SUSCEPTIBILITY OF FUNGI, MAINLY CHOCOLATE SPOT (*Botrytis fabae* SARD.), TO GAMMA IRRADIATION IN THE FABA BEAN CROP (*Vicia faba* L.)

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ABSTRACT

Fungi cause considerable losses in the faba bean crop in many regions of the world. The aim of the current research was to evaluate the susceptibility of faba bean to fungi, mainly chocolate spot, and its effect on yield, using seeds exposed to gamma irradiation. Faba bean seeds were cultivated in three regions: Hidalgo, Puebla and Tlaxcala, Mexico; the seeds were irradiated with a dose rate of 4.90 Gy·min^{-1} using a Gammacell 220 irradiator. The irradiation doses were 0, 20, 40, 60, 80, 160, 250 and 350 Gy of gamma rays with ^{60}Co radioisotopes. The irradiated seeds showed acceptable germination (75.70 %) without visible damages, and survival was 53.62 % until 118 days after sowing. When unirradiated seed was used, the susceptibility of the crop could reach 60 % and the disease severity up to 34 %, with a disease progress rate of 0.006 units·day^{-1}. The 40 Gy dose offered the best response to control the disease; up to 58.80 g of dry weight was obtained for every 100 seeds and a production of 4,442 kg·ha^{-1}. In faba bean crop, up to 77 % of the variation in yield was due to severity of fungi, mainly the chocolate spot.

Additional keywords: Area under disease progress curve, mutagenesis, mutagenic effectiveness, radiosensitivity

RESUMEN

Susceptibilidad de los hongos, en especial, la mancha chocolate (*Botrytis fabae* Sard.) a la irradiación gamma en *Vicia faba* L.
Las enfermedades fungosas causan considerables pérdidas en muchas regiones productores de haba en el mundo. El objetivo de la presente investigación fue evaluar la susceptibilidad del cultivo de haba a estas afectaciones, en especial, la mancha chocolate, y su efecto en el rendimiento, a partir del uso de semillas expuestas a irradiación gamma. Las semillas de haba se cultivaron en tres regiones de México: Hidalgo, Puebla y Tlaxcala, y fueron irradiadas con el Gammacell 220 a una tasa de 4,90 Gy·min^{-1}. Las dosis de irradiación fueron: 0, 20, 40, 60, 80, 160, 250 y 350 Gy de rayos gamma con una fuente de radioisótopos ^{60}Co. Las semillas irradiadas mostraron aceptable germinación (75,70 %), sin daños visibles, con una supervivencia de 53,62 % hasta los 118 días después de la siembra. Cuando se utilizó semilla sin irradiar, la susceptibilidad del cultivo alcanzó el 60 % y la severidad de la enfermedad hasta 34 %, con una tasa de progreso de 0,006 unidad·día^{-1}. La dosis a 40 Gy es la que mejor respuesta ofreció para el control de la enfermedad, además de que se obtuvo hasta 58,80 g de peso seco por cada 100 semillas y una producción de 4442 kg·ha^{-1}. En el cultivo de haba, hasta el 77 % de la variación en el rendimiento se debió a la severidad del ataque de hongos, en especial, la mancha chocolate.

Palabras clave adicionales: Área bajo la curva de progreso, mutagénesis, efectividad mutagénica, radiosensibilidad

INTRODUCTION

The faba bean (*Vicia faba* L.) is an annual herbaceous plant (Fabaceae), cultivated in temperate climates. It has a high protein content besides carbohydrates, B vitamins, and minerals (Crépon et al., 2010). In Mexico, it is a seasonal crop with a planted area near 28,000 ha, and average yield of 1.23 Mg·ha^{-1}.

Among the diseases that affect the crop, the chocolate spot caused by the fungus *Botrytis fabae* Sard. is the most important in Mexico (Rojas et al., 2012) and other producing regions of the world (Etemadi et al., 2019). Globally, a reduction in bean production is observed because this crop is very susceptible to the disease (Terefe et al., 2015). It can cause a reduction in yield of 10 to 90 % (Tivoli et al., 2006), since producers

generally use susceptible local varieties and do not apply fungicides or some other control method (Kora et al., 2017). The chocolate spot is a fungal disease, economically important because it damages the foliage and all green tissues, limiting the photosynthesis and causes losses by reducing the yield and quality of the affected seed (Kora et al., 2017).

The disease is activated in lengthy periods of high humidity and temperature, promoting the change from a non-aggressive phase (chocolate color in green tissues) to an aggressive phase (gray-brown color) (Terefe et al., 2015). Field recognition of the fungus in its non-aggressive and aggressive phases makes it possible to easily distinguish it since it is dominant over other fungi. The chocolate spot affects practically all faba bean producing regions in the world and has been reported by growers of haba production areas of Mexico in the last 30 years; therefore, efficient strategies for the integrated management of the disease should be sought, such as the development of resistant varieties (Bouhassan et al., 2004).

