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Inheritance of Resistance to Bean Yellow Mosaic Virus in Faba Bean Plants

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ABSTRACT

Bean Yellow Mosaic Virus (BYMV) is one of a devastating pathogen that reduces faba bean crop production in Ismailia. Resistance is limited and resistance-breaking isolates are becoming problematic. Effective BYMV disease control strategies will come from a better understanding of inheritance the resistance to BYMV disease in the crop considering. In this article, we consider the determination role of the inheritance mode of the resistance to BYMV in F₁, F₂ and F₃ faba bean populations. The F₁, F₂ and F₃ progeny of resistant and susceptible faba bean plants were investigated under artificial infection by BYMV in the green house during two successive seasons (2008/09 and 2009/010). Faba bean population's plants were evaluated for the resistance to BYMV using a four-class scale of increasing susceptibility to the BYMV disease which took into account the infected main percentage and disease severity for the faba bean population's plants. Our results analysis confirms that the F₁'s was completely resistant and the F₂'s was segregated a clear to been 3 resistant: 1 susceptible, while the F₃'s families confirmed the F₂'s families segregation which suggests as a dominant character controlled by a single locus. This resistance character in faba bean considering has a good potential for direct use in commercial faba bean breeding or for transfer to other faba bean genotypes.

Key words: *Vicia faba* L., disease resistance, BYMV, virus infection

INTRODUCTION

Faba bean, *Vicia faba* L. is one of the major pulse crops grown in Egypt. It is a multi-purpose crop that plays an important role in the socio-economic life of farming communities (Agegnehu and Fessehaie, 2006). Improvement of seed yield of faba bean via hybridization, mutation and selection are being hampered by diseases. Since faba bean crop is cultivated in different seasons with different genotypes, the crop may respond differently to economic traits including diseases resistance (Torres *et al.*, 2006).

In general the production of faba bean, has been constrained by several biotic and abiotic factors as riverward by EL-Bramawy and Abdul Wahed (2005) and Agegnehu and Fessehaie (2006). Diseases are among the most important biotic constraints limiting the production of faba bean. A virus disease caused by Bean Yellow Mosaic Virus (BYMV) is one of the economically important diseases that damages the foliage, limits photosynthetic activity (depending on the infection stage) and reduces faba bean production globally (Cheng *et al.*, 2002; Miteva *et al.*, 2005). In the Arabic region loss due to BYMV disease can be reached up to 30% on susceptible cultivars

of faba bean (Khalil and Erskine, 2001). In Egypt region, a specific Ismailia Governorates, whereas working considering, the loss due to BYMV infection could be around 55.6 and 81.00% due to different environmental conditions and the wide distribution range of insects in the Ismailia district (EL-Beshehy, 1999).

The disease can also cause total crop failure under severe epidemic conditions (Cheng *et al.*, 2002). Therefore, BYMV is a major limiting factor in the main faba bean growing regions of Egypt. Therefore, using of resistant cultivars is the most desirable control method, because it provides a practical, long-term and environmentally benign means of limiting the damage from this disease (Wang *et al.*, 2001). Most studies of resistance to diseases in faba bean crop have concentrated on fungal diseases resistance but virus disease (BYMV) resistance is so few than others. Hence, it is so necessary to search toward sources of the resistance to BYMV in faba bean and knowing the mode of inheritance the resistance to virus disease (BYMV) in faba bean crop.

From these studies, faba bean populations could be probably enough to evaluate their resistance to BYMV under green house conditions to know the bearing/attitude with this pathogen. For this reason, therefore, since a little work had been done on faba bean virus diseases in Egypt, especially the inheritance of the resistance to this disease (BYMV), the objective of this study was to determine the inheritance of the resistance to BYMV in the crop populations (F_1 's, F_2 's and F_3 's considered and their relation with the biochemical changes which associated with virus incidence under artificial infection conditions by BYMV.

MATERIALS AND METHODS

BYMV source: Samples of faba bean plants exhibiting venial yellowing, followed by obvious green or yellow mosaic, vein banding with yellowish line patterns were collected from eight different fields of Ismailia Governorate. These samples were tested serologically against BYMV, using specific polyclonal antibodies by direct ELISA test, according to the method described by Clark and Adams (1977). Leaf samples which gave positive reaction with specific BYMV antibodies, were extracted and diluted to be used for inoculation faba bean cv. Giza 461 (Radwan *et al.*, 2008).

Parental selected and background of genetic populations: Since few years ago (2003 and 2004), a breeding program for disease resistance in faba bean was started in the Agronomy Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. EL-Bramawy and Abdul Wahed (2005). This breeding program was started by using twenty faba bean genotypes which were varied between domestic and introduced genotypes.

Some of these faba bean genotypes were tested in earlier with BYMV (Radwan *et al.*, 2008). According the previous studies for the potential high yield and BYMV diseases resistance, we selected a six faba bean genotypes as a parents materials using as a basic for breeding program via diallel cross in the forma of half diallel cross of the six faba bean genotypes, two faba bean plants (BPL 710 and Giza 3) were determined and considered as appeared as a cornerstone of this research work. The first one (BPL 710) was resistance to BYMV but the second one (Giza 3) was susceptible to BYMV.

Parental determination cross and obtaining generations: The genotypes of the two faba bean plants (BPL 710 and Giza 3) were selfed three times, retested in the field between each generation of selfing and the resulting S_3 individuals used as resistant and susceptible faba bean parents. The resistant (BPL 710) and susceptible (Giza 3) cultivars were crossed to create F_1 hybrids

(First generation). Then ten F₁ individuals from two different parental crosses were selfed to produce the F₂ populations (Second generation). Twenty-six resistant or susceptible F₂ individuals, from four different families, were selfed to produce F₃ populations (Third generation). These hybridizations for obtaining the three generations (F₁, F₂ and F₃), were carried out in the experimental Farm, through three seasons (2005/06, 2006/07 and 2007/08), respectively.

Green house testing of the faba bean generation's resistance: F₁, F₂ and F₃ faba bean generations plants were tested at the green house in 2008/09 and 2009/2010. Seeds of the faba bean generations (F₁, F₂ and F₃) were sown in pots (40 cm in diameter) by rate of 10 seeds per pot. Experimental plants of the F₁, F₂ and F₃ were arranged in a completely randomized block design with three replications to account for eventual differences in BYMV incidence development. At planting the seedlings of every F₁, F₂ and F₃ populations were split into three equal groups and each group assigned to a different block. We tested 30 F₁, 225 F₂ and 315 F₃ faba bean plants. Every generation's plant was evaluated after three weeks of inoculation using the interaction faba bean phenotype with BYMV.

Infection of BYMV inoculum on faba bean plants: The inoculum was prepared from BYMV infected top faba bean leaves, ground in a mortar containing 0.1 M phosphate buffer, pH 7.0 (1:2 w:v). The homogenate was filtrated through two layers of muslin and the leaves of healthy plants were dusted with carborundum and rubbed gently with a cotton swab previously dipped into the suspension of virus inoculums. The faba bean plants were kept at 100% water-holding capacity.

Infection percentage and disease severity calculation: The inoculated faba bean plants through the six faba bean genotypes as a parents materials and the non-inoculated (control) were counted and calculated as percentage of BYMV incidence in relation to total plant in each one. Hence, three weeks after inoculation, the percentage of infection and disease severity the following rating scale was used to determine, 0 = No symptoms; 1 = Light Leaf roll in upper leaves; 2 = Yellow and Leaf roll in upper leaves; 3 = Mosaic, Yellow and Leaf roll in upper leaves and 4 = Severe Mosaic, Yellow and Leaf roll in upper leaves. Percentage of Disease Severity (DS) values were calculated using the following formula according to Yang *et al.* (1997).

$$Ds (\%) = \frac{\sum (\text{Disease grade} \times \text{No. of plants in each grade})}{(\text{Total No. of plants} \times \text{highest disease grade})} \times 100$$

Non-inoculated pots of faba bean plants from each generation were used as a control. All plants tested were checked for BYMV by direct-ELISA test and no chemical treated were applied to the plants during the green house assay.

Statistic analysis: Chi-square tests were used to determine good ness of fit to hypothesized models based on observed and expected numbers of resistant and susceptible individuals in F₂ and F₃ populations.

RESULTS

Reactions of the six faba bean genotypes (Tribe White, Giza 3, Giza 714, Giza 429, Sakha 1 and BPL 710) as a original parents materials to BYMV infection were evaluated, hence select and determine the two parents considering. The results presented in Table 1 show the symptoms

Table 1: Interaction between faba bean parental plants and BYMV

Code No.	Parents cultivars	Symptoms
P ₁	Tribe White	Yellow and Leaf roll in upper leaves
P ₂	Giza 3	Severe Mosaic, Yellow and Leaf roll in upper leaves
P ₃	Giza 714	Mosaic, Yellow and Leaf roll in upper leaves
P ₄	Giza 429	Light Leaf roll in upper leaves
P ₅	Sakha 1	Yellow and Leaf roll in upper leaves
P ₆	BPL 710	No symptoms

Table 2: Calculated percentage of infection and disease severity with BYMV in faba bean parental cultivars

Code No.	Parents cultivars	S/T * replicates			Percentage of infection	Percentage of disease severity (DS%)
		R ₁	R ₂	R ₃		
P ₁	Tribe White	7/10	7/10	6/10	66.67	33.33
P ₂	Giza 3	9/10	7/10	9/10	83.33	83.33
P ₃	Giza 714	8/10	7/10	7/10	73.33	55.00
P ₄	Giza 429	6/10	7/10	5/10	60.00	15.00
P ₅	Sakha 1	7/10	5/10	7/10	63.33	48.33
P ₆	BPL 710	-	-	-	0.00	0.00

*No. of symptomatic plant (S)/No. of total treated plants (T)

accompanied with genotypes as a result of their interaction with BYMV, whereas, the faba bean genotype Giza 3 exhibited severe mosaic, yellow and leaf roll in upper leaves, BPL710 exhibited no clear symptoms.

Data concerning the percentage of infected plants and the diseases severity were and presented in Table 2.

The results showed that parent cultivar Giza 3 revealed highest percentage of artificial infection (83.33%) followed by parent cultivar Giza 714 (73.33%). On the contrary, the lowest percentages of artificial infection was obtained by parent cultivar BPL 710 (0%). On the other parent cultivar Giza 3 gave the highest percentage of disease severity. The lowest percentage of disease severity was noticed in parent cultivar BPL 710.

Regarding to the inheritance of resistance to BYMV in *Vicia faba* varieties and these were genetically characterized, so we are considering presenting our results and discussion aiming this approach.

The F₁'s crosses which resulted between the resistant and susceptible faba bean parents were exhibited uniformly resistant and presented in Table 3. Also, it can be conducted that all ten F₂'s populations were segregated close to be at the ratio of 1 susceptible:3 resistant. When all F₂'s generations were pooled the chi-square analysis value (χ^2) indicated a very close fit (p = 0.96) to the 3:1 ratio (Table 3).

The F₃ families as a progenies originated from the resistant F₂ families plants were either uniformly resistant or segregated 3. Resistant:1, susceptible, distinguishing the homozygous from the heterozygous F₂ parent. However, the only exception was the susceptible F₃ progeny family (No. 20) from a putative resistant F₂ plant which was obviously a false resistant (Table 4). The F₃ families from the susceptible F₂ plants were uniformly susceptible. Also the resistant plants in F₃'s number 4, 13, 16, 18 and 24 are probably escapes due to irregular infection incidence by BYMV in one block.

Table 3: Faba bean segregation for green house resistance to BYMV in the F₁ hybrids and ten F₂ populations

Cross	Observed plant	IP class				Observed ratio R:S	1 Gene hypothesis		
		0	1	2	3		Expected ratio R:S	χ^2	p value
*F ₁	17	17	-	-	-	17 : 0	1 : 0 (17 : 0)	-	-
*F ₁	13	13	-	-	-	13 : 0	1 : 0 (13 : 0)	-	-
F ₂	27	19	3	3	2	22 : 5	3 : 1 (20.25 : 6.75)	0.605	0.212
F ₂ ^a	21	14	2	4	1	16 : 5	3 : 1 (15.80 : 5.20)	0.001	0.943
F ₂ ^b	20	13	2	2	3	14 : 6	3 : 1 (15.00 : 5.00)	0.267	0.482
F ₂	22	16	2	3	1	15 : 7	3 : 1 (16.50 : 5.50)	0.545	0.451
F ₂	24	17	1	2	4	16 : 8	3 : 1 (18.00 : 6.00)	0.889	0.284
F ₂ ^c	22	12	4	2	4	16 : 6	3 : 1 (16.50 : 5.50)	0.060	0.451
F ₂ ^d	23	14	3	5	2	16 : 7	3 : 1 (17.25 : 5.75)	0.362	0.772
F ₂	26	15	3	4	4	20 : 6	3 : 1 (19.50 : 6.50)	0.051	0.134
F ₂	17	11	3	1	2	13 : 4	3 : 1 (12.75 : 4.25)	0.005	0.441
F ₂	23	16	3	1	3	18 : 5	3 : 1 (17.25 : 5.75)	0.130	0.374
F ₂ Total	225	147	25	28	25	110 : 37	3 : 1 (110.50 : 36.50)	0.009	0.961

F₁ from a resistant × susceptible cross. a, b, c, d F₂'s originating the F₃ families

Table 4: Faba bean segregation for green house resistance to BYMV in 26 F₃ families from four different F₂ populations

F ₃ family	F ₃ phenotype	Observed plant	Observed ratio R : S	Gene hypothesis		
				Expected ratio RS	χ^2	p value
1	0	16	13 : 3	3 : 1 (12.05 : 3.95)	0.313	0.301
2	0	15	12 : 3	3 : 1 (11.25 : 3.75)	0.200	0.473
3	0	10	9 : 1	1 : 0 (10.00 : 0.00)	-	-
4	2	12	2 : 10	0 : 1 (0.00 : 12.00)	-	-
5	0	16	16 : 0	1 : 0 (16.00 : 0.00)	-	-
6	0	9	5 : 4	3 : 1 (6.75 : 2.25)	1.815	0.042
7	0	12	9 : 3	3 : 1 (8.25 : 2.75)	0.091	0.624
8	0	11	10 : 1	1 : 0 (11.00 : 0.00)	-	-
9	0	5	5 : 0	1 : 0 (5.00 : 0.00)	-	-
10	0	16	16 : 0	1 : 0 (16.00 : 0.00)	-	-
11	0	14	13 : 1	1 : 0 (14.00 : 0.00)	-	-
12	3	13	0 : 13	0 : 1 (0.00 : 13.00)	-	-
13	2	8	3 : 5	0 : 1 (0.00 : 8.00)	-	-
14	0	8	8 : 0	1 : 0 (8.00 : 0.00)	-	-
15	0	12	12 : 0	1 : 0 (12.00 : 0.00)	-	-
16	3	13	1 : 12	0 : 1 (13.00 : 0.00)	-	-
17	0	13	13 : 0	1 : 0 (13.00 : 0.00)	-	-
18	4	12	4 : 8	0 : 1 (12.00 : 0.00)	-	-
19	0	16	10 : 6	3 : 1 (11.95 : 4.05)	1.257	0.024
20	0	14	0 : 14	1 : 0 (14.00 : 0.00)	-	-
21	0	12	12 : 0	1 : 0 (12.00 : 0.00)	-	-
22	0	11	11 : 0	1 : 0 (11.00 : 0.00)	-	-
23	0	12	12 : 0	1 : 0 (12.00 : 0.00)	-	-
24	2	9	2 : 7	0 : 1 (0.00 : 9.00)	-	-
25	0	11	11 : 0	1 : 0 (11.00 : 0.00)	-	-
26	2	15	0 : 15	0 : 1 (0.00 : 15.00)	-	-

F₃ 1-5 from F₂ a ; F₃ 6-13 from F₂ b; F₃14-18 from F₂ c and F₃19-26 from F₂ d (cf. Table 4)

DISCUSSION

The potential loss of effective pesticides has created strong incentives to develop effective alternative methods for virus, BYMV control. Therefore, the use of resistant cultivars is the most desirable control method because it provides a practical, long-term and environmentally benign means of limiting the damage from the diseases in general (Wang *et al.*, 2001).

In respect to the findings in Table 1 and 2, we selected the resistant plants of faba bean genotype 'Parent No. 20, P₆' (BPL 710.) with no disease symptoms (R, resistance) as well as susceptible plants of fabe bean 'Parent No. 2, P₂' (Giza 3) showing highest infection percentage by BYMV accompanied with highest symptoms severity. The susceptible cultivar (Giza 3) was used earlier as indicator host for board bean stain virus (BBSMV) by Omar *et al.* (1990) and the second cultivar (BPL 710) was detected before as a resistant one in previous work by Jellis *et al.* (1982). Therefore, it's supporting to our selection and good considering as a basic for inheritance of resistance to BYMV of the crop consider. For this reason, we choosed the both parents (BPL 710 and Giza 3) to be as a basic materials in this study.

The segregation ratios found suggest that this gene is dominant in this inbreeding faba bean species. These findings were harmony with results reported by Cheng and Jones (2000) and Jones (2005).

Ideally, final confirmation of the existence of gene responsible would require inoculation of F₃ generation. Progeny of F₂ plants grown from seed to individual plants to determine whether they are homozygous or heterozygous for the susceptibility trend or are purely non-susceptible (resistant) in their response (Shankar *et al.*, 2002). Unfortunately, such tests were impossible because the F₂ plants infected with BYMV were either positive response or were so stunted that no seed formed negative response (Jones and Smith, 2005). At the same regard, thought study on inheritance of hypersensitive resistance to BYMV in narrow-leafed lupin (*Lupinus angustifolius*) was reported by Jones and Smith (2005).

A model where a plant resistance is may be controlled by a single dominant locus can explain the segregation presented in Table 3 and 4. This model is in agreement with the inheritance of the adult-plant resistance described by Barnes (1968) in broccoli and cabbage and by Jones and Smith (2005) in *Lupinus angustifolius*. In the same connection, Mahajan *et al.* (1995) also found during their studies on inheritance of downy mildew resistance in Indian cauliflower that resistance character, controlled by a single dominant locus in one cross of Indian cauliflower. In the other studies related inheritance to diseases resistant, the resistance to downy mildew (*Peronospora parasitica*) at a four-to five-leaf stage explained by a single recessive gene in a broccoli line from the USA or at a three-to four-leaf stage by two complementary dominant genes in a doubled-haploid line developed from Everest' broccoli (Wang *et al.*, 2001) whereas, probably represent a different types of the resistance which expressed at an early stage of the growth development.

In regarding to our background in inheritance of the resistance to diseases, we do not know how the resistance described in our research work relates to that reported earlier in different crops by other authors, because the characterization of current races of Bean Yellow Mosaic Virus (BYMV) and its relationship with previous studies is impossible, since there is no collection of differential lines to identify physiological races of BYMV. However, there is evidence of high virulence for the BYMV pathotype present in the evaluating the field cultivated area at Ismailia Governorate regains. In generally, faba bean, *Vicia faba* accessions could be resistant to BYMV at stage of potent or specific of age plant and then transferee to be susceptible and may be the opposite. This depends on fierce located the nurse (BYMV) and the situation preparedness plant the breadwinner,

where the capacity to the resistance or vulnerability to infection under the umbrella of the environmental conditions surrounding the area that is or grow plant. In the respect, Coeiho and Monteiro (2000) found during their studied on pathotype specific resistance to downy mildew in *Brassica oleracea* at cotyledon and adult plant stages that *Tronchuda cabbage* var. Murciana is resistant to isolate P005 from the UK and susceptible to isolate P523 collected at Batalha at cotyledon and adult-plant stages. However, natural source of extreme resistance to BYMV is found and cultivars with this resistance bred, it is important to ensure that no new narrow-leafed lupin cultivar is released that lacks the necrotic trait (Jones and Smith, 2005).

The resistance character in the faba bean materials which obtained in the present work, was confirmed by the results detected by Jones and Smith (2005). They found through their work on inheritance of hypersensitive resistance to Bean yellow mosaic virus in narrow-leafed lupin, that four possible combinations of crosses between the different necrotic and non-necrotically reacting genotypes, segregation for the necrotic response in F₂ progeny plants always fitted a 3:1 ratio (necrotic: non-necrotic). Also, other resistance has high value, because it is expressed in broccoli plants in field conditions (Wang *et al.*, 2001). However, the responses to inoculation and segregation ratios which found provide evidence for the existence of one gene among the genes control the resistance to BYMV (Jones and Smith, 2005). Finally, the control of this resistance by a single dominant gene has good promise for its direct use in faba bean plant breeding or for transfer to other field crops.

CONCLUSION

To our knowledge, this is the first study in our faba bean breeding program in the Agronomy Department, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, to focus on the inheritance of BYMV resistance. The inheritance of resistance to BYMV confirmed the resistance character in faba bean as has a good considering potential for direct use in commercial faba bean breeding for transfer to other materials genotypes. Hence, it is possibility consequently become a one of the most encouraging options for the efficient management of viral/virus diseases in the near future.

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