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RESEARCH ARTICLE

Evaluation of disease resistant and high yielding faba bean germplasm in India

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Abstract. Faba bean (*Vicia faba* L.) is one of the earliest domesticated food legumes after chickpea and pea in the world. It is been produced in many countries including China, Ethiopia, Egypt, northern Europe, the Mediterranean region, central Asia, East Asia, Latin America and as a minor crop in India. The crop is affected by many diseases and alternaria leaf blight (*Alternaria* spp.) is one of the serious threat to faba bean production. Twenty-five lines of faba bean were selected from three international nurseries and were evaluated at ICARDA-FLRP-Amlaha during 2016–2017 and 2017–2018, to identify resistant lines against alternaria blight disease. A wide range of variation to disease reaction was observed among faba bean genotypes. One faba bean line (S2011-134) found tolerant, six genotypes (S2011-116, FLIP15-139, FLIP15-156, FLIP15-159, FLIP15-164-S2 and FLIP15-169) were found moderately tolerant and 16 genotypes were found susceptible to alternaria blight. The faba bean genotypes showed resistance to the disease scoring (0–9) with high yield as compared to the checks, Giza and Gwalior local. The identified sources of resistance can be utilized in faba bean breeding programmes for the development of disease tolerant cultivars with high yield.

Keywords. faba bean; alternaria leaf blight; phenotyping; genetic variability.

Introduction

Faba bean (*Vicia faba* L.) is one of the earliest domesticated food legumes in the world. Globally, the total production of faba bean is 4.9 Mt on a harvested area of 2.6 Mha (FAOSTAT 2018 retrieved from http://www.fao.org/faostat/en/#data/QC). Due to its high nutritional value (protein content of 24–30%), and high energy, this crop is grown for human consumption and animal feed in many parts of the world (Maalouf *et al.* 2019). Faba bean is consumed as a green vegetable (whole pods) and sometimes as split seeds. Faba bean is grown in rotaion with cereal crops and improve soil fertility through nitrogen fixation. In India, the crop is minor and mainly grown as a garden crop in Bihar, Uttar Pradesh, Rajasthan, Madhya Pradesh, West Bengal, Assam, Manipur and Nagaland (Singh *et al.* 2012a, b). It is mainly a rabi pulse in plains of India, although it is successfully grown during kharif (rainy season) in hilly and mountainous regions (Singh and Bhatt 2012).

The major diseases of faba bean are chocolate spot (*Botrytis fabae* and *B. cinerea*), rust (*Uromyces fabae*), ascochyta blight (*Ascochyta fabae*), alternaria leaf blight (*Alternaria* spp.), cercospora leaf spot (*Cercospora zonata*), downy mildew (*Peronospora viciae*) (Mahmoud 1996; Stoddard *et al.* 2010; Singh *et al.* 2012b). Alternaria blight is caused by two species, namely *A. alternata* and *A. tenuissima*, and cause significant yield losses when there is high humidity and temperature in some countries (Honda *et al.* 2001; Yang *et al.* 2018).

The occurrences of alternaria blight were first reported in India on faba bean by Gurha *et al.* (1981) and later in Himachal Pradesh, India, by Gupta *et al.* (1992). This disease generally occurs late in the growing season as the plants near to maturity. The main disease symptoms appear as dark brown spots which have a zoned brown ring with dark

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Table 1. List of faba bean genotypes evaluated for agronomic, disease resistance and yield at ICARDA, Amlaha, India.

Entry	Pedigree
FLIP14-021	5/08/F8/7054/06-HBP/S0D/2000
FLIP14-035	94/08/F8/7712/06-S 97112 (ILB 4365 X BPL2282)
FLIP15-139	BPL 5061-33005/TR2010
FLIP15-176	Sel TerWI11-161-2/2012
FLIP15-156	S 88134-3-1-1-32146/TR2010
FLIP15-158	S 88094-8-1-32233-1/TR2010
FLIP15-159	S 88092-4-3-32301-1/TR2010
FLIP15-164-S1	Giza 4-32087-2/TR2010
FLIP15-164-S2	Giza 4-32087-2/TR2010
FLIP15-169	S 88134-3-1-1-32221-2/TR2010
FLIP15-171	S 88094-6-4-32111/TR2010
FLIP15-172	S 88100-11-1-1-32307-1/TR2010
S2011,040	Luz de OtonoxSel. 2008 Latt. 212-3
S2011,073	Sel. F7/8985/05xSel. 2010 Latt. 1120-1
S2011,088	Sel.2010-3709-1xSel. 2010 Latt. 1120-2
S2011,104	Luz de OtonoxWRB 1-2
S2011,116	T.WxSel. 2010 TER.192-2 Heat T.
S2011,134	India-2xSel. 2008 Latt. 778-4
Syn8	S2011-111xS2011-112xSel2011-107xSel.Br./20640-1/2010xNubaria 2xWRB 767-3-1-2-08xSel. F7/8975/05x Sel.2010-Cold/265-4
Syn11	S2011-111xS2011-112xSel2011-107xSel.Br./20640-1/2010xNubaria 2xWRB 767-3-1-2-08xSel. F7/8975/05xSel 2010-Cold/265-4xAtunaxMisr2xSuperaquadolce
FLIP15-163FB	S 88094-8-2-32334/TR2010
FLIP15-167FB	Selection from ILB1270
Syn-4-FB	S2011-111xS2011-112xSel2011-107xSel.Br./20640-1/2010
Giza	ICARDA Check
Gwalior Local Check	Local Check

margins, the fungus probably survives on other hosts and crop residues (Zahran 1982). Initially, lesions appeared as brown, water soaked, circular to irregular shape on the stem, pod and other parts of the plant. Mostly the older leaves were attacked first and then the disease progresses upward to the younger leaves. In the later stage, the affected leaves become blighted from the margin to the centre and most of them defoliated completely (Rahman *et al.* 2001; Singh *et al.* 2012b). The conidia are detached easily and are carried by air currents. The germinating spores penetrate the susceptible plant tissue directly or through wounds and soon produce new conidia that are further spread by wind, splashing rain, etc. (Singh *et al.* 2012b). The use of chemicals against the disease has good results but often leads to resistance against the disease (Sillero *et al.* 2010).

Research on faba bean is being carried out by a few institutions in India. With its global mandate, ICARDA is engaged in faba bean improvement research at the Food Legume Research Platform (FLRP), Amlaha. Production of the crop is, however, constrained by various biotic factors and alternaria blight (*Alternaria alternata* (Fr.) *Keissler*), is one of the serious threats to faba bean production in many countries including India. Therefore, an experiment was conducted to identify faba bean germplasm resistance to alternaria leaf blight with quantitative traits.

Material and methods

Isolation of pathogen

Pathogen isolation was done from randomly collected diseased leaves of faba bean plants. The diseased leaves were washed thoroughly with tap water, small pieces of infected leaves were cut with sterilized blade. These pieces were surface sterilized with 1:1000 mercuric chlorides ($HgCl_2$) solution for 1 min followed by three changes in sterilized water to remove the trace of $HgCl_2$. The pieces were then transferred aseptically to Petri plates containing potato dextrose agar (PDA). The inoculated Petri plates were incubated at 25±1°C for 7–8 days and examined at frequent intervals to check the growth of the fungal culture. The identification was based on morphology under the microscope (Leica DM LB).

Screening of faba bean genotypes

Twenty-five faba bean genotypes were selected from three ICARDA international faba bean nurseries and planted during the 2016–2017 and 2017–2018 crop seasons in simple lattice (5 x 5) design with two replications (table 1). Each genotype was grown in two rows of 4 m length with 50 cm spacing between rows. Disease development was based on natural infections. Plants were irrigated for two to three times as needed to ensure optimum plant growth and development. The experiment was monitored regularly to observe the onset of the disease. The disease was scored using 0–9 rating for disease severity (Mayee and Datar 1986).

Statistical analysis

The means of disease scoring and all the morphological characters were subjected to statistical analysis using CROPSTAT (v. 8.5) statistical package. Pearson's correlation matrix among the traits was generated by employing GenSTAT v. 16.1. The factorial and clusters analysis based on disease and morphological traits was done by using DARwin5 software 5.0.158.

Results

Pathogen identification

The characteristic symptoms of alternaria leaf blight were observed on diseased leaves as leaf spot symptoms. Initially, lesions were brown, water-soaked, circular to irregular in shape, also appeared on stems, pods and other parts of the plant. In the later stage of the disease, the leaves became blighted from the margin to the centre, the circular to irregular spots enlarge and coalesce, the whole plant turned black and most of the diseased plants defoliated completely (figure 1a). The mycelial growth of fungus on PDA was black coloured and effuses. Initially, the mycelium was hyaline that turned to brownish to black, multicelled, septate and irregularly branched (figure 1b). Conidiophores arised singly or in clusters and were erect, long or short that bear single or branched chains of conidia which were dark, long or pear-shaped and multicellular, with both transverse and longitudinal cross walls (figure 1c).

Table 2. Range, mean, variation, standard deviation and coefficient of variation for all agronomic traits and disease scoring.

	DTF (days)	DTM (days)	PH (cm)	YLD (g)	Alternaria blight (0–9 rating)
Range	47–58	116–122	63–100	143–481	2–6
Mean	51	119	84	332	5
Variation	9.37	2.48	65.92	8553.06	1.15
Standard deviation	3.06	1.58	8.12	92.48	1.07
Coefficient of variation (%)	6.01	1.33	9.64	27.83	22.70

DTF, days to flowering; DTM, days to maturity; PH, plant height; YLD, plot yield.

Figure 1. (a) Disease affected plants in the field, (b) *Alternaria* spp. culture on PDA; and (c) conidia of *Alternaria* spp.

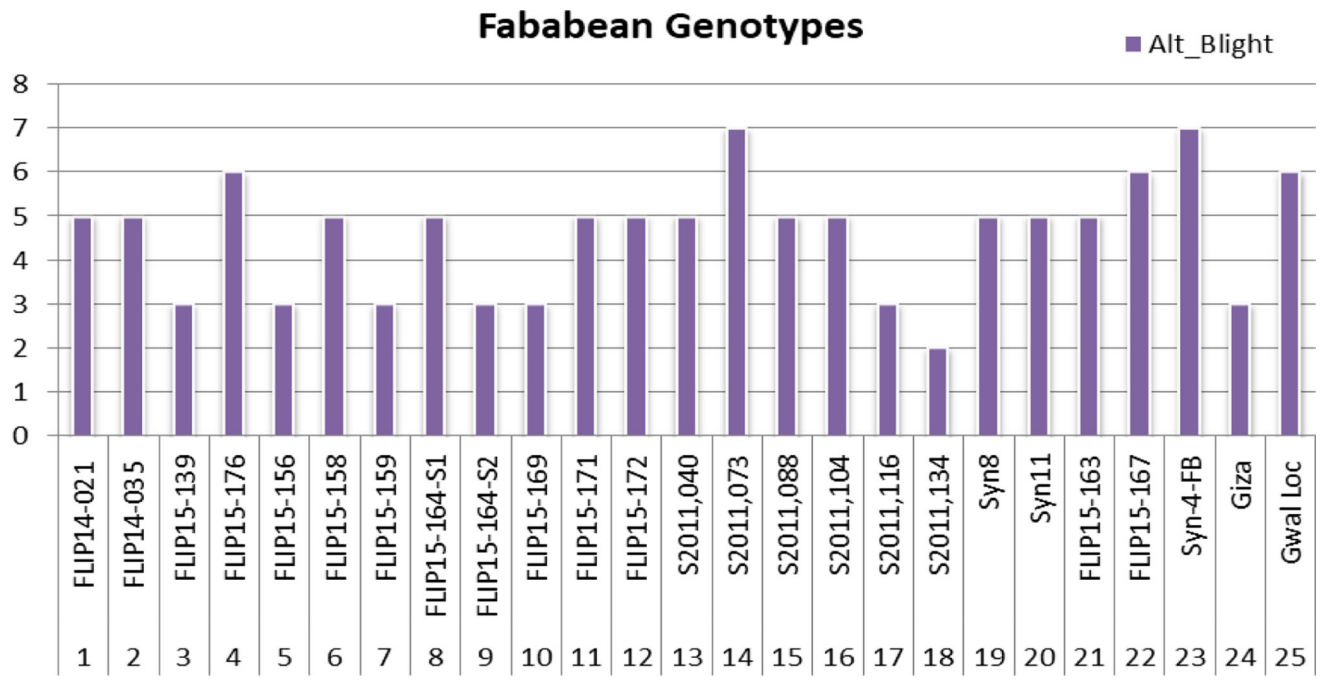

Figure 2. Graphical representation of alternaria blight disease scoring of faba bean genotypes.

