata of the maturing pod (Burgess and Fleming 1973) and has

Fig. 6 Neoplastic growth on pea pod under greenhouse condition

been reported in some field pea genotypes (Nuttall and Lyall 1964; Dodds and Matthews 1966). This trait is due to a dominant gene referred to as *Np*, and plants with this trait were proved to be less susceptible to pea weevil attack as compared to those without the gene, due to low success rate of larval penetration through neoplastic pods (Berdnikov et al. 1992; Doss et al. 2000). In the present study, neoplastic growth might have contributed to the relative resistance of neoplasm producing genotypes against the pea weevil.

This study revealed that cream-colored seeds, regardless of their origin, are more susceptible to pea weevil than seeds with other colors. Although seed color may not have direct link with pea weevil resistance in field peas, as suggested by Aryamanesh et al. (2012), the extremely high damage recorded in cream-colored seeds suggests that other traits associated with seed color might have played a role in the susceptibility of such plants. Most field pea varieties with cream-colored seeds that are currently grown in Ethiopia have round seeds and comparatively large cotyledons. The large cotyledons guarantee excess food during larval development and augment survival and fecundity at later stages. It is also likely that round seeds give more space to the larvae as compared to wrinkled seeds. In contrast, the less damaged accessions in our study have wrinkled green or variegated green seeds. Hence, the search for pea weevil resistance in the field pea gene pool should focus on accessions with wrinkled green seeds.

An interesting observation made during the present study was that pea weevil infestation led to a change in seed color in field pea, with few exceptions. The most distinct color changes were observed in green and variegated green seeds, which changed their color to brown and variegated brown, respectively, after infestation. Although it was not as distinct as the case in green seeds, brown seeds also showed slight color change by becoming deep brown upon infestation. Generally, the change in color was observed in all seed color types except in the case of cream-colored seeds, which remains the same after infestation. These seed color changes could be of scientific interest as part of efforts to develop resistance against the pea weevil. In other legumes, e.g. *Sesbania drummondii*, it has been reported that attacks by *Hyalymenus tarsatus* cause seed color change that also causes alteration in the seed coat physiology that triggers early germination (Ceballos et al. 2002). In the present study, there was no evidence that changes in seed color due to pea weevil attack are associated with any fitness or physiological mechanisms. However, it is an important phenotypic marker that farmers can use to get rid of infested seeds for the following planting season and hence has a vital importance in pea weevil management.

Farmers have difficulties identifying symptoms of pea weevil infestation in field pea, particularly at its early stages, and hence fail to take necessary measures. Recently, a new method to classify healthy and pea weevil infested seeds based on reflectance data was reported (Nansen et al. 2014). Another study indicated that density based separation of infested seeds of *P. fulvum* lines with 30 % caesium chloride was possible (Aryamanesh et al. 2012). However, such approaches are unaffordable and difficult to implement for small-scale farmers in developing countries. Hence, simple color-based separation of infested seeds from healthy seeds with the naked eye could be a viable option. Selecting healthy seeds for planting would enhance germination rate and may reduce pest pressure during flowering. Observation of color change is also a simple approach for postharvest inspection. Training farmers with this approach would reduce economic losses due to this pest, as fumigating

already infested seeds at its early stages can prevent further damage. Overall, the screening work conducted on a large number of field pea accessions for resistance against pea weevil resulted in some accessions and genotypes with a moderate level of resistance, which should be incorporated in field pea breeding programs. Supplying local farmers with seeds from moderately resistant accessions after seed multiplication could be an alternative short-term approach in the fight against pea weevil. In parallel, screening field pea accessions for resistance against pea weevil should be continued as there are more than 1,500 *P. sativum* accessions at EIB not yet evaluated.

