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Gene action determining grain yield and chocolate spot (*Botrytis fabae*) resistance in faba bean

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Abstract Production of faba bean (*Vicia faba* L.) is hampered by chocolate spot disease caused by *Botrytis fabae*. Studies of the genetics of resistance is vital to devise a viable strategy for faba bean breeding. The present study was carried out with the objective to determine the mode of inheritance and maternal effect for chocolate spot resistance and yield. A 10 × 10 full diallel mating design was used to generate 90 F1 hybrids which were evaluated at three sites in Ethiopia with two replications under natural and with artificial inoculation of the pathogen. There was significant variation for chocolate spot resistance and yield among the genotypes (P ≤ 0.001). The general combining ability (GCA) and specific combining ability (SCA) effects were both highly significant (P ≤ 0.001) for chocolate spot resistance. However, the GCA effect was predominant (84.5 %) suggesting that additive gene effects were more important than non-additive effects and that subsequent selection would be effective to enhance disease resistance. In contrast, the SCA effects were predominant (89.3 %) for grain yield suggesting that non-additive gene action was more important which may provide transgressive segregants. Reciprocal effects were generally negligible (<10 %) for both yield and disease resistance. The line ILB-4726, which combined good disease resistance with high grain yield potential would be recommended for faba bean breeding.

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Introduction

Faba bean (*Vicia faba* L.) is one of the leading staple legumes in many countries, spanning from Africa to the Middle East. However, there is a reduction in global production of faba bean in many countries (Torres et al. 2006; Pe'rez-de-Luque et al. 2010) which can worsen the gap between production and consumption. Faba bean production is threatened by chocolate spot disease among other constraints. Chocolate spot disease caused by *Botrytis fabae* Sardina compromises faba bean yields by damaging the foliage and limiting photosynthetic activity. The disease affects crop productivity worldwide, but

especially in the north east and southern Africa (Akem and Bellar 1999; Bouhassan et al. 2004; Tivoli et al. 2006). Severe epidemics of chocolate spot can be devastating resulting in over 90 % yield reduction. For example, extensive damage has been reported in Australia with yield loss of 90 %, United Kingdom (59 %), China (>50 %) and in Ethiopia (>61 %) (Gorfu and Yaynu 2001; Elad et al. 2004). Therefore, breeding for chocolate spot resistance should be important goal to enhance productivity.

An integration of cultural methods with host plant resistance would be effective to manage the disease, especially in developing countries. Integrating disease control options, such as early sowing, sprays of mancozeb and chlorothalonil fungicides, use of moderately resistant cultivars, and intercropping with cereals can reduce the disease epidemics (Gorfu 2000; Sahile et al. 2008). However, smallholder farmers involved in faba bean production in developing countries cannot afford to buy fungicides. Additionally, completely resistant cultivars for chocolate spot have not been developed. The use of resistant cultivars and incorporating favorable resistance genes into the locally adapted susceptible varieties is one of the most effective ways of controlling chocolate spot disease (Sillero et al. 2010).

Knowledge on the mode of inheritance and gene action for important traits in faba bean is important to device breeding strategies (Lithourgidisa et al. 2005). In studies of faba bean resistance to ascochyta blight, additive gene effects were found predominant to non-additive effect (Kharrat et al. 2006). In faba bean populations and diallel F_1 hybrids, additive gene action was more important in determining the response to rust disease resistance (Stoddard and Herath 2001). El-Bramawy and El-Beshehy (2011) reported that additive gene effect was more important than dominance effect for the inheritance of resistance to bean yellow mosaic virus of faba bean. In a study of the inheritance of chocolate spot and rust disease tolerance in faba bean, the mode of inheritance was suggested as dominant character controlled by a single gene (Noorka and EL-Bramawym 2011). This study generated information which is applicable for the two diseases, but only two germplasms were considered. In addition, there is no information on inheritance of chocolate spot resistance in faba bean for using divergent breeding lines which is fundamental to devise viable chocolate spot resistance breeding strategies in Ethiopia. Therefore, the objective of this study was to determine the mode of inheritance and type of gene action which govern chocolate spot resistance and yield in faba bean.

Materials and methods

Experimental sites and germplasm

The experiments were conducted at three locations in Ethiopia. The first site was Holetta Agricultural Research Centre (HARC) (09°03′N, 38°30′E, and 2390 meter above sea level, i.e., m a. s. l) with average maximum and minimum temperatures of 22.5 and 8.5 °C, respectively, and average rainfall of 533.6 mm, and soil pH of 4.62. The second site was the Kulumsa Agricultural Research Centre (KARC) (08°00′N; 39°09′E; 2211 m a. s. l). The average maximum and minimum temperature at KARC was 22.5 and 10.9 °C, respectively. The average rainfall received was 584.1 mm and the soil pH of 5.2. The third site was the Kofele (07°04′N; 38°48′E; 2620 m a. s. l.) and the average maximum and minimum temperatures were 18.3 and 2.3 °C, respectively. The average rainfall received was 1077.4 mm and the soil pH of 4.37.