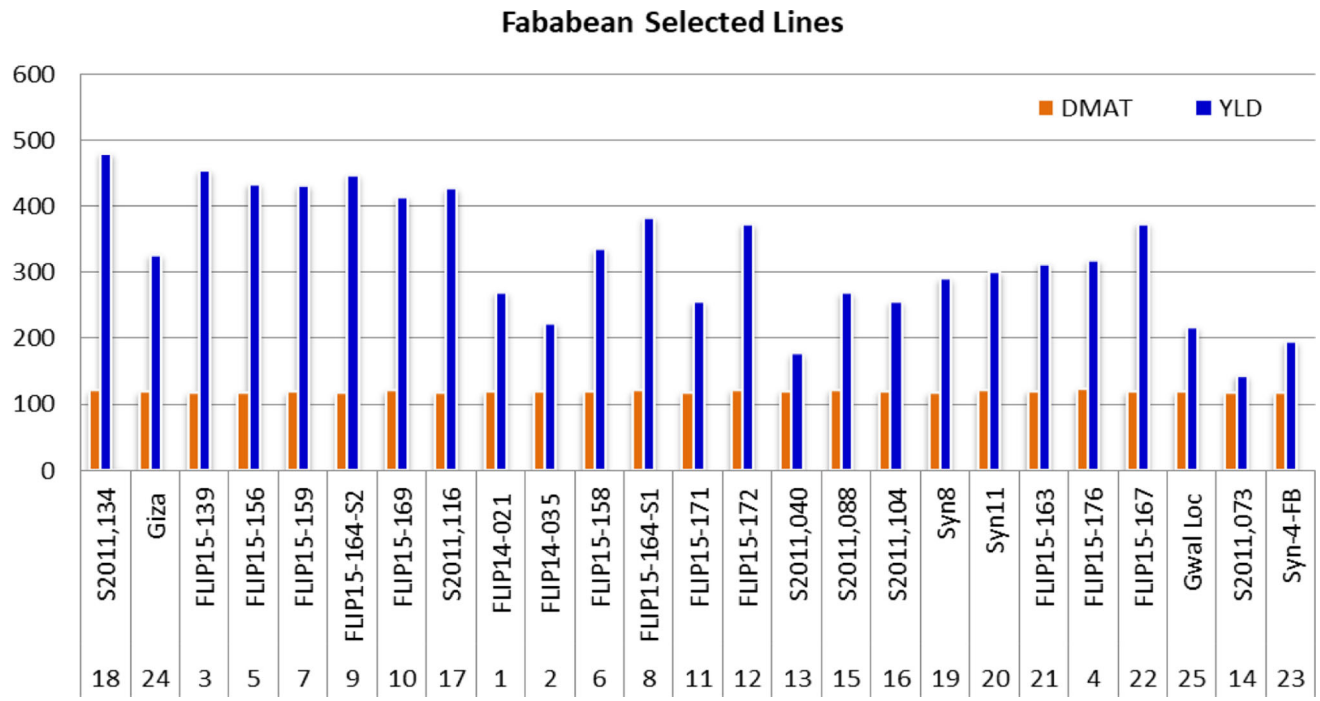

Figure 3. Graphical representation of days to maturity with comparison to yield.

Agronomic traits and yield

Range, mean, variation, standard deviation and coefficient of variation were highly significant for all agronomic traits among genotypes (table 2). Alternaria blight scoring ranged from 2–6 with the mean of 5. Standard deviation was 1.07 with the coefficient of variation 22.7% (figure 2). Similarly, days to flowering ranged from 47–58 days with a mean of 51 days, the standard deviation was 3.06 and the coefficient of variation was 6.01%. Plant height ranged from 63–100 cm with the mean of 84 cm, the standard deviation was 8.12 and the coefficient of variation was 9.6%. Days to maturity ranged between 116 and 122 days with a mean of 119 days, the standard deviation was 1.58 and the coefficient of

Table 3. Pearson's correlation matrix for all the morphological characters obtained.

	DTF	PH	DTM	YLD	AltB
DTF	1				
PH	–0.846**	1			
DTM	0.741**	–0.669**	1		
YLD	0.271	–0.326**	0.174	1	
AltB	–0.132	0.059	–0.28*	0.127	1

*And** is the significance at 5% and 1%, respectively.

variation was 1.33%. Yield varied from 143–481 g with a mean of 332 g. Standard deviation was 92.48 and the coefficient of variation was 27.83% observed for the genotypes (figure 3).

Pearson's correlation among the traits

Pearson's correlation matrix shows that plant height is negative and significantly correlated with days to flowering. Days to maturity is positive and significantly correlated to days to flowering but negative and significantly correlated to plant height.

Yield was negative and significantly correlated with plant height. Alternaria blight is negatively correlated to days to flowering and also negative but significantly correlated to days to maturity (table 3).

Dendrogram generated from an unweighted pair group method analysis (UPGMA) for all the characters

The means of all the quantitative characters were statistically analysed and Euclidean distances were calculated for all the morphological characters and the genotypes grouped as per their characters (figure 4). There are two distinct groups A and B which were formed. The two groups were separated mainly based on the disease tolerance and yield. Group A was divided into A-I and A-II; group A-I contains highly susceptible and low-yielding genotypes. Group A-II contains moderately susceptible genotypes. Further, group B was divided into B-I and B-II; group B-I contains tolerant