Acknowledgments We would like to dedicate this paper to the late Dr. Emiru Seyoum who was one of the major collaborators behind this project and a co-author. The authors would like to thank Swedish International Development Agency (Sida) for funding this research project. Our thanks also go to Ethiopian Institute of Biodiversity (EIB) and Holeta Agricultural Research Centre (HARC) for providing pea accessions and varieties, and Amhara Agricultural Research Institute and Adet Agricultural Research Centre for cooperation during the field trials.

References

Abate T (2006) IPM in Ethiopia: the current status. In: Bekele E, Azerefegne F, Abate T (eds) Facilitating the implementation and adoption of integrated pest management (IPM) in Ethiopia, Melkassa Agricultural Research Center, Ethiopia. DCG Proceedings, pp 3–15

Aryamanesh N, Byrne O, Hardie DC, Khan T, Siddique KHM, Yan G (2012) Large-scale density-based screening for pea weevil resistance in advanced backcross lines derived from cultivated field pea (*Pisum sativum* L.) and *Pisum fulvum*. Crop Pasture Sci 63:612–618

Assayehegne B (2002) The biology and ecology of pea weevil (*Bruchus pisorum*). In: A National workshop on the management of pea weevil (*Bruchus pisorum*). Bahir Dar, Ethiopia, pp 37–45

Bajwa WI, Kogan M (2004) Cultural practices: springboard to IPM. In: Koul O, Dhaliwal GS, Cuperus GW (eds) Integrated pest management: potential, constraints and challenges. CAB International, UK, pp 21–38

Baker GJ (1998) Pea weevil. Fact sheet. Primary Industries and Resources SA and the South Australian Research and Development Institute

Berdnikov VA, Trusov YA, Bogdanova VS, Kosterin OE, Rozov SM, Nedel'kina SV, Nikulina YN (1992) The neoplastic pod gene (Np) may be a factor for resistance to the pest *Bruchus pisorum* L. Pisum Genet 24:37–39

Bousquet Y (1990) Beetles associated with stored products in Canada: an identification guide, vol 1837. Agriculture Canada, Ottawa

Byrne OMT (2005) Incorporation of pea weevil resistance from wild pea (*Pisum fulvum*) into field pea (*Pisum sativum* L.). PhD thesis, The University of Western Australia, Perth, Australia

Burgess J, Fleming EN (1973) The structure and development of a genetic tumour of the pea. Protoplasma 76:315–325

Ceballos L, Andary C, Delescluse M, Gibernau M, Mckey D, Hossaeart-Mckey M (2002) Effects of sublethal attack by a sucking insect, *Hyalymenus tarsatus*, on *Sesbania drummondii* seeds: impact on some seed traits related to fitness. Ecosceince 9:28–36

Clement SL, Evans MA, Lester DG (1996) Settling and feeding responses of pea weevil (Coleoptera; Bruchidae) to flowers of selected pea lines. J Econ Entomol 89:775–779

Clement SL, Hardie DC, Elberson LR (2002) Variation among accessions of *Pisum fulvum* for resistance to pea weevil. Crop Sci 42:2167–2173

Clement SL, McPhee KE, Elberson LR, Evans MA (2009) Pea weevil, *Bruchus pisorum* L. (Coleoptera: Bruchidae), resistance in *Pisum sativum* × *Pisum fulvum* interspecific crosses. Plant Breed 128:478–485

CSA (2011) Report on area and production for major crops, statistical bulletin, vol 1. Central Statistical Authority, Addis Ababa

Dodds KS, Matthews P (1966) Neoplastic pod in the pea. J Hered 57:83–85

Döring TF, Knapp S, Kovacs G, Murphy K, Wolfe MS (2011) Evolutionary plant breeding in cereals-into a new era. Sustainability 3:1944–1971

Doss RP, Oliver JE, Proebsting WM, Potter SW, Kuy S, Clementi SL, Williamson RT, Carney JR, DeVilbiss ED (2000) Bruchins: insect-derived plant regulators that stimulate neoplasm formation. Proc Natl Acad Sci USA 97:6218–6223

