

Genetic evaluation on molecular level for some faba bean lines(*vicia faba* L.)

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This study was carried out in the green house and Laboratories of the Genetics Department, Faculty of Agriculture, Benha Branch, Zagazig University and Laboratories of the Department of Genetics, Faculty of Agriculture, Ain shams University during the period from 2002 to 2004. Twelve faba bean (*Vicia faba* L.) genotypes were used in this study. The genotypes G3, G461 and Pakistani were supplied by Leguminous Crops Research Department (LCRD), Field Crops Research Institute Agricultural Research Center, Giza, Egypt. And the new accessions Scirocco, Condor, Gloria and Columbo were obtained from Agricultural Research Center, Plant Nutrition & Soil Science Institute, Braunschweig, Germany. The five determinate lines were obtained from 7th generation of Dr. I. El-Shawaf program at Dept. of Genetics, Moshtohor, Fac. Agric., Zagazig Univ. Twelve faba bean genotypes were separately seeded in pots No.25, filled with natural field soil, each was planted with eight seeds and irrigated regularly. Germinated seeds were thinned to five seeds per pot. Each genotype was seeded in 10 pots where five pots contained the artificial inoculation experiment and the other five pots serve as control. The twelve faba bean genotypes were evaluate under chocolate spot infection. Six yield-related traits were studied including plant height, branches number/ plant, pods number/ plant, pods weight/ plant, seeds number/ plant and seeds weight/ plant. Chemical analysis were performed to determined sugars (reducing, nonSummary 104reducing and total) and phenols (conjugated, free and total) concentration in healthy and infected leaves. Molecular studies including SDS-PAGE for seed storage protein (water-soluble fraction), and RAPD-PCR were applied to study the genetic diversity of the ten faba bean genotypes. Besides, the efficiency of using the molecular data to identify the resistant faba bean genotypes for chocolate spot, and to determine (determinate growth habits as indeterminate). The results could be summarized as follows: 1- Statistical analysis (LSD) of the agronomic performance data under chocolate spot infection in greenhouse experiment for the twelve faba bean genotypes revealed that Pakistani, Giza3, Giza461, Scirocco and DH6 are resistant genotypes, Condor is moderately resistant genotype, Gloria and Columbo are moderately susceptible genotypes and DH1, DH3, DH4 and DH5 are susceptible genotype. 2- Statistical analysis (LSD) of the agronomic performance data concerned with yield related traits and its relationship with the infection. in greenhouse experiment for the twelve faba bean genotypes revealed that Highly significant differences were obtained among all genotypes for all studied characters. Highly significant differences were also found for infection and genotype X infection for number of pod / plant, weight of pod / plant, seed number / plant and seed yield / plant. The presence of highly significant values of mean of squares for number of pods and pod weight / plant as well as seed number and seed yield / plant indicated that these characters are highly affected by infection with chocolate spot spore disease. Meanwhile, plant height and number of branches / plant are the less influenced characters by disease. Meanwhile, highly significant mean of squares of infection and genotype X infection for pod and seed characters could indicate presence of differences between infected and non infected plants for such influenced traits such as number of pods, pod weight, seed number, and seed yield / plant. Moreover, high significant mean of squares of genotype X infection could indicated that genotypes behaved differently under infection by chocolate spot disease for these traits. Pakistani genotype (undeterminate) was the shorter one 33.56 cm, while Condor (undeterminate) was the tall genotype 65.32 cm. Data showed

insignificant differences for infection and genotype X infection in plant height among uninfected and infected plants within the same genotype. Means of number of branches / plant indicated that significant differences were exhibited among genotypes. It was found that the mean of branches / plant in undetermined genotypes were low and ranged from 1.6 branch for Condor to 4.4 branch in Pakistani. Meanwhile, all determined genotypes exhibited higher number of branches / plant and ranged from 4.1 branch in DH4 up to 5.4 in DH6. Meanwhile, data showed no significant differences for number of branches / plant between non infected and infected one within the same genotypes. The effect of genotypes X infection interaction was not significant. Number of pods / plant was significantly affected by *Botrytis fabae* infection. Data showed a reduction in number of pods / plant in the infected plants in each genotype as compared with uninfected plants. The reduction was severe in susceptible genotypes than resistant ones. For example Pakistani genotype gave 23.0 pods / plant in uninfected population and 16.0 pods / plant in the infected one. These genotype were considered resistant one. The susceptible genotype i.e. DH1 showed reduction in number of pods / plant from 5.8 to 3.2. Pod weight / plant showed reduction due to infection with *Botrytis fabae*. The decrease in pod weight per plant was great in susceptible genotype, i.e. Gloria which showed mean value of 0.49 gram per plant, under infection. Data on seed number / plant showed that this character is influenced by the artificial inoculation of *Botrytis fabae*. Resistant genotypes were less influenced as compared with susceptible genotypes. Severe reduction were observed in DH3, DH4, and DH5 which showed 50% reduction in seed number / plant under infection treatment. Data showed the effect of inoculation of *Botrytis fabae* on seed yield / plant in grams. Seed yield decreased significantly in infected genotypic plants as compared with uninfected ones. However, the resistant genotypes exhibited less loss in seed yield / plant. Meanwhile, susceptible genotypes showed severe reduction in seed yield / plant. This susceptible genotypes are, Gloria, Columbo, DH1, DH3, DH4, and D1-15 which showed about 50% reduction in yield.