Crosses and mating design

A full diallel mating design developed by Hayman (1954) and Jinks (1955) was used for this study. It provides reliable mechanism to properly understand the nature of gene action and create sufficient genetic variability for the purpose of developing transgressive segregants (Sharma 2006). The characteristics and sources of the 10 faba bean parental lines used for the 10 × 10 full diallel mating with reciprocal crosses are indicated in Table 1. These lines are genetically different and form three genetic clusters established using 30 SSR markers (data not presented).

Experimental design and management

The 100 entries consisting of 90 F_1 reciprocal progenies (i.e., 45 direct crosses and 45 reciprocals) developed from the 10 × 10 full diallel mating design and the 10 parental lines were planted in a 10 × 10 α-lattice design in single row plots of 3 m long at 0.4 m inter-row and 0.06 m intra-row spacing. This resulted

Table 1 Description of the 10 faba bean lines used in a diallel cross

Parents	Source/pedigree	Seed size (1000 seed weight/gram)	Resistance status	Maturity period
ILB-4726	ICARDA	Large (1127)	Resistant	Late
ILB-938	ICARDA	Large (930)	Resistant	Late
BPL-710	ICARDA	Large (845)	Resistant	Late
Moti	HARC/ILB-4432 × kuse-2-27-33	Medium (781)	Moderately resistant	Medium
Dosha	HARC/Landrace collection	Medium (704)	Moderately resistant	Medium
Gebelcho	HARC/Tesfa × ILB-4726	Medium (797)	Moderately resistant	Medium
Kasa	HARC/Landrace collection	Small (428)	Susceptible	Early
NC58	HARC/Landrace collection	Small (449)	Susceptible	Early
CS-20-DK	HARC/Landrace collection	Small (476)	Susceptible	Early
Bulga-70	HARC/Landrace collection	Small (440)	Susceptible	Early

ICARDA International Center for Agricultural Research in the Dry Area, *HARC* Holetta Agricultural Research Center, *HARC/ Landrace collection* refers to the variety developed by HARC from landrace collection

in 51 plants per plot. To minimize inter-plot interference each test genotype was bordered by a common susceptible faba bean variety NC 58, which also acted as a disease spreader. Diammonium phosphate (DAP) fertilizer was applied at 100 kg ha^{-1} with 18 kg ha^{-1} Urea at planting. The trials were kept weed-free by hand weeding. In addition, trials were artificially inoculated with *Botrytis fabae* isolate with a spore concentration of 5×10^5 ml^{-1} at 60 days from planting, which coincided with the flowering stage at all sites. This practice was recommended by previous researchers (Bernard et al. 2006). Inoculation was made during the late afternoon to avoid the effect of sunlight on spore viability.

Data collection

Genotype reaction to *Botrytis fabae* was evaluated 7 days after inoculation and disease severity scoring was taken six times with weekly interval from the same five randomly pre-tagged plants per genotype. In addition, general disease assessment of the genotypes for disease severity of chocolate spot was recorded from the whole plot once at 88 days after planting. The severity of chocolate spot was recorded as a percentage of leaf area infected using the rating of Bernier et al. (1993) and Bernard et al. (2006) scale as follows: 1 % no disease symptoms or very small specks (highly resistant); 3 % few small disease lesions (highly resistant); 6 % small coalesced lesions with some defoliation (resistant); 12 % large coalesced sporulating

lesions, 20 % defoliation (moderately resistant); 25 % large coalesced sporulating lesions, 50 % defoliation and some dead plants (susceptible); and 50 % extensive, heavy sporulation, stem girdling, blackening and death of more than 80 % of the plants (highly susceptible). Grain yield t ha^{-1} data was taken from the entire plot. Grain yield was adjusted to 10 % moisture content following the oven drying method. The total grain yield recorded on a plot basis was converted to t ha^{-1} for statistical analysis.

Data analysis

The analyses of variance (ANOVA) was performed using the PROC GLM in SAS (SAS Institute 2012). The severity data collected weekly in the field was used to calculate the area under the disease progress curve (AUDPC) in Microsoft excel. The AUDPC was calculated for each genotype according to the following formula proposed by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^{n} 1/2[(yi+1+yi)(xi+1-xi)]$$

Where: yi = the cumulative disease severity percentage of infected plants at the ith observation (day i), xi = time (days) at the ith observation, n = total number of symptom observations. The estimate of AUDPC was normalized by dividing with the total area of the graph (i.e. the number of days between the first and the last readings multiplied by maximum potential AUDPC), for a better visual comparison

among genotypes over location (Mohapatra et al. 2008). The normalized AUDPC was referred to as the relative area under disease progress curve (rAUDPC).

