New faba bean germplasm with multiple resistance to Ascochyta blight, Chocolate spot and rust diseases

Fouad Maalouf[1*], Seid Ahmed[2], Khalil Shaaban[4], Bayaa, Bassam[5], Fawzi Nawar[2], Murari Singh[3], Ahmed Amri[2]

[1]International Center for Agricultural research in Dry Areas (ICARDA). Terbol, Bekaa Valley Lebanon. *corresponding author email: f.maalouf@cgiar.org; phone number: 009618955127; FAX: 009618955728

[2]International Center for Agricultural research in Dry Areas (ICARDA). B.P. 6299, Rabat-Institutes, Rabat, Morocco

[3]International Center for Agricultural research in Dry Areas (ICARDA). P.O. Box 950764 Amman 11195, Jordan

[4]Agricultural Research Center (ARC) - Cairo – Egypt.

[5]Aleppo University, Aleppo, Syria

ABSTRACT

Faba bean is one of the oldest crops originated in Fertile Crescent and distributed around the world and produced under different agro climatic conditions. It mainly cultivated in high rainfall and irrigated areas favoring the development of the foliar diseases causing severe loses in the crop. The purpose of this study is to identify new sources of resistance for the three key foliar diseases. 2000 accessions, breeding lines and populations were screened for different diseases at Lattakia station, Syria from 2005 to 2012. The results showed that multiple disease resistance for two or three disease were identified. 30 lines combining chocolate spot, Ascochyta blight and rust resistance were selected and evaluated for three seasons from populations collected from China, Italy, Jordan, Lebanon and Spain. 10 improved lines derived from breeding program at ICARDA were developed for disease resistance. These improved sources for resistance will enhance the development of faba bean breeding lines combining the three foliar diseases with other economical traits.

Key words: Ascochyta blight, chocolate spot, faba bean, multiple disease resistance, Germplasm, rust.

Introduction

Faba bean (*Vicia faba* L.) is the third most important cool season food legume crop in many countries worldwide. It was domesticated at the beginning of agriculture in the Fertile Crescent of the Near East following the Neolithic era around 9000–10,000 BC. Faba bean center of diversity includes Iraq, Iran, Georgia, Armenia, Azerbaijan, Syria and Turkey (Maxted, 1995, Tanno & Willcox, 2006). Subsequently, its cultivation has spread around the world (Cole, 1970; Tanno & Willcox, 2006) through different routes from the Near East to Europe and to other parts of the world (Cubero, 1974). Secondary centers of diversity are postulated to be located in Afghanistan, China, and Ethiopia. The movement of faba bean towards South America, especially the Andean region, probably occurred in the fifteenth century through Spanish and Portuguese travelers. This resulted in the development of Peruvian and Bolivian landraces displaying a large variability in seed size, color and shape (Duc et al., 2010). Currently Faba bean is cultivated under rainfed and irrigated conditions in more than 55 countries covering are about 2.7 million ha with 4.2 million tons of dry grain and 1.7 million tons of green pods yields (FAOSTAT, 2015).

Faba bean yields remained low in many countries due to susceptibility of the crop to different biotic and abiotic stresses. The dominant foliar diseases affecting faba bean are chocolate spot (*Botrytis fabae* Sard), rust (*Uromyces viciae fabae*) and Ascochyta blight (*Ascochyta fabae* Speg.). Ascochyta blight can cause up to 90% yield losses on susceptible cultivars when environmental conditions are conductive to disease development (Hanounik, 1980). Chocolate spot is especially severe in humid areas, having been reported to be the cause of heavy reductions in yields in places such as the Maghreb countries, Southern China, Egypt, UK and France (Bouhassan et al., 2004; Tivoli et al., 2006). Faba bean rust can cause moderate to substantial yield losses (Rashid and Bernier, 1991; Jellis et al., 1998). Application of agronomical practices such as crop rotations, delayed date of planting, use of chemicals and use of certified seeds may provide partial protection agianst chocolate spot and ascochyta blight; however, effective disease control is possible with host resistance. The first effective resistant sources for ascochyta blight and chocolate spot were identified at the International Center for Agricultural Research in the Dry Areas (ICARDA) (Robertson 1984; Hanounik and Robertson 1988, 1989). ICARDA in close collaboration with National Agricultural Research Systems (NARS) continued in developing foliar disease resistance combined with high yield potential breeding lines and faba bean varieties with good levels of foliar disease resistance and high yield potential. As a result, varieties were released in Ethiopia, Australia, Canada, China Egypt and Spain (Hanounik and Robertson 1989; van Leur et al. 2000; Sillero et al, 2001; Sillero et al, 2010; Trivoli et al. 2006; Redden et al. 2008; Villegas-Fernández et al. 2009; Rubiales et al 2012; Villegas-Fernández et al. 2012; Maalouf et al., 2013; Temesgen et al., 2015). Although most breeding programs are focusing on developing resistant genotypes for a single disease of economic importance, faba bean lines with multiple disease resistance have been identified (Villegas-Fernández et al. 2011). This work presents results of identification of new sources of resistance to Ascochyta blight, chocolate spot and rust for three key foliar diseases of faba bean from ICARDA breeding program, genebank collections and from different donors. The field screening method was also evaluated.