3- Chemical content and its relationship with the infection: Leaf sugar content indicate that the leaves of all *Vicia faba* genotypes under conditions of infection by *Botrytis fabae*. contain much higher reducing, non reducing and total sugars than those of healthy ones. The content of free and total phenols in infected leaves exudates of the 12 *Vicia fabae* genotypes record much higher values than the healthy leaves.

4- The electrophoretic banding patterns of proteins extracted from the seeds of the twelve faba bean genotypes was different. The results of SDS-PAGE revealed a total number of 27 bands with molecular weights (MW) ranging from about 11.7 to 328.7 kDa. Data revealed 11 common bands (monomorphic), while the remaining 16 bands were polymorphic with 59.25 % polymorphism. The densitometric analysis of the SDS-protein banding patterns of the studied genotypes were found to be useful in varietal identification of the studied faba bean genotypes. The twelve genotypes showed different patterns. The maximum number of bands (21) appeared in Gloria and the minimum number (18) was present in Giza 3 genotype. However, there common bands between many genotypes, not yet each genotype was characterized by a unique fingerprint. At the same time, there was a marker band (s) for some genotypes such as band number 14 at MW 97.3 kDa for Pakistani. However there were some negative marker bands such as band 5 at 230.2 kDa for Pakistani, band 18 at 59.4 kDa for Scirocco and band number 12 at 116.1 kDa for Pakistani and Giza 3.

5- All the eight random primers successfully amplified DNA fragments for all genotypes. A total number of 78 fragments were visualized across the twelve investigated genotypes. Primers produced band numbers ranging from five (primer OP-B 17) to 15 (primer OP-F09) fragments across genotypes. There were a marker bands for some genotypes in RAPD-PCR with all primers such as OP-A07 which exhibited some markers like band number 1 at 2255 bp and band 6 at 870 bp for DH4 and DH1, respectively, and band 3 at 1305 bp for Condor and DH4. However, there were marker for Giza 3 at band 9 with 595 bp.

6- Combined analysis based on SDS-protein and RAPD-PCR showed that the highest similarity index recorded (0.96) was between the two genotypes DH6 and DH5. Meanwhile, the lowest similarity index 0.63 was observed between genotypes DH3 and Pakistani.

7- SDS protein markers SDS protein electrophoresis exhibited some molecular genetic markers for chocolate spot disease resistance. Band number 19 at MW 52.4 was exhibited only in the three resistant faba bean genotypes Pakistani, Giza3 and Giza461. Therefore, it may be considered as protein marker for chocolate spot resistance.

8 - RAPD analysis indicated

some molecular genetic markers for chocolate spot resistance. For example, primer OP-F01 showed fragment number 8 at 400 by which appeared only in the four resistant genotypes Pakistani, Giza3, Giza461 and Condor and band number 11 at 275 by which appeared in the three resistant genotypes Giza3, Giza461 and Scirocco and disappeared in the others. Therefore, could be considered as a positive molecular marker for chocolate spot resistance.9- SDS protein markers SDS protein electrophoresis exhibited some molecular genetic markers for determinate growth character. Bands number 20, 25 and 26 at MW 49.0, 20.5 and 15.5 KDa, respectively, appeared in all determinate genotypes DH1, DH3, DH4, DH5 and DH6. and were absent in all indeterminate genotypes It may be considered as a positive protein marker for determinate growth character.10- RAPD analysis indicated some molecular genetic markers for determinate growth character. For example in primer OP-F09, band number 15 at 215 by appeared only in the three determinate genotypes DH4, DH5 and DH6, therefore, it could be used as positive molecular markers for determinate growth character.