GCA effects of the parents and SCA of the crosses, as well as their mean squares in each environment for chocolate spot resistance and grain yield were estimated in the 10 × 10 full diallel crosses following Griffing's analysis, method 1, model 1 (Griffing 1956) using DIALLEL-SAS05 program (Zhang et al. 2005) in SAS software version 9.3 (SAS Institute 2012). In addition, the role of maternal effects of chocolate spot resistance in faba bean was assessed from the reciprocal effects. The relative sum of squares was calculated for each element of the crosses: GCA, SCA and reciprocal effects as a percentage of the entry sum of squares.

Genetic variance ($\hat{\sigma}^2$GCA, $\hat{\sigma}^2$GCA and $\hat{\sigma}^2$REC) were estimated using Diallel SAS Griffing's random-effect model method 1 diallel analysis using DIALLEL-SAS05 (Zhang et al. 2005). The variances were then used to estimate the following parameters: the general predicted ratio (GPR) Baker's (1978) $\frac{2\sigma^2\text{GCA}}{2\sigma^2\text{GCA}+\sigma^2\text{SCA}}$; broad-sense heritability (h_b^2): $\frac{\hat{\sigma}^2\text{GCA}+\hat{\sigma}^2\text{SCA}+\hat{\sigma}^2\text{REC}}{\hat{\sigma}^2\text{phenotypic}}$; narrow-sense heritability (h_n^2): $\frac{\hat{\sigma}^2\text{GCA}}{\hat{\sigma}^2\text{phenotypic}}$; and Coefficient of genetic variation (CVg): $\frac{\sqrt{\sigma^2}gca+\sigma^2sca+\sigma^2REC}{\mu}$ where μ is the mean value of the trait while coefficient of error variation (Kao and Mcvetty 1987) calculated as $\frac{\sqrt{\sigma^2}e}{\mu}$. (Habarurema et al. 2012; Vieira et al. 2012).

Results

Analysis of variance

There was significant ($P \leq 0.001$) variation among the genotypes for chocolate spot general disease severity, area under disease progress curve values and grain yield (Table 2). Genotype by environment interaction was significant ($P < 0.05$) for general disease severity and grain yield ($P \leq 0.001$).

General and specific combining ability

The combining ability data is presented in Table 3. The GCA and SCA effects were highly significant ($P < 0.001$) for resistance to chocolate spot based on

the two disease assessments. Reciprocal effects (REC) were also significant for GDS ($P < 0.05$) and for the rAUDPC ($P < 0.01$). The GCA effects contributed most of (84.9 % GDS) the sums of squares and (54.9 % rAUDPC value) for the crosses. The SCA contributed 8.7 % for the GDS and 29.9 % for the rAUDPC. The REC effects were small and contributed only 4.9 % for the GDS and 15.2 % for the rAUDPC. There was significant interaction for GCA x Environment ($P < 0.001$) for both disease assessments. However, contribution of GCA x Environment was low at 24 and 25.9 % for GDS and rAUDPC, respectively. Significant ($P < 0.05$) REC x Environment effects were observed for the GDS and its contribution to the variation of chocolate spot severity in crosses accounted for 49.7 and 43.3 % based on GDS and rAUDPC, respectively.

The GCA and SCA effects were highly significant ($P < 0.001$) for grain yield but the reciprocal effects (REC) were not significant (Table 3). Although the GCA effects were highly significant, the % GCA (SS) contribution was only 5.84 % while the SCA effect contributed for most of (89.3 %) the sums of square for grain yield. The SCA × Env effect was highly significant ($P < 0.001$) for grain yield. The GCA × Env accounted for 6.8 %; the REC accounted for 29.3 % while SCA × Env accounted for 64 % of the G × E.

Performance of the parents and F₁ progenies of faba bean for chocolate spot resistance

The most resistant (top 25) and most susceptible (bottom 10) F₁ faba bean genotypes and parental lines are presented in Table 4. The chocolate spot general severity for the parental lines ranged from 3.8 % for the resistant variety (ILB-4726) to 32.2 % for the susceptible faba bean variety (Kasa). The disease severity for the F₁ genotypes ranged from 2.2–25.7 %. All F₁ genotypes from crosses of the resistance parent lines; R × R (ILB 4726, ILB 938 and BPL 710) had the lowest disease severity of 2.2–4.1 % and the lowest rAUDPC values ranging from 0.20 to 0.28. Similarly, the crosses of ILB-4726 (R) × Gebelcho (MR) resulted in the lowest general disease severity of 9.4 % across the three environments. On the contrary, the F₁ genotype of crosses involved the susceptible parents NC58, Kasa, CS-20-DK and Bulga-70 had the highest disease severity of 21.1–25.7 % and high

Table 2 Combined analysis of variance for chocolate spot general disease severity (GDS), relative area under disease progress curve (rAUDPC) and grain yield from 10 × 10 full diallel cross over three environments

Source of variation	d.f	Mean squares		
		General disease severity score	Relative area under disease progress curve value	Grain yield
Rep	1	72.17*	403,112***	17.97***
Row	9	153.23***	175,219***	8.19***
Col	9	180.79***	53,702***	12.37***
Genotype	99	99.89***	47,710***	11.16***
Environment	2	3675.02***	2,453,637***	257.74***
Genotype × environment	198	14.98*	15,102	1.92***
Error	281	11.34	14,662	1.26
Total	599	44.19	32,060	4.27

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

rAUDPC values 0.35–0.40. The F_1 family derived from Kasa × NC58 had the highest diseases severity of 25.7 % and NC58 × Kasa recorded the highest rAUDPC value of 0.40. In general, the majority (86.6 %) of F_1 genotypes exhibited highly resistant to moderately resistant reactions. On the other hand, 13.3 % of F_1 progenies displayed a susceptible disease reaction to the chocolate spot severity. The 10 parental lines showed significant differences (P ≤ 0.001) in reaction to the chocolate spot disease and yield (Table 4).

Genetic parameters

The variance components, namely GCA ($\hat{\sigma}^2$GCA), SCA ($\hat{\sigma}^2$SCA) and REC ($\hat{\sigma}^2$REC) for chocolate spot severity, exhibited a similar trend for both the GDS and rAUDPC (Table 5). The GCA variance was predominant over the SCA variance and the REC variance for both disease assessments. The general predicted ratio Baker's (1978) $\frac{2\hat{\sigma}^2\text{GCA}}{2\hat{\sigma}^2\text{GCA}+\hat{\sigma}^2\text{SCA}}$ was greater than 0.5 (0.99 for GDS and 0.75 for rAUDPC). Heritability in the broad sense and narrow sense was high for the GDS (93.4 and 82.6 %) and for rAUDPC (86.4 and 48.9 %). However, the narrow sense heritability of chocolate spot was lower for the rAUDPC than the GDS. Moreover, the ratio of the coefficient of genetic variation to coefficient of error variation was greater than one in both assessments. The SCA variance were predominant than GCA variance and the REC variance for grain yield implying non-

additive genetic effects were predominant (Table 5). In addition, general predicted ratio Baker's (1978) was less than 0.5 (0.21). Heritability in the broad sense was high (94.95 %), but heritability in the narrow sense was low (9.1 %).

Combining ability and specific combining ability effects

The GCA effects of the lines for chocolate spot severity, rAUDPC and grain yield are presented in Table 6. The GCA effects for chocolate spot severity were highly significant (P ≤ 0.001) across the three environments. Based on the disease scale used, negative GCA effects were desirable for chocolate spot resistance. Negative and significant (P ≤ 0.001) GCA effects for disease resistance were recorded for parents ILB-4726, ILB-938 and BPL-710 in GDS and rAUDPC. On the other hand, the lines Kasa, Bulga-70, NC58, CS-20-DK, and Moti displayed significant (P ≤ 0.001) and positive GCA effects for chocolate spot across locations. For grain yield, the desirable positive GCA effects were significant (P ≤ 0.001) for the parental line ILB-4726, while the line Gebelcho exhibited significant (P ≤ 0.05) negative GCA effects for grain yield potential.

The SCA effects of crosses for the chocolate spot for GDS and rAUDPC are presented in Tables 7. Significant (P ≤ 0.05) and positive SCA effects for chocolate spot were observed for the crosses Kasa × CS-20-DK, CS-20-DK × Bulga-70, ILB-938 × Bulga 70 and Kasa × BPL 710 (Table 7).

Table 3 Mean squares and significant tests of faba bean genotypes from 10 × 10 full diallel cross for chocolate spot disease severity and grain yield tested across three locations in Ethiopia

Source of variation	MS	DF	Grain yield	General disease severity (GDS)	RAUDPC
ENV	257.74	2	257.74***	7350.05***	2453637.3***
REP (ENV)	11.99	3	11.99***	630.41***	536797.3***
ENTRY	12.88	99	12.88***	12186.59***	61210.3***
ENV * ENTRY	1.92	198	1.92***	2965.39*	15102.3*
GCA	8.28	9	8.28***	1149.67***	369381.7***
SCA	25.29	45	25.29***	23.54***	40287.9***
REC	1.38	45	1.38	17.34*	20498.5**
MAT	0.8074	9	0.81	11.90	27229.6*
NMAT	1.5257	36	1.53	18.70*	18815.7*
GCA * ENV	1.4094	18	1.41	40.35***	49213.2***
SCA * ENV	2.705	90	2.705***	8.52	9001.0
REC * ENV	1.2395	90	1.24	16.36*	14381.4
MAT * ENV	1.2127	18	1.21	25.32**	10514.0
NMAT * ENV	1.2462	72	1.25	14.12	15348.22
Error			1.18	11.24	12244.04
Trial Statistics					
CV (%)			31.45	21.75	21.70
R^2			86.25	87.39	81.10
Mean			3.46	15.41	508.83
% GCA (SS)GCA * ENV			6.67	24.49	29.62
% SCA (SS)SCA * ENV			64.00	25.85	27.09
% RCA (SS)REC * ENV			29.33	49.66	43.28
% GCA (SS) contribution			5.84	84.90	54.86
% SCA (SS) contribution			89.28	8.69	29.92
% REC (SS) contribution			4.88	6.40	15.22

ENV environment, *REP* replication, *GCA* general combining ability, *SCA* specific combining ability, *REC* reciprocal effect, *MAT* maternal effect, *NMAT* non maternal effect, *CV* coefficients of variation, *SS* Sum of square, *rAUDPC* relative area under disease progress curve value

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

Significant (P ≤ 0.001) SCA effects were observed for grain yield (Table 7). Fifteen hybrids had significant and positive SCA effects for grain yield.

Association between grain yield and disease resistance

A simple regression analysis of grain yield on chocolate spot general disease severity showed significant (P ≤ 0.001) relationships across the three environments. The equation of grain yield (t ha^{-1}) on chocolate spot severity had a negative slope with the following linear regression model: Yield = 5.12 − 0.11x, where x is the faba bean disease severity. There was a significant (P ≤ 0.001) and negative correlation (r = −0.32) between grain yield and chocolate spot general disease severity.

Discussion

Performance of parents and F$_1$ progenies of faba bean for chocolate spot severity

The parental lines ILB-4726, ILB 938 and BPL 710 showed a consistently resistant reaction for the

Table 4 The top 25 resistant to moderately resistant 10 susceptible F_1 genotypes 10 and parental lines to chocolate spot disease and grain yield across three environments

Genotype	GDS (%)	Disease reaction	rAUDPC (AUDPC)	Grain yield (t ha^{-1})	Rank based on GDS
Resistant crosses					
ILB-938 × BPL-710 (R × R)	2.184	R	0.28 (491.7)	1.11	1
BPL-710 × ILB-4726 (R × R)	2.782	R	0.20 (346.4)	1.31	2
BPL-710 × ILB-938 (R × R)	2.934	R	0.25 (443.0)	1.35	3
ILB-938 × ILB-4726 (R × R)	3.035	R	0.21 (363.6)	1.92	4
ILB-4726 × ILB-938 (R × R)	4.129	R	0.24 (421.5)	1.62	7
ILB-4726 × Gebelcho (R × MR)	9.429	MR	0.22 (389.3)	1.81	9
ILB-938 × Gebelcho (R × MR)	10.140	MR	0.28 (491.7)	2.09	10
ILB-4726 × CS-20-DK (R × S)	10.189	MR	0.19 (338.3)	5.66	11
ILB-4726 × Bulga-70 (R × S)	10.339	MR	0.20 (351.8)	6.90	12
ILB-938 × Dosha (R × MR)	10.861	MR	0.30 (520.2)	3.27	13
Dosha × ILB-4726 (MR × R)	10.888	MR	0.23 (407.4)	4.35	14
BPL-710 × Gebelcho(R × MR)	11.004	MR	0.26 (452.5)	1.62	15
BPL-710 × CS-20-DK (R × S)	11.087	MR	0.24 (417.1)	5.46	16
ILB-4726 × Dosha (R × MR)	11.417	MR	0.27 (470.2)	3.66	17
Dosha × BPL-710(MR × R)	11.475	MR	0.28 (481.7)	3.34	18
Gebelcho × ILB-938(MR × R)	11.517	MR	0.24 (416.9)	3.05	29
Bulga-70 × ILB-4726 (S × R)	11.626	MR	0.26 (459.9)	5.63	20
Dosha × Gebelcho (MR × MR)	11.747	MR	0.23 (407.4)	4.38	21
CS-20-DK × Gebelcho (S × MR)	11.984	MR	0.26 (448.7)	4.21	22
NC58 × BPL-710 (S × R)	11.987	MR	0.23 (401.9)	6.22	23
Moti × ILB-4726 (MR × R)	12.205	MR	0.26 (461.6)	4.58	24
NC58 × ILB-4726 (S × R)	12.280	MR	0.25 (434.8)	5.95	25
CS-20-DK × ILB-938(S × R)	12.308	MR	0.30 (528.8)	6.11	26
ILB-4726 × Moti (R × MR)	12.420	MR	0.27 (466.9)	5.91	27
Bulga-70 × BPL-710(S × R)	12.451	MR	0.26 (458.9)	5.04	28
Susceptible crosses					
Moti × Kasa (MR × S)	21.170	MS	0.33 (583.0)	1.51	87
CS-20-DK × NC58 (S × S)	21.182	MS	0.35 (606.6)	2.51	88
Kasa × Dosha (S × MR)	21.448	MS	0.36 (621.6)	3.33	89
NC58 × Moti (S × MR)	21.534	MS	0.33 (575.8)	2.70	90
Moti × Bulga-70 (MR × S)	22.749	MS	0.36 (627.7)	1.95	91
Kasa × Bulga-70 (S × S)	22.856	MS	0.36 (632.9)	1.54	92
CS-20-DK × Kasa (S × S)	23.156	S	0.39 (689.7)	1.59	93
NC58 × Kasa (S × S)	24.123	S	0.40 (693.2)	2.35	95
Kasa × CS-20-DK (S × S)	24.673	S	0.39 (680.9)	1.73	98
Kasa × NC58 (S × S)	25.650	S	0.39 (682.4)	2.03	99
Parent lines					
ILB-4726	3.817	R	0.07 (122.7)	2.03	5
ILB-938	4.012	R	0.08 (142.1)	2.29	6
BPL-710	5.427	R	0.12 (211.1)	2.35	8

Table 4 continued

Genotype	GDS (%)	Disease reaction	rAUDPC (AUDPC)	Grain yield (t ha^{-1})	Rank based on GDS
Gebelcho	14.848	MR	0.30 (516.6)	3.99	47
Moti	16.493	MR	0.36 (629.5)	4.69	57
Dosha	17.707	MR	0.33 (584.3)	4.09	66
CS-20-DK	23.468	S	0.39 (680.9)	2.68	94
NC58	24.554	S	0.38 (662.5)	2.59	96
Bulga-70	24.557	S	0.37 (649.8)	3.42	97
Kasa	32.251	S	0.40 (698.9)	2.66	100
Trial mean	15.402			3.43	

R resistant (<9.5 % disease severity), *MR* moderately resistant (10–20 % disease severity score), *MS* moderately susceptible (20–23 %), *S* susceptible (>23 % disease severity score), *rAUDPC* relative area under disease progress curve value, number in bracket AUDPC values, *GDS* general disease severity score

Table 5 Estimates of genetic parameters for resistance to chocolate spot over three environments with general disease severity, rAUDPC value and yield

Genetic parameters	GDS	rAUDPC	GY
$\hat{\sigma}^2 GCA$	70.38	20568.36	1.06
$\hat{\sigma}^2 SCA$	6.15	14021.95	12.06
$\hat{\sigma}^2 REC$	3.05	4127.21	0.10
$\hat{\sigma}^2 error$	5.62	6122.02	0.59
$\hat{\sigma}^2 phenotypic$	85.21	44839.54	11.69
$\frac{2\hat{\sigma}^2 GCA}{2\hat{\sigma}^2 GCA + \hat{\sigma}^2 SCA}$	0.99	0.75	0.21
$h_b^2 (\%)$	93.41	86.35	94.95
$h_n^2 (\%)$	82.60	45.87	9.10
CV g	57.89	38.67	96.20
CV e	15.38	15.38	22.20
CV g/CV e	3.76	2.51	4.34

GDS general disease severity, *rAUDPC* relative area under disease progress curve value, *GY* grain yield, $\hat{\sigma}^2 GCA$ variance of general combining ability, $\hat{\sigma}^2 SCA$ variance of specific combining ability; $\hat{\sigma}^2 REC$ reciprocal variance; $h_b^2 (\%)$ percentage of broad sense heritability, $h_n^2 (\%)$ percentage of narrow sense heritability, *CVg* coefficient of genetic variation, *CVe* error coefficient of variance

Table 6 General combining ability (GCA) effects for chocolate spot general disease severity score (GDS), relative area under disease progress curve value (rAUDPC) and grain yield of 10 faba bean lines across three sites

Parent	GDS[a]	rAUDPC	GY (t ha^{-1})
Moti	1.89***	45.08***	0.21
ILB-938	−3.65***	−49.54***	0.16
NC58	2.74***	28.69*	−0.09
Dosha	0.42	19.38	−0.20
ILB-4726	−5.06***	−117.07***	0.59***
CS-20-DK	1.65***	30.33*	0.01
Kasa	3.50***	49.11***	−0.25
BPL-710	−3.47***	−44.94***	−0.01
Gebelcho	−0.88*	−13.69	−0.26*
Bulga-70	2.85***	52.67***	−0.17

GDS general disease severity, *rAUDPC* area under disease progress curve value, *GY* grain yield (t ha^{-1})

[a] Negative GCA effects were desirable for Chocolate spot resistance

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

chocolate spot disease across the three environments. These genotypes were reported as resistant in a study to identify resistance (Villegas-Ferna'ndez et al. 2009). This suggested that the four lines; ILB-4726, ILB 938, BPL 710 and Gebelcho would be best sources for chocolate spot resistance. There was no cross between two susceptible lines that resulted in a progeny with better resistant reaction indicating the absence of transgressive segregants. In general, resistance of the crosses was greater when both parents were resistant which is consistent with additive gene effects.

Gene action for faba bean chocolate spot severity and grain yield

Highly significant GCA and SCA effects implied that both additive and non-additive gene effects were important in determining resistance to chocolate spot

Table 7 Specific combining ability (SCA) effects of the 90 F_1 genotypes (direct and reciprocal crosses) for chocolate spot disease severity, relative area under disease progress curve value and for grain yield (t ha^{-1})

Cross	Severity		rAUDPC		GY	
	Direct	Reciprocal	Direct	Reciprocal	Direct	Reciprocal
Moti × ILB-938	1.54	−0.60	49.7	−33.93	1.36***	−0.07
Moti × NC58	−0.62	−2.11	−75.01	−68.21	0.09	0.35
Moti × Dosha	0.72	0.38	22.32	14.60	−0.59	−0.23
Moti × ILB-4726	0.07	−0.11	27.4	−2.61	0.99*	−0.67
Moti × CS-20-DK	−1.29	0.54	−38.04	−24.38	−0.63	0.73
Moti × Kasa	−0.53	0.89	−8.88	−11.14	−0.51	0.16
Moti × BPL-710	0.84	0.83	60.86	16.83	−0.13	0.05
Moti × Gebelcho	−0.66	−0.45	−61.06	−8.61	−0.57	0.54
Moti × Bulga-70	1.35	1.95	−38.29	28.93	−1.62*	0.01
ILB- 939 × NC58	−1.01	0.31	−10.43	−38.14	1.33***	0.02
ILB- 938 × Dosha	0.22	−1.54	58.06	−16.53	−0.07	−0.08
ILB- 938 × ILB-4726	1.78	−0.46	50.32	−28.94	−2.44***	0.15
ILB- 938 × CS-20-DK	−0.35	0.76	23.95	−15.24	2.19***	−0.29
ILB- 938 × Kasa	−0.77	−0.18	−0.27	−29.63	0.87*	−0.13
ILB- 938 × BPL-710	1.45	0.40	52.98	24.35	−2.39***	−0.12
ILB- 938 × Gebelcho	−0.75	−1.40	−44.26	−15.54	−0.78*	−0.48
ILB- 938 × Bulga-70	6.40*	0.26	355.3***	5.66	2.91***	0.04
NC58 × Dosha	−0.43	0.77	−19.90	50.99	−1.03*	−0.08
NC58 × ILB-4726	−0.64	−0.17	−19.27	33.65	2.09***	−0.10
NC58 × CS-20-Dk	0.62	−0.76	−3.88	−42.64	−1.90***	−0.02
NC58 × Kasa	1.23	1.24	16.18	20.38	−1.47***	−0.07
NC58 × BPL-710	−0.80	−1.90	−26.65	−64.03	−2.38***	0.47
NC58 × Gebelcho	0.01	0.88	40.72	−2.74	0.80*	0.83
NC58 × Bulga-70	−3.66	−0.09	−94.44	−16.14	−0.91	−0.09
Dosha × IlB-4726	0.39	−0.26	27.68	−31.40	0.16	0.34
Dosha × CS-20-DK	0.46	0.78	−16.96	−7.49	−0.08	−0.72
Dosha × Kasa	−0.04	−2.16	−30.11	−74.33	−0.05	0.45
Dosha × BPL-710	−0.35	−0.53	−4.67	3.12	0.13	−0.05
Dosha × Gebelcho	−2.46	−0.74	−75.49	−31.60	1.02*	0.37
Dosha × Bulga-70	−1.43	0.96	−34.36	−3.29	−1.56*	0.19
ILB-4726 × CS-20-DK	−0.06	−1.75	−25.09	−58.65	1.67***	−0.08
ILB-4726 × Kasa	−1.11	−0.07	−11.75	−27.08	2.05***	0.30
ILB-4726 × BPL-710	1.38	1.19	21.80	22.27	−2.69***	0.05
ILB-4726 × Gebelcho	1.68	−1.71	79.51	−68.29	−1.61***	−0.36
ILB-4726 × Bulga-70	−0.95	−0.64	73.42	−54.04	4.99***	0.64
CS-20-DK × Kasa	−0.65	3.24*	−2.99	104.40*	−1.28**	−0.40
CS-20-DK × BPL-710	−0.57	1.94	9.17	86.26	1.91***	−0.07
CS-20-DK × Gebelcho	−2.36	−1.83	−32.34	−44.39	0.65	0.34
CS-20-DK × Bulga-70	5.31*	1.11	−136.68	22.77	−0.93	0.06
Kasa × BPL-710	1.56	0.71	102.40*	−12.51	1.29***	−0.47
Kasa × Gebelcho	1.12	−0.26	11.16	11.06	0.65	0.11
Kasa × Bulga-70	−0.49	1.74	−19.48	69.99	−0.97	0.17
BPL-710 × Gebelcho	0.80	−0.85	18.36	−16.03	−1.23**	−0.34

Table 7 continued

Cross	Severity		rAUDPC		GY	
	Direct	Reciprocal	Direct	Reciprocal	Direct	Reciprocal
BPL-710 × Bulga-70	1.77	1.07	181.48	31.20	2.95***	0.09
Gebelcho × Bulga-70	0.22	−1.57	−6.99	−83.55	−1.03	0.27

rAUDPC area under disease progress curve value, *GY* grain yield (t ha^{-1})