Material and Methods

Screening faba bean accessions against chocolate spot, Ascochyta blight, and rust were conducted at Jableh (35.36°N, 35.93°E) site in Lattakia province, Syrian from 2005 to 2012. The weather (November to May), was characterized by high precipitation (average 1000 mm/year), high humidity (45 to 90%) and mild temperature (7 to 28 °C) during the cropping season (Weather Spark, 2015). During dry spells, mist irrigation was applied to ensure good disease development of the three foliar diseases. A total of 2000 genotypes (germplasm accessions and breeding lines), genotype including landrace accessions and breeding lines were screened (Table 1).

Methods of disease resistance screening:

Chocolate spot resistance screening: Eight aggressive isolates of *B. fabae* collected from different parts of Syria were used to inoculate the disease nursery every season. To maintain the pathogenicity of the isolates; each season, isolations were made after artificially inoculating seedlings at fourth leaf stage of susceptible faba bean genotype. Inoculum of the eight isolates was multiplied on faba bean dextrose agar and kept under 12/12 light regime under room temperature at Food legume Pathology Laboratory at ICARDA Tel Hadya Research Station,; 10-12 days cultures were blended and spore suspensions were adjusted to $4\text{-}5\times10^5$ spores/ml and Tween-20 (0.03% v/v) was added to the suspension. Infested seeds were prepared in growth room and distributed in the field to induce the infection. The first artificial inoculations were done during February and March at vegetative growth to early flowering stages to supplement natural inoculum sources. To secure homogenous distribution of the inoculum automatic sprayer was used with fixed pressure.

Ascochyta blight resistance screening: Seven aggressive isolates of *A. fabae* collected from different parts of Syria were used to inoculate the disease nursery every season. To maintain the pathogenicity of the isolates; each season, isolations are made after artificially inoculating seedlings of susceptible faba bean genotype in the greenhouse. Inoculum of the seven isolates was re-isolated from infected leaves and multiplied on faba bean dextrose agar and kept under 12/12 light regime under room temperature at Food legume Pathology Laboratory at ICARDA Tel Hadya Research Station. 10-15 days cultures (equal number of Petri dishes/isolate) were blended and spore suspensions were adjusted to 5×10^5 spores ml^{-1}. Each season, 2-3 artificial inoculations were done during January, February and March of each season at vegetative and early flowering stages of the crop.

Rust resistance screening: Faba bean genotypes were planted late (early January), in order to coincide with favourable weather conditions for rust development under natural infection.

Experimental design and disease measurements

Chocolate spot resistance screening nurseries were arranged in a randomized complete block design with two replications and with repetitive susceptible and resistant checks after eight test entries. Ascochyta blight and rust screening nurseries from 2009 to 2012, where arranged in a replicated design with repetitive resistant and susceptible checks after 8 test entries. The resistant check for Ascochyta blight was ILB1593, susceptible check Giza 4, for chocolate spot resistant check was BPL710 and susceptible check Rebeya40 and for rust, resistant check was BPL710 and the susceptible check ILB1814. Single plants from resistant sources were selected and evaluated for three seasons. Each disease was evaluated using 1-9 disease rating scale where 1 = highly resistant and 9 = is highly susceptible (Hanounik and Robertson, 1988, 1989).

Identification of origin of disease resistance:

A database was created using MySQL (My acronym stands for Monty Widenius's daughter, SQL for Structured Query Language, https://www.mysql.com) for faster data manipulation/processing where about 10000 observation points were added to the database (Fig. 1). A list of unique germplasm identifier was extracted with their scores; then each entry was traced back to its original Genebank accession record in the Genebank's database to find its geographical coordinates or site description. In case the longitude and latitude of some germplasm accessions were not found in the database, site description was used to derive coordinate values using Biogeomancer (www.biogeomancer.org) and gazetteer within +/- 5 km radius. The software program for mapping and geographic data analysis (DIVA-GIS) (http://www.diva-gis.org) was then used to convert the extracted coordinates to Environmental Systems Research Institute (ESRI) shape file format to create distribution map.

Data analyses and efficiency of methods of screening

For identification of sources of resistance from the disease measurements, the reactions of the accessions was scored using the following resistant/susceptible classes: HR= highly resistant, R= resistant, MR=moderately resistant, MS= moderately susceptible and S=susceptible. For Ascochyta blight and chocolate spot: A line is classified as HR if Score is = 1; R if Score <= 2, 3; MR if Score =4; MS if Score =5; and S if Score is >5. Note that the class R also includes lines in class HR. For rust a line is classified as R if Score <= 1, 2, 3; MR if Score = 4 or 5; MS if Score = 6 or 7; and S if Score > 7.

The efficiency of disease screening was examined in terms of the distribution of scores for susceptible and resistant repeated checks by calculating the probability: Probability, Prob (Score for Susceptible check = i) = number of plots with the susceptible check score i divided by the total number of plots under the susceptible check. Similar calculation was carried out for resistant check. The probability was computed for the susceptible check falling in S class and probability of resistant check in R class will be the indicators of the efficiency of the screening.

Results

The disease scores varied from 1 to 9 for Ascochyta blight, chocolate spot and rust during all cropping seasons. The probability of susceptible check scored above 6 [$P(S \geq 6)$] and of resistant checks $P(R \leq 3)$ were estimated (Table 2) The highest probability indicated that disease developments were uniformly distributed throughout the experimental fields. Trials with low probability of susceptible checks were not considered for selection of resistant sources. For chocolate spot, the score for resistance check BPL710 varied from 1 to 3 from 2005 to 2012. $P(S \geq 6)$ for chocolate spot varied between 0.17 to 0.94 over seasons and values above 0.60 were reported in 2005, 2006, 2009 and 2010. $P(R \leq 3)$ for resistant chocolate spot check varied from 0.85 to 1 and screening conducted in 2008 and 2012 were not considered for selection (Table 2). For Ascochyta blight susceptible check P(S≥6) varied from 0.81 over the years except in 2012 and for the resistant check P(R≤3) varied among the years, except 2011/2012 cropping season, where disease development was low. The results indicated that disease was high and uniformly distributed in the disease nurseries (Table 2). For rust screening the probability of infection of susceptible check was high during the three cropping seasons indicating uniform disease development.

Among the 2000 samples, 424 faba bean samples identified resistant to chocolate spot, 136 showed resistance to Ascochyta blight and 52 for were resistant to rust. The generated maps by MySQL showed that resistance donors, scored below 4, were geographically distributed (Fig. 2 and Fig. 3) for Ascochyta blight and chocolate spot, respectively.

The geographical distribution of major donors of resistance to chocolate spot were from Turkey, Bangladesh, Iran, Iraq, Tunisia, Italy, Morocco, Egypt, Ethiopia, China, Columbia, Ecuador and Peru (Fig.2); while for Ascochyta blight, the resistance was mainly in the Mediterranean costal area of Lebanon, Syria, Turkey, Egypt, Algeria, Tunisia, Morocco, and in Ethiopia, China, Ecuador, Peru and Columbia (Fig. 3).

Thirty accessions with resistance to Ascochyta blight and chocolate spot were IG11199, IG11233, IG11359, IG11404, IG11458, IG11463, IG11479, IG11630, IG11805, IG12404, IG12406, IG13114, IG13626, IG13635, IG13681, IG14041, IG14046, IG14227, IG14267, IG14275, IG14278, IG14281, IG14301, IG70592, IG70593, IG70628, IG70637, IG70650, IG70660, IG72243 obtained from 11 countries: Canada, Ethiopia, Iraq, Jordan, Lebanon, Turkey, Italy, Spain, United kingdom, Ecuador, Columbia, Peru. The following seven faba bean landraces collected from Ethiopia, Cyprus and Syria were identified as resistant to Ascochyta blight and Rust: IG12682, IG12685, IG12686, IG12691, IG13549, IG72370, and IG72401. The accessions identified with combined resistance to Ascochyta blight, chocolate spot and rust were IG13132, IG13599, IG13625, IG13630, IG13681, IG14301, IG14331 originated from Spain, Columbia and China.

Utilization of sources of disease resistance in the faba bean breeding program

Single plants with multiple diseases' resistances and good agronomic performance were identified and re-evaluated for their disease resistance for three seasons for further use in hybridization breeding program. Seven new sources resistant Ascochyta blight originated from Morocco, Syria and Spain, two lines for chocolate spot lines originated from Ecuador and Turkey; 19 lines with combined resistance to Ascochyta blight and Chocolate spot originated from Canada, Columbia, Algeria, Spain, Morocco, Lebanon, Tunisia and Turkey; three lines resistant to rust and chocolate spot were derived from landraces received from Canada and Columbia. Thirty lines combining chocolate spot, Ascochyta blight and rust resistance were selected and evaluated for three seasons from populations collected from China, Italy, Jordan, Lebanon and Spain (Table 4). Ten improved lines derived from breeding program at ICARDA were developed for disease resistance (Table 5)

Discussion

Faba bean is usually grown under high rainfall and irrigation conditions in the Mediterranean and sub-tropical environments with cool, warm and humid weathers, which favor the development of foliar diseases that cause severe crop losses throughout the world (Tivoli et al., 2006). Measures to control these diseases have relied on identification of adequate sources of resistance and their use in the development of resistant varieties through screening in the field and in controlled environments. Field screening is commonly used to evaluate faba bean germplasm against Ascochyta blight, chocolate spot and rust (Tivoli et al., 2006). Results obtained indicated that field screening techniques was efficient as shown by the uniform reactions of the susceptible and resistant checks showing homogeneity of infections and avoiding the probability of escapes. Villegas-Fernandez et al. (2009) and Tivoli et al. (1986 and 1988) considered that the distribution of the inoculum in the soil to secure homogenous distribution of the disease is better than spraing spore suspension over the plants. However, their assumption needs to be supported by experiments. Since results obtained in the present study showed homogenous distribution of the suspensor, we suggest further experimental studies be carried out in order to compare the efficiency of the two mentioned screening techniques. In the field screening some row-column designs allowing a denser coverage of the layout by the checks and more replications of the test lines (Sarker and Singh, 2015)

The uses Biogeomancer for identifying the distribution of faba bean germplasm based on the obtained results, with different reaction to the two major diseases, namely Ascochyta blight and chocolate spot. It indicated focal areas where faba bean germplasm with low severity for both diseases was originated. The two maps indicated that the main area for sources for resistance to chocolate spot was the Fertile Crescent coinciding with center of origin of this crop (Cubero, 1973) with wide distribution along the routes of faba bean migration to the secondary centers of diversity and new world.