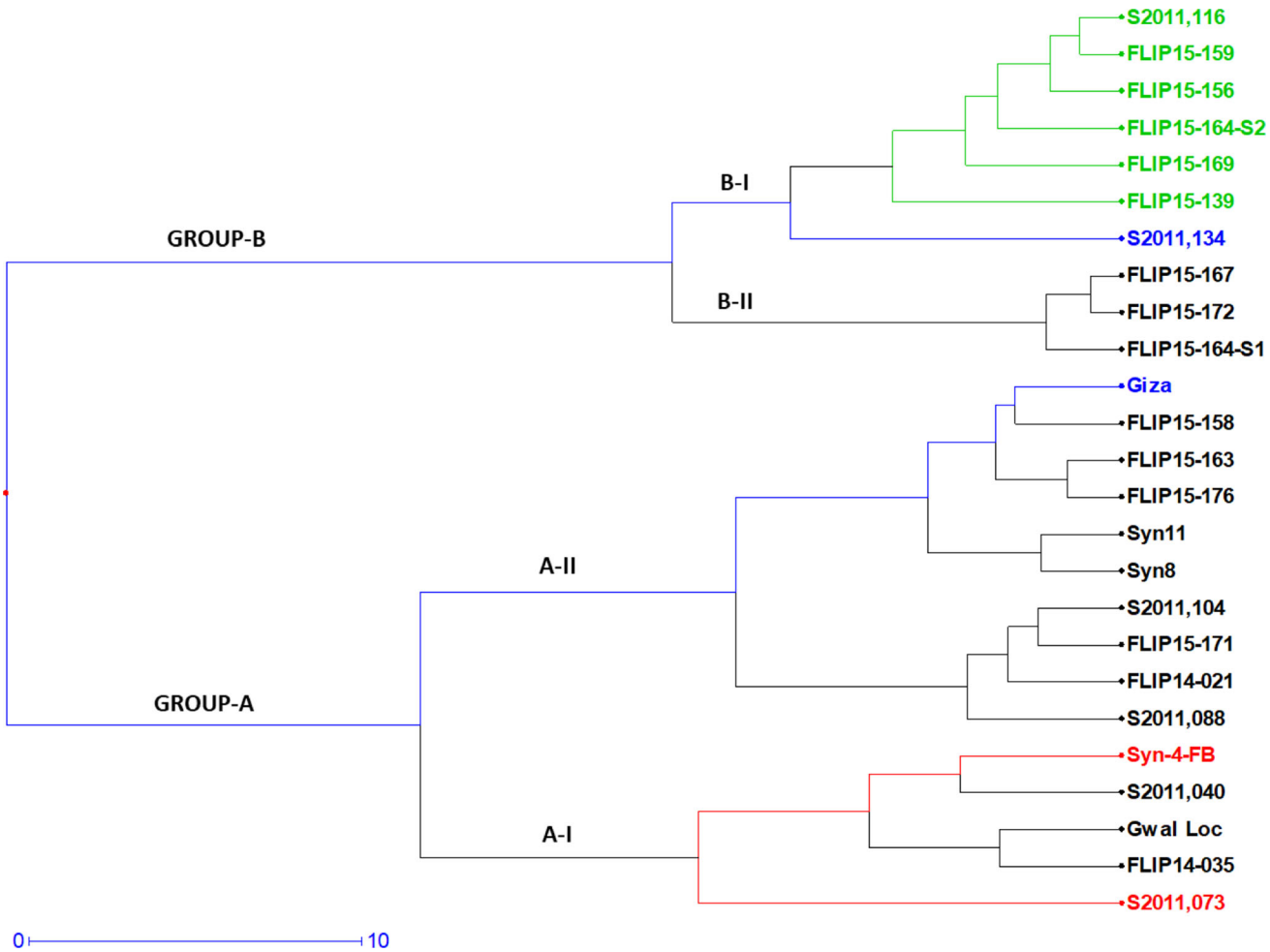

Figure 4. Dendrogram generated from an UPGMA cluster analysis based on all the traits.

Table 4. Clustering based on UPGMA for all the morphological characters.

Major cluster	Minor cluster	Number of genotypes	Name of genotypes
Group-A	A-I	05	Syn4-FB, S2011-040, Gwalior Local (LC), FLIP14-035 and FLIP15-173
	A-II	10	Giza (IC), FLIP15-158, FLIP15-163, FLIP15-176, Syn11, Syn8, S2011-104, FLIP15-171, FLIP14-021 and S2011-088
Group-B	B-I	07	S2011-116, FLIP15-159, FLIP15-156, FLIP15-164-S2, FLIP15-169, FLIP15-139 and S2011-134
	B-II	03	FLIP15-167, FLIP15-172 and FLIP15-164-S1

(S2011-134) to moderately tolerant (S2011-116, FLIP15-159, FLIP15-156, FLIP15-164-S2, FLIP15-169 and FLIP15-139) with high-yielding genotypes. Group B-II contains moderately susceptible but having good yielding genotypes (table 4).

Principal component analysis (PCoA)

A data matrix plot based on all the morphological characters was subjected to PCoA for estimating genetic differentiation among the 25 genotypes of faba bean. The scatter plot based on these components disclosed a pattern of mainly two groups which were distinctively separated based on disease and yield. The plot showed that the high-yielding genotypes with disease tolerance formed a separate group (figure 5).

Discussion

Faba bean is considered as an important source of dietary protein for human and animal nutrition. Besides its ability to grow on diverse agroclimatic conditions, it is still an underutilized crop in some part of the world including India. Fungal diseases are among the key biotic factors responsible for yield loss in faba beans. Crop losses due to diseases in species of the family Fabaceae can reach up to 15% or even 80% (Horoszkiewicz-Janka *et al.* 2013; Kaur *et al.* 2014). The yield and quality of faba bean seeds are affected by soil type, climatic conditions, and agronomic factors (Ksiezak *et al.* 2009; Kulig *et al.* 2011; Barlog *et al.* 2018). Alternaria leaf blight disease has been predominantly associated with faba bean as a consequence of global climate change, especially temperature in Egypt and in other countries (Reis *et al.* 2007; Juroszek and von Tiedemann 2011).

Lack of suitable varieties are one of the major bottlenecks to adopt this crop in India (Verma *et al.* 2015). Farmers are bound to cultivate low-yielding disease susceptible local landraces of faba bean. The potentiality of faba bean is around 6.0–7.0 t/ha whereas in India its average productivity is 1.5 t/ha (Singh *et al.* 2012c). Fungal pathogens cause major losses to economically important legume crops, including faba beans, by suppressing the growth and development of plants and, in extreme cases, by causing wilting and plant death. Some fungal pathogens can cause epidemic outbreaks, thus contributing to the closure of seed plantations (Horoszkiewicz-Janka *et al.* 2013; Deneke 2018).