*, **, *** Significant at 0.05, 0.01, and 0.001 probability levels, respectively

disease of faba bean. However, the contribution of GCA to the crosses sums of squares was 84.9 % for chocolate spot disease. This suggested predominance of additive effects. The higher values of GCA variance ($\hat{\sigma}^2$GCA) compared with the SCA ($\hat{\sigma}^2$SCA) and REC ($\hat{\sigma}^2$REC) variance indicating the greater relative importance of genes with additive effects. Further, general predicted ratio Baker's (1978) were close to unity signifying a predominant role of additive gene action. This indicated that additive gene action played significant role in the inheritance of chocolate spot disease than non-additive gene action. Previous studies reported that resistance was quantitative (Hartwell et al. 2008), which is consistent with observation of large additive effects in the current study. Reciprocal effects (REC) were also observed yet the REC contributed only <15 % of the sums squares for crosses for chocolate spot disease. Therefore, the low magnitude of reciprocal effect indicates that maternal effects or cytoplasmic inheritance were less influential (Kearsey and Pooni 1996) for chocolate spot resistance. The estimates of broad sense heritability for chocolate spot resistance was high suggesting reasonable progress can be achieved in selection for disease resistance to faba bean.

There were highly significant differences among the parents and the F_1 genotypes for grain yield. The importance of both GCA and SCA effects were evident with predominance of the latter, which is sufficiently reflected from higher magnitude (89.3 %) of its contribution to the crosses sums of squares for grain yield. This implies the importance of non-additive gene action for grain yield. Further, the general predicted ratio of 0.096 showed predominance of non-additive gene action in the inheritance of grain yield. Moreover, the predominance of SCA variance ($\hat{\sigma}^2$SCA) indicated the importance of non-additive gene action. This suggests that breeding gain can be

realized through hybridization followed by selection at advanced generations. This could provide substantial genetic gain in grain yield of faba bean. Since GCA effects are the manifestation of additive genetic effects, parents selected based on GCA effects will be useful for developing breeding lines with high grain yield. The parental line ILB-4726 with significant and positive GCA effects could be used in the breeding programmes which emphasises chocolate spot resistance. The large heritability estimates obtained in the current study are in agreement with broad sense heritability from 90 to 99 % reported by El-Badawy et al. (2012). Toker (2004) reported large broad sense heritability of 97 % for grain yield, which is comparable to the 94.95 % in the current study. However, the estimate of narrow sense heritability for grain yield was low (9.1 %) suggesting the significant role of environmental effects and large SCA effects in modifying this trait. The SCA × Env effects were highly significant with high values of variance components across the environments indicating the genotypes responded differently in different environments. Consequently, multi-location evaluation of genotypes for yield could be essential.

General and specific combining ability effects for chocolate spot severity and grain yield

Significant estimates of GCA effects for chocolate spot disease were observed in this study. The parental lines ILB-4726, ILB-938 and BPL-710 had negative estimates for chocolate spot resistance. Therefore, these lines were decreasing the overall chocolate spot severity. Based on their combining ability effects these lines may be used in intra-population breeding with focus on chocolate spot resistance. Parental line Gebelcho also exhibited a negative and significant estimate for chocolate spot severity and this could be

due to its pedigree from ILB-4726. In general, based on GCA effects, parental line ILB-4726 was found to be a good combiner for improving chocolate spot resistance and grain yield. Non-significant negative estimates of SCA effect were observed for the disease. However, significant SCA effects would not be appreciated since in the short-term the faba bean breeding programme does not aim at producing F_1 progenies (hybrids).

Regression analysis and correlations
among genotypic means and genetic effects

The linear regression model of grain yield on chocolate spot severity across the three locations showed a negative slope and indicated that grain yield of faba bean decreased by 0.11 t ha^{-1} per unit increase of chocolate spot severity. However, the regression model accounted for less than 20 % of the total variation, implying that chocolate spot disease was not the only factor affecting the grain yield. In general, there was negative correlation between the mean of chocolate spot severity and grain yield and GCA effect of the disease and GCA effect of grain yield.

Conclusion

The parental lines ILB-4726, ILB-938, BPL-710 and Gebelcho had negative GCA effects indicating good combining ability for chocolate spot resistance. These lines would be exploited as sources of resistance to chocolate spot disease in faba bean breeding. Genes with additive effects were predominant over the non–additive gene effects for chocolate spot resistance. The predominance of additive effect indicates that resistance to faba bean chocolate spot disease can be improved through selection. On the contrary highly significant non-additive gene effects were predominant over additive gene effects for grain yield, indicating that development of hybrids and selection would enhance productivity. The reciprocal effects were negligible (<20 %), indicating that cytoplasmic gene effects did not play a major role in modifying the resistance of the diallel cross to chocolate spot disease. The materials produced from this diallel cross will be further used as source of the genetic base for the development of resistant varieties through selection in the breeding program.

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