The first sources for resistance to chocolate spot (ILB 438 and ILB 938) were from Columbia, outside the center of origin (Hanounik, 1982; Robertson 1984; Khalil *et al.*, 1984). Additional accessions with resistance to chocolate spot were identified by Hanounik and Roberston (1988) and originated from Ecuador. Rhaeïm et al. (2002) identified four landraces resistant to chocolate spot originated from Tunisia and considered the BPL710 originated from Ecuadorian collection as susceptible to chocolate spot in Tunisia. Villegas-Fernandez et al. (2009) evaluated selected 46 lines issued from landraces collected in Spain and showed also that improved sources for resistance from ICARDA International Nurseries showed low severity and these were direct selection from landraces collected from Ecuador and Ethiopia. Our results indicated diverse geographical distribution of the resistant sources from Latin America, Columbia Ecuador and Peru. Four countries around the Mediterranean basin: Morocco, Tunisia and Turkey and Lebanon. Sources for resistance were also found in Bangladesh, China, Ethiopia and Iran.

Sources for resistance to Ascochyta blight were reported in different countries by different researchers. Tÿmchenco (1964) reported four cultivars with resistance to Ascochyta blight in Moscow region. Resistance was also found in England by Bond and Pope (1980) and Lockwood et al. (1985) and in the gene bank collection at ICARDA (Hanounik and Roberston 1989, Rashid et al., 1991 and Bayaa et al. 2004) and by NARS partners (Kharrat *et al*. 2006; Ondrej and Hunady, 2007). Our results indicate diverse sources of resistance to Ascochyta blight which includes germplasm collected from the Fertile Crescent. The genetic basis of resistance in the identified donors was not yet studied although there is information about the genetic basis of the resistance. It was reported as both polygenic (Román *et al.*, 2003) and major gene inheritance (Rashid *et al*. 1991, Kohpina *et al*. 2000). Therefore, studies of genetic basis of major donors for resistance need to be conducted to generate information useful for the implementation of appropriate breeding scheme. Several sources of resistance against rust have been reported in faba (Tymchenco 1964). Several resistant lines have been identified from ICARDA genetic resources (Maalouf et al., 2013). Our study showed that newly identified sources for rust collected from different origins.

Most of disease resistant program focuses on single sources of resistance but two or more foliar disease caused high yield losses in many countries. The first combined disease resistant lines were reported (ILB938 and ILB 438) from accessions collected in Columbia and were resistant to chocolate spot and rust (Khalil *et al*. 1984) and later, Villeagas Fernandez *et al.* (2011) reported many lines for chocolate spot and rust resistance. Our results allowed us to identify germplasm with combined resistances to the three major diseases from accessions collected and kept at ICARDA gene bank to narrow yield gaps in many countries when used in their high yielding cultivars.

The breeding efforts are relatively slow due the nature of the mechanism of resistance for these major diseases. To accelerate breeding cycles, there is a need to use the emerging biotechnological tools such as marker assisted selection which remain not applicable besides the significant achievements on QTLs studies associated with Ascochyta blight, rust and chocolate spot resistance (Torres et al., 2010) and the development of consensus maps in faba bean (Webb et al., 2015; Satovic et al., 2013). Hopefully, knowledge generated in the model legumes *Medicago truncatula* and *L. japonicus* will help to overcome these gaps and speed the process (Dita et al., 2006; Rispail et al., 2010). Gene expression, including large-scale approaches and functional analyses performed in the models will help to understand plant/pathogen interactions. Although relevant progress is made in tissue culture, genetic transformation of faba bean can be used to introgression sources for resistance in the faba bean cultivars (Hanafy *et al.*, 2013)

Acknowledgments: This work was supported by Australia Center for International Agricultural Research (*Project number CIM/2004/003 on Plant Health Management for Faba Bean, Chickpea and Lentils*) and the CGIAR Research Program on Grain Legumes. We also acknowledge the contribution of the research assistants: Basem Attar, Munzir Kabakebji, Lynn Abou Khater, Omar Labban, Siham Kabbabeh, and Sawsan Tawqaz in nursery management, data collections and data processing.

References

Bayaa B, Kabakebji M, Khalil S, Kabbabeh S, Street K (2004) Pathogenicity of Syrian isolates of *Ascochyta fabae* Speg. and *Botrytis fabae* Sard and sources of resistance to both pathogens in a germplasm collection from central Asia and Caucasia. In: European Association for Grain Legume Research-AEP (Eds.), Legumes for the Benefit of Agriculture, Nutrition and the Environment: their Genomics, their Products, and their Improvement. Proceeding of the 5th European Conference on Grain Legumes. INRA, Dijon, p. 308.

Bond DA, Pope M (1980) *Ascochyta fabae* on winter beans (*Vicia faba*): pathogen spread and variation in host resistance. Plant Pathol 29: 59–65.

Bouhassan A, Sadiki M, Tivoli B (2004) Evaluation of a collection of faba bean (*Vicia faba* L.) genotypes originating from the Maghreb for resistance to chocolate spot (*Botrytis fabae*) by assessment in the field and laboratory. Euphytica 135: 55–62.

Cole S (1970) The Neolithic revolution. London, UK: Trustees of the British Museum of Natural History.

Cubero JI (1973) Evolutionary trends in *Vicia faba*. *Theoretical and Applied Genetics 43*:: 59–65.

Cubero JI (1974) On the evolution of *Vicia faba* L. *Theoretical and Applied Genetics*, *45*:47–51.

Dita MA, Rispail N, Prats E, Rubiales D, Singh KB (2006) Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica 147:1–24.

Duc G, Bao SY, Baum M, Redden B, Sadiki M, Suso MJ (2010) Diversity maintenance and use of *Vicia faba* L. genetic resources. Field Crops Research, *115*: 270–278.

FAOSTAT (2015) *World statistics on faba bean*. Rome, Italy: Food and Agriculture Organization of the United Nations. Available at: <http://faostat.fao.org/>.

Hanounik SB (1980) Effect of chemical treatments and host genotypes on disease severity/yield relationships of ascochyta blight in faba beans. FABIS Newsletter 2: 50.

Hanounik SB (1982) Resistance in faba beans to chocolate spot. FABIS Newsletter 5: 24–26.

Hanounik SB, Robertson LD (1988) New sources of resistance in *Vicia faba* to Chocolate Spot Caused by *Botrytis fabae*. Plant Disease 72: 696-698.

Hanounik, SB, Robertson LD (1989) Resistance in *Vicia faba* germplasm to blight caused by *Ascochyta fabae*. Plant Disease 73: 202-205.

Jellis GJ, Bond DA, Boulton RE, (1998) Diseases of faba bean. In: Allen, DJ Lenne´, JM (Eds.), The Pathology of Food and Pasture Legumes. CAB International, pp 371–422.

Khalil SA, Nassib AM, Mohamed HA, Habib WF (1984) Identification of some sources of resistance for chocolate spot and rust in faba beans. In Chapman GP, Tarawali SA (eds.) Systems for Cytogenetic Analysis in *Vicia Faba* L.Martinus Nijhoff Dr W, Junk Publishers. pp 80-94

Kharrat M, Le Guen J, Tivoli B (2006) Genetics of resistance to 3 isolates of *Ascochyta fabae* on faba bean (*Vicia faba* L.) in controlled conditions. Euphytica 151: 49–61.

Kohpina S, Knight R, Stoddard FL (2000) Evaluating faba beans for resistance to Ascochyta blight using detached organs. Australian Journal of Experimental Agriculture 40: 707–713.

Link W, Ghaouti L (2012) Faba bean: breeding for organic farming systems organic crop breeding. In: van Lammerts BE, Myers JR eds Organic crop breeding. Wiley, Oxford, pp 215–225

Lockwood G, Jellis GJ, Aubury RG (1985) Genotypic influences on the incidence of infection by *Ascochyta fabae* in winter hardy varieties of faba bean (*Vicia faba*). Plant Pathology 34: 341–346

Maalouf F, Nawar M, Hamwieh, A, Amri A, Zong X, Bao S, and Yang T (2013) Faba bean In: Mohar Singh, HDU, Ishwari SB (Eds) Genetic and Genomic Resources of Grain Legume Improvement. Elsevier Inc 935-3. ISBN: 978-0-12-397. Pp 113-136

Maxted N (1995) An ecogeographical study of *Vicia* subgenus *Vicia*: *Systematic and ecogeographic studies on crop genepools 8*. Rome, Italy: International Plant Genetic Resources Institute. Available at: <http://pdf.usaid.gov/pdf_docs/PNABU773.pdf/>.

Ondrej M, Hunady I (2007) Faba bean (*Vicia faba* L.) breeding for resistance toAnthracnose *Ascochyta fabae* Speg. in the Czech Republic. Czech J Genet Plant Breeding 43: 61–68.

Rashid KY, Bernier CC, Conner RL (1991) Evaluation of faba bean for resistance to *Ascochyta fabae* and development of host differentials for race identification. Plant Disease 75: 852–855.

Rashid KY, Bernier CC (1991) The effect of rust on yield of faba bean cultivars and slow-rusting populations. Canadian Journal of Plant Science 71:967–972.

Redden R,, van Leur J, Zong X, Bao S, Yujioa L, Paull J, Leonforte T (2008) Evaluation and utilisation of pea and faba bean germplasm from China. In 'Global issues, paddock action. Proceedings of the 14th Australian Ed. M Unkovich Australian Society of Agronomy/The Regional Institute Ltd: Gosford, NSW Available at: www.regional.org.au/au//pdf/asa/2008/5804_redden.pdf

Rhaïem A, Cherif M, Kharrat M, Harrabi M (2002) New faba bean genotypes resistant to chocolate spot caused by *Botrytis fabae*. Phytopathologia. Mediterranea. 41: 99–108

Rispail N, Kalo´ P, Kiss GB, Ellis THN, Gallardo K, Thompson RD, Prats E, Larrainzar E, Ladrera R, Gonza´ lez EM, Arrese-Igor C, Ferguson B,J, Gresshoff PM (2010) Model legumes to contribute to Faba bean breeding. Field Crops Research 115: 253–269.

Robertson LD (1984) A note on the I.L.B. source of Botrytis fabae resistance. In. Chapman G.P,. Tarawali S.A (eds.) Systems for Cytogenetic Analysis in *Vicia Faba* L. Martinus Nijhoff Dr W, Junk Publishers. Pp 80

Román B Satovic, Z. Avila, C.M. Rubiales, D. Moreno, M.T. Torres, A.M. 2003. Locating genes associated with Ascochyta fabae resistance in Vicia faba L. Australian Journal of Agriculture Research 54: 85–90.

Rubiales D, Avila CM, Sillero JC Hybl M, Narits L, Sass O, Flores F (2012) Identification and multi-environment validation of resistance to *Ascochyta fabae* in faba bean *Vicia faba*. Field Crops Research 126: 165–170.

Sarker A, Singh M (2015) Improving breeding efficiency through application of appropriate experimental designs and analysis models: a case of lentil (*Lens culinaris* Medikus subsp. *culinaris*) yield trials. Field Crops Research 179: 26-34. (doi:10.1016/j.fcr.2015.04.007)

Satovic Z, Avila CM, Cruz-Izquierdo S. Díaz-Ruíz R, García-Ruíz GM, Gloria M Palomino1 Carmen, Gutiérrez1 Natalia, Vitale1 Stefania, Ocaña-Moral S. Victoria Gutiérrez= M. Cubero JI. Torres AM (2013) A reference consensus genetic map for molecular markers and economically important traits in faba bean *Vicia faba* L. BMC Genomics 14: 932

Sillero J C, Avila CM, Moreno MT, Rubiales D (2001) Identification of resistance to *Ascochyta fabae* in Vicia faba germplasm. Plant Breeding 120: 529-531.

Sillero JC, Villegas-Fernandez Angel M, Thomas J, Rojas-Molina MM, Emeran AA, Fernandez-Aparicio M, Rubiales D (2010) Faba bean breeding for disease resistance. Field Crops Research 115: 297–307.

Tanno K, Willcox G (2006) The origins of cultivation of *Cicer arietinum* L. and *Vicia faba* L.: Early finds from Tell el-Kerkh, north-west Syria, late 10th millennium B.P Vegetation History and Archaeobotany, 15:197–204

Temesgen T, Keneni G, Sefera T, and Jarso M (2015) Yield stability and relationships among stability parameters in faba bean (*Vicia faba* L.) genotypes. The Crop Journal. Special Issue: Breeding to Optimize Agriculture in a Changing World 3: 258-269

Tivoli B Berthelem D, Le Guen J Onfroy C (1986) Comparison of some methods for evaluation of reaction of different winter faba bean genotypes to *Botrytis fabae*. FABIS Newsletter 16: 46–51.

Tivoli, B. Berthelem,DJ, Le Guen J Onfroy C., 1988. A study of the performance of certain faba bean genotypes in relation to *Botrytis fabae* and *Ascochyta fabae* in France. FABIS Newsletter 21: 36–39.

Tivoli B, Baranger A, Avila CM, Banniza S, Barbetti M CW, Davidson J. Lindeck K and Kharrat M Rubiales D, Sadiki M, Sillero JC, Sweetingham M, Muehlbauer FJ (2006) Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. Euphytica 147: 223–253.

Torres AM, Avila CM, Gutierrez N, Palomino C, Moreno MT, Cubero JI (2010) Marker-assisted selection in faba bean (*Vicia faba* L.). Field Crops Research 115: 243–252.

Tÿmchenco LF (1964) Diseases of fodder beans in the Moscow region and the effectiveness of seed disinfectants. Review of applied mycology 43: 446.

Van Leur JAG, Marcellos H, Paull J, Rose I, (2000) Higher resistance to chocolate spot in faba bean. Biological and Cultural Tests for Control of Plant Diseases 15: 28.

Villegas-Fernández A M, Sillero JC, Rubiales D (2012) Screening faba bean for chocolate spot resistance: evaluation methods and effects of age of host tissue and temperature. European Journal of Plant Pathology 132: 443-453.

Emeran AA, Flores F, Rubiales D (2011) Multiple-disease resistance in *Vicia faba*: Multi-environment field testing for identification of combined resistance to Rust and chocolate spot. Field Crops Research 124: 59-65.

Villegas-Fernández A M, SilleroJC, Emeran AA, Winkler J, Raffiot B, Tay J (2009) Identification and multi-environment validation of resistance to Botrytis fabae in *Vicia faba*. Field Crops Research 114: 84–90.

Webb A, Cottage A, Wood T, Khamassi K, Hobbs D, Gostkiewicz K, White M, Khazae H, Ali M, Street D, Duc G, Stoddard FL, Maalouf F, Ogbonnaya F, Link W, Thomas J, O' Sullivan DM (2015) A SNP-based consensus genetic map for synteny - based trait targeting in faba bean *Vicia faba* L Plant Biotechnology Journal doi: 10.1111/pbi.12371. pp. 1–9

Table 1: Number of faba bean accessions evaluated for three foliar diseases, Lattakia, 2004/05 -2011/2012 cropping season

Cropping seasons	Breeding lines	Germplasm accessions
Ascochyta blight and chocolate spot		
2004/2005	53	196
2005/2006	3	247
2006/2007	9	243
2007/2008	69	191
Ascochyta blight, chocolate spot and rust		
2008/2009	100	150
2009/2010	101	149
2010/2011	9	241
2011/2012	0	250

Table 2: Efficiency of field screening for the three foliar diseases using repeated resistant and susceptible checks

Year	Ascochyta blight nursery			Chocolate spot nursery			Rust nursery		
	N	Prob (S≥6)	Prob (R≤3)	N	Prob(S≥6)	Prob (R≤ 3)	N	Prob (S ≥6)	Prob(R ≤ 3)
2005	80	0.98	0.53	80	0.69	1.00			
2006	82	0.96	1.00	82	0.94	1.00			
2007	82	0.98	1.00	82	0.48	1.00			
2008	82	0.84	1.00	82	0.11	1.00			
2009	82	0.96	0.52	82	0.65	1.00	41	0.95	0.05
2010	42	0.81	0.80	82	0.77	0.92	42	0.57	0.02
2011	41	1.00	0.70	41	0.32	1.00	41	1	0.78
2012	41	0.29	0.63	41	0.17	0.85			