The screened faba bean genotypes in the present study differ significantly from one another with respect to disease score and yield under field condition. A significant variation was observed for most of the investigated characters including disease tolerance. It is concluded from the results that the genotypes S2011-134, S2011-116, FLIP15-159, FLIP15-156, FLIP15-164-S2, FLIP15-169 and FLIP15-139 were not only tolerant to disease but also out-yielded local check. These findings are in agreement with the investigations of other studies based on high-yielding disease resistant varieties of faba bean (Dubey and Patel 2000; Singh *et al.* 2015).

Alternaria spp. are ubiquitous fungal pathogen reported globally on different hosts. In India, the pathogen is reported from different host like *Punica granatum*, *Stevia rebaudiana, Pistia stratiotes*, *Gloriosa superba*, *Aloe vera*,

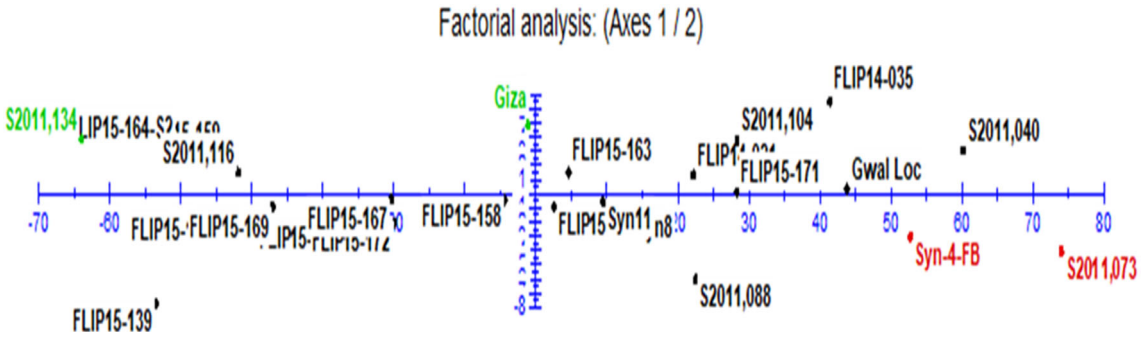

Figure 5. Representation of the 1–2 plane of factorial analysis based on all morphological traits.

Adhatada vasiac, *Calotropis gigantean*, *Basella alba*, *Rumex vesicarius*, *Morinada citrifolia* and *Withania coagulans* (Singh and Suhag 1983; Gautam 2013). This crop also appears to be an agronomically viable alternative to cereal grains (Klimek-Kopira *et al.* 2015). Plant genetic resources from Mediterranean regions are the reservoirs of natural genetic variations for many important agronomic traits which can be utilized for the crop improvement (Bains *et al.* 2012). These Mediterranean genotypes were evaluated to estimate for disease tolerance, early maturity, genetic variability as well as high yielding among the selected faba bean genotypes under natural field conditions.

Alternaria blight could be one of the major disease causing responsible for yield loss in faba bean crop in the future. Exploring the Mediterranean faba bean accessions would be very important to develop new varieties. These identified promising faba bean genotypes tolerant to the disease with good yield will be very important towards the development of alternaria leaf blight resistance faba bean varieties in India and can be utilized in faba bean breeding programmes for the development of disease resistant as well as high yielding cultivars for the commercial cultivation purpose also.

In conclusion, in India, faba bean is a minor legume crop and is grown mainly in eastern Indian states. The result of this study clearly demonstrated that faba bean genotypes from ICARDA may play an important role in adaptation and development of new varieties for the Indian subcontinent to well establish the underutilized crop due to biotic and abiotic factors. The present study provides an opportunity to select adapted and disease tolerant genotypes with good yield. These tolerant sources could save the extra cost of plant protection chemicals and help to ensure a disease free crop. Also, these selected genetic materials will be shared with national institutions to promote and for the utilization in breeding programmes.

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