FAOSTAT (2012) Food and Agriculture Organization of the United Nations. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor. Accessed February 03, 2014

Fikere M, Tadesse T, Gebeyehu S, Hundie B (2010) Agronomic performances, disease reaction and yield stability of field pea (*Pisum sativum* L.) genotypes in Bale highlands, Ethiopia. Aust J Crop Sci 4:238–246

Gantner R, Stjepanović M, Popović S, Greger Ž (2008) Resistance of field pea genotypes (*Pisum sativum* L.) to the occurrence of pea weevil (*Bruchus pisorum* L.) in Seed. In: 43rd Croatian and 3rd International Symposium on Agriculture. Opatija, Croatia, pp 322–325

Hardie DC, Baker GJ, Marshall DR (1995) Field screening of *Pisum* accessions to evaluate their susceptibility to the pea weevil (Coleoptera: Bruchidae). Euphytica 84:155–161

Keneni G, Jarso M, Wolabu T (2003) Eco-geographic distribution and microcenters of genetic diversity in faba bean (*Vicia faba* L.) and field pea (*Pisum sativum* L.) germplasm collections from Ethiopia. E Afr J Sci 1:1–15

Lehmann C (1954) Das morphologische system der Saaterbsen (*Pisum sativum* L. sens. lat. GOV. ssp. *sativum*). Züchter 24:316–337

McDonald G (1995) Pea weevil. Department of Environmental and Primary Industries (DEPI), Victoria

Nansen C, Zhang X, Aryamanesh N, Yan G (2014) Use of variogram analysis to classify field peas with and without internal defects caused by weevil infestation. J Food Eng 123:17–23

Ninkovic V, Åhman I (2009) Aphid acceptance of *Hordeum* genotypes is affected by plant volatile exposure and is correlated with aphid growth. Euphytica 169:177–185

Ninkovic V, Olsson U, Pettersson J (2002) Mixing barley cultivars affects aphid host plant acceptance in field experiments. Entomol Exp Appl 102:177–182

Nuttall VW, Lyall LH (1964) Inheritance of neoplastic pod in the pea. J Hered 55:184–186

Pesho GR, Muehlbauer FJ, Harberts WH (1977) Resistance of pea introductions to pea weevil. J Econ Entomol 70:30–33

Ratnadass A, Fernandes P, Avelino J, Habib R (2012) Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agron Sustain Dev 32:273–303

R Core Team (2013). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org/

Schroeder HE, Gollasch S, Moore A, Tabe LM, Craig S, Hardie DC, Chrispeels M, Spencer JD, Higgins TJV (1995) Bean α-amylase inhibitor confers resistance to the pea weevil (*Bruchus pisorum*) in transgenic peas (*Pisum sativum* L.). Plant Physiol 107:1233–1239

Smith JH, O'Keeffe LE, Muehlbauer FJ (1982) Methods of screening dry peas for resistance to the pea weevil (Coleoptera: Bruchidae): variability in seed infestation levels. J Econ Entomol 75:530–534

Stenvovic V, Dukic D, Mandic L (2005) Productive and quantitative traits of pea fodder and grain depending on nitrogen nutrition. Biotech Anim Husb 21(5–6):287–291

Teka W (2002) The importance and distribution of pea weevil (*Bruchus pisorum* L) in the Amhara region. In: A national workshop on the management of pea weevil, *Bruchus pisorum*, Bahir Dar, Ethiopia, November 25–27, pp 30–36

Tesfaye A, Dawd M, Degene A, Getinet S (2002) Suggested management options of pea weevil, *Bruchus pisorum* L. (Coleoptera: Bruchidae). In: A national workshop on the management of pea weevil, *Bruchus pisorum*, Bahir Dar, Ethiopia, November 25–27, pp 47–59

Tooker JF, Frank SD (2012) Genotypically diverse cultivar mixtures for insect pest management and increased crop yields. J Appl Ecol 49:974–985