N= Number of repeats of the susceptible/resistant checks in each screening nursery from 2005 to 2012. Prob =Prob (S) = Susceptible check; Prob (R)= Resistant check

Table 3: Origin of selected single resistant line and combined two diseases

Selection	Origin	IG	Pedigree	Traits	Cropping seasons
Sel . 2006 latt.(ILB 3555)	SYR	72433	OT-74-1	AB	2007-2010
Sel . 2000 latt.12327	ESP	102181	ILB 265	AB	2005-2007
Sel .1997 latt.98264-1	ESP	105831	ILB2549	AB	2005-2007
Sel .1997 latt.98302-2	ESP	105860	ILB 2558	AB	2005-2007
Sel . 97 latt.98305-6	MAR	105863	ILB 2559	AB	2005-2007
Sel . 2009 latt.(BPL 4104)	MAR	105863	ILB 2559	AB	2007-2010
Sel . 2006 latt.(BPL 4164)	MAR	106583	ILB2559	AB	2007-2010
Sel .1999 latt.11135-2	TUR	102068	ILB 187	CS	2007-2010
Sel . 2008 latt. 202-1	ECU	102468	ILB 437	CS	2009-2011
Sel . 2008 latt. 212-1	CAN	102639	ILB 611	CS + RT	2009-2011
Sel . 2008 latt. 212-4	CAN	102639	ILB 611	CS + RT	2009-2011
Sel . 2008 latt. 767	COL	106583	ILB 3161	CS + RT	2009-2011
Sel . 99 latt.11350	DZA	11562	ILB 0368	CS + AB	2005-2007
Sel . 2001 latt.219	CAN	11761	2N 15	CS + AB	2005-2007
Sel . 99 latt.10247-1	SWD	11946	S.V. 0720	CS + AB	2005-2007
Sel . 99 latt.11289	DZA	12111	ILB 0917	CS + AB	2005-2007
Sel . 2000 latt.12449	TUN	12116	Malti	CS + AB	2005-2008
Sel . 2006 latt.(ILB 2583)	MAR	13777	F 329	CS + AB	2007-2010
Sel . 2006 latt.(ILB 2921)	TUR	14115	LH 209	CS + AB	2007-2010
Sel . 2000 latt.12672	ESP	102180	ILB 264	CS + AB	2006-2008
Sel . 2000 latt.12094-1	ESP	102182	ILB 266	CS + AB	2007-2010
Sel . 2000 latt.12094 -2	ESP	102182	ILB 266	CS + AB	2005-2007
Sel . 2009 latt. (BPL 471)	LBN	102230	ILB 287	CS + AB	2005-2007
Sel . 99 latt.10418	COL	102938	ILB 937	CS + AB	2005-2007
Sel . 97 latt.97382	ESP	105761	ILB 2526	CS + AB	2005-2007
Sel . 2004 latt. 47-2	ESP	105762	ILB 2526	CS + AB	2005-2007
Sel . 99 latt.10460	ESP	105831	ILB2549	CS + AB	2005-2007
Sel . 97 latt.98265-5	ESP	105832	ILB2549	CS + AB	2005-2007
Sel . 2004 latt. 69-1	TUR	105847	ILB 2554	CS + AB	2007-2010
Sel . 99 latt.10135-1	MAR	105862	ILB 2559	CS + AB	2005-2007
Sel . 99 latt.10135-2	MAR	105862	ILB 2559	CS + AB	2005-2007

IG: Genbank identification; CA: Canada, COL: Columbia, DZA: Algeria, ECU Ecuador, ESP Spain, LBN Lebanon, MAR Marroco, SWD Sweeden, SYR: Syria; TUN Tunisia,, TUR Turkey. AB: Ascochyta blight, CS: chocolate spot, RT: Rust

Table 4: Origin of combined resistant lines for three foliar diseases: Ascochyta blight, chocolate spot and rust evaluated for three consecutive seasons (2010 - 2012)

Selection	IG	Pedigree	Origin
Sel 2009lat.554	101763	ILB 3	JOR
Sel 2009lat.513-2	102449	ILB 418	GER
Sel 2009lat.307-4	104286	ILB 1700	UNK
Se l2009lat.307-5	104286	ILB 1700	UNK
Se l2009lat.373-3	104310	ILB 1713	UNK
Sel 2009 latt. 750-1	104545	ILB 1816	LBN
Sel 2009lat.750-1	104545	ILB 1816	LBN
Sel 2009lat.750-2	104545	ILB 1816	LBN
Sel 2009 latt. 750-2	104545	ILB 1816	LBN
Sel 2009 latt. 73	104545	ILB 1816	LBN
Sel 2009lat.438-1-1	105247	ILB 2417	ESP
Sel 2009lat.438-1-2	105247	ILB 2417	ESP
Sel 2009lat.444-1	105251	ILB 2417	ESP
Sel 2009lat.444-2	105251	ILB 2417	ESP
Sel 2008 latt.49-1	105303	ILB 2432	ESP
Sel 2008 latt.49-2	105303	ILB 2432	ESP
Sel 2009lat.519-2	105473	ILB 2480	ESP
Sel 2009lat.684-2	105499	ILB 2487	ESP
Sel 2009lat.620-1-1	105612	ILB 2777	CHN
Sel 2009lat.620-1-2	105612	ILB 2777	CHN
Sel 2009lat.443-1-1	105643	ILB 2781	CHN
Sel 2009lat.443-1-2	105643	ILB 2781	CHN
Sel 2009lat.443-1-3	105643	ILB 2781	CHN
Sel 2009lat.524-1-1	105696	ILB 2779	CHN
Sel 2009lat.524-1-2	105696	ILB 2779	CHN
Sel 2009lat.524-1-3	105696	ILB 2779	CHN
Sel 2009lat.737	105974	ILB 2606	MAR
Sel 2009lat.737-2	105974	ILB 2606	MAR
Sel 2008 latt.368-1	106668	ILB 3227	ITA
Sel . 2008 latt. 368-2	106668	ILB 3227	ITA

IG: Genbank identification; CHN: CHINA, ESP Spain, GER: Germany; ITA: Italy, JOR: JORDAN; LBN Lebanon, MAR Marzocco, UNK: Unknown

Table 5: Elite lines from faba bean breeding combining resistance to major diseases and having good agronomical traits.

Line	Selection Name	Pedigree	Target diseases	Seasons
Sel .1997 latt.98134-1	B8833	74TA63 X 78S49694	Ascochyta	2005-2007
Sel .1997 latt.98129-1	B8838	L82009	Ascochyta	2005-2007
Sel .1997 latt.98129-3	B8838	L82009	Ascochyta	2005-2007
Sel . 99 latt.11230-1	L 83 117	L 83 117	Ascochyta	2005-2007
Sel .1997 latt.98170-2	S95003	S95003	Ascochyta	2005-2007
Acc 0735-1	Acc 0735-1	ILB466	Botrytis+ Ascochyta	2005-2007
Sel . 97 latt.98133-1	L 82 003	74TA63 X 78S49694	Botrytis+ Ascochyta	2005-2007
Sel . 97 latt.98154-3	L 82 003	74TA63 X 78S49694	Botrytis + Ascochyta	2005-2007
Sel . 97 latt.98114-1	L 82 004	L.L.L x 78S49694	Botrytis + Ascochyta	2005-2007
Sel . 97 latt.98114-2	L 82 004	L.L.L x 78S49694	Botrytis + Ascochyta	2005-2007
Sel . 2009 latt. 10268-3	L 83 129	L 83 129	Botrytis + Ascochyta. + Rust	2010-2012

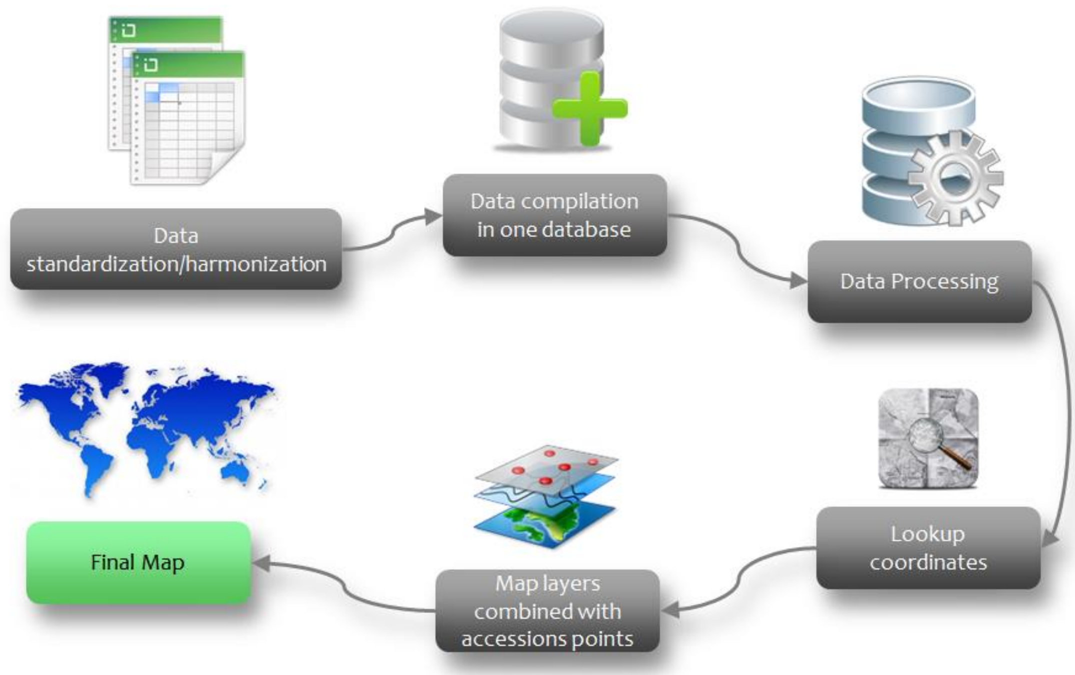

Fig. 1: Method used for the creation of maps to locate sources for of resistance to ascochyta blight and chocolate spot in faba bean

Fig. 2 : Distribution of the origin of different faba bean accessions with different reaction to chocolate spot. Highly resistant to Resistance denotes P(1≤R≤3), moderately resistant P(4≤S≤6), susceptible to high susceptible P(7≤S≤9)

Fig.3: Distribution of different faba bean accessions with different reaction to Ascochyta blight. Highly resistant to Resistance denotes P(1≤R≤3), moderately resistant P(4≤S≤6), susceptible to high susceptible P(7≤S≤9