Mutagenesis is an important tool to increase variability in autogamous plants, without altering their original genetic composition (Ambavane et al., 2015). It can increase the genetic variability of certain characters, both useful and harmful (Ambavane et al., 2015); making selection and the probability of getting the desirable genotypes more effective,

compared to conventional breeding methods (Raina et al., 2016).

Although there are several procedures used in mutagenesis, the use of gamma rays (^{60}Co) is one of the most used (Ulukapi and Ozmen, 2018). Gamma rays are less risky to human health than chemical mutagens (Khan and Tyagi, 2010); they are not toxic and do not require detoxification after use (Mba, 2013). Also, they have been used to develop tolerance to biotic-abiotic stress in plants grown from seed (Jain, 2010). Therefore, the objective of this work was to evaluate the susceptibility of the faba bean crop to fungal diseases, mainly chocolate spot and its effect on yield from the use of seeds exposed to gamma irradiation (^{60}Co).

MATERIALS AND METHODS

Faba bean seeds (with 12 % humidity and 5 months of storage) were collected in the town of Tenzoncuahuictic, municipality of Ixtacamaxtitlan, Puebla, Mexico. We worked with the botanical 'Major' variety and the experiment was carried out in winter 2018, at three sites (Table 1). The seeds (approximately 600) were irradiated in the facilities of the National Institute for Nuclear Research, in Mexico with Gammacell 220 irradiator at a dose rate of 4.90 Gy·min^{-1} (1 Gy = 1 J·kg^{-1}). The irradiation doses were 0, 20, 40, 60, 80, 160, 250 and 350 Gy of gamma rays with a source of ^{60}Co radioisotopes.

Table 1. Experimental sites with faba bean production and incidence of fungi, mainly chocolate spot, in Mexico, winter 2018

Site, State	N	W	ALT (m)	MAT (°C)	MAP (mm)
Apan, Hidalgo (AH)	19°41'09"	98°24'45"	2514	12.7	610
Tetela de Ocampo, Puebla (TP)	19°49'01"	97°47'36"	1764	13.9	971
Españita, Tlaxcala (ET)	19°27'41"	98°25'23"	2640	12.9	743

N: north latitude. W: west longitude. ALT: altitude. MAT: mean annual temperature. MAP: mean annual precipitation

At each site, 24 furrows (9 m each, 60 cm apart) were prepared, and one seed per hole was sown 30 cm between them, using three rows for each of the eight irradiation doses. The seeds were placed on both sides of the furrow, to promote the highest incidence of fungi in the plants. The management traditionally applied to the crop in each zone was followed.

The variables evaluated were the following:

Germination and survival. Observations of these variables were recorded from the seventh day after sowing, until harvest. For this purpose a total of 30 plants per treatment-site were selected randomly.

Mutagenic effectiveness and efficiency. Observations of these variables were recorded until the harvest in 30 random plants per treatment-site. Then, the following formulas,

suggested by Ambavane et al. (2015), were used:

$$Mutagenic\ effectiveness = \frac{M}{Doses\ of\ irradiation\ (Gy)}$$

$$Mutagenic\ efficiency = \frac{M}{L}$$

where M is the frequency expressed as percentage of chlorophyll mutation in M_2 generation, estimated on M_1 plant basis, Gy is a measure of the absorbed dose, and L is the percentage of lethality or reduction in survival.

Susceptibility and severity. Observations of these variables were recorded until harvest, in 30 plants randomly selected per treatment-site. For susceptibility, the scale proposed by Tivoli et al. (2006) was used and for severity (mean infection severity of the whole plant) the method of Hanouike and Hasanain (1986):

$$Susceptibility\ (\%)\ = \frac{diseased\ plants}{total\ number\ of\ plants}(100)$$

$$Severity\ (\%)\ = \frac{\Sigma\ (NxV)}{9\ N}(100)$$

Where N is the number of infected leaves. V is the numerical grade, and 9 is the higher degree in the category.

At all three sites, the prevalence of *B. fabae* was high and dominant over other fungi. It was easily recognized in its non-aggressive phase with chocolate color in green tissues, and gray- brown in its aggressive phase.

Area under disease progress curve (AUDPC). Disease severity values were recorded on 30 pre-tagged plants at weekly interval per treatment-site. They were calculated using the following formula, adapted by Jeger and Viljanen-Rollinson (2001):

$$AUDPC\ =\ D\ \left[\frac{1}{2}\ (Y_1 + Y_k) + (Y_2 + Y_3 + \ldots + Y_{k-1})\right]$$

where: Y_1, Y_2 … Y_k are the k disease scores at a constant interval of D days.

Also, regression equations were developed to describe the disease progress rate (DPR) at the different irradiation doses.

Yield and its components. In 30 randomly selected plants per treatment-site, seed yield (SY) and hundred-seed weight (HSW) were quantified. The relative percent yield and yield losses by component were determined using the following formula:

$$RYL\ (\%)\ = \frac{(Y_1 - Y_2)}{Y_1}(100)$$

where RYL is the relative yield loss. Y_1 is the

mean of the respective parameter in plants with irradiation. Y_2 is the mean of the respective parameter in plants without irradiation.

Also, a regression analysis between losses in seed yield and severity index of fungi was performed.

The experimental design was completely randomized with eight treatments and three repetitions. The data was subjected to one-way ANOVA, and the mean comparison tests were performed by the Tukey's ($P{\leq}0.05$) using Minitab Statistical Software v.18 ⟨State College, PA, USA⟩.

RESULTS AND DISCUSSION

Germination and survival. Faba bean germination started on day seven in TP and ended until day 16 in ET and AH (sites with drier conditions); the average value for Mexico, represented by these three sites was 75.70 %. In TP, the percentages were higher regardless of the irradiation dose, as it was a rainier and warmer site (971 mm and 13.9 °C, respectively). In Figure 1, it is observed that the doses of 20 to 350 Gy had a significant effect ($P{\leq}0.05$) on the germination percentage; but from 20 to 60 Gy the germination remained constant with respect to the seeds without irradiation; with an average of 90.83 %. As the irradiation dose was increased from 80 Gy, the germination percentage was reduced, up to 30.25 %, for the three sites. This indicates that doses lower than 60 Gy do not affect germination. In AH, the conditions less favorable for germination were found with only 50 % at 350 Gy, being up to 43 % lower, compared to TP (from 0 to 60 Gy). It is worth mentioning that, in non-irradiated seed, the percentages were high, from 89 to 93 % and it remained constant at low doses of 20 to 60 Gy. Up to 16 days after sowing (DAS), no visible symptoms of damage were observed in hypocotyl, cotyledon, epicotyl and nomophyles due to fungi attack in any site and irradiation dose.

The results showed that as the irradiation dose increased from 80 Gy, the germination percentage decreased. Various studies show that germination is affected with the increase in the irradiation dose, like in *Eleusine coracana* (Ambavane et al., 2015), in *Phaseolus vulgaris* (Ulukapi and Ozmen, 2018) and *Triticum monococcum* since germination results from a combination of many

cellular and metabolic events coordinated by a complex regulatory network of reactions (Well et al., 2018). In non-irradiated seeds, germination was 93 %. (Kiong et al., 2008) found that

irradiation influenced germination since it caused delays in nutrient mobilization to the embryo, decreased respiratory rate and reduced the number of internal regulators.
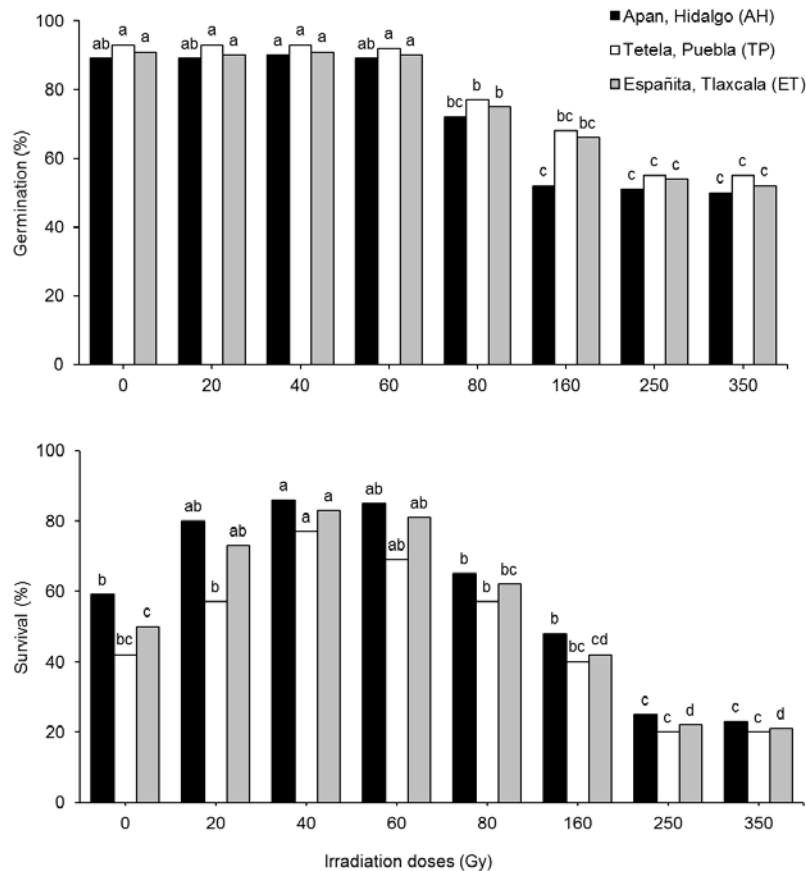

Figure 1. Germination and survival of faba beans from seeds irradiated with ^{60}Co (mutant M_1 generation) in three sites of Mexico. Means with the same letter in a column are not significantly different according to Tukey test ($P \leq 0.05$)

Survival of faba bean plants varied ($P \leq 0.05$) with the production environment and irradiation dose. Until harvest, the average value for Mexico represented by three sites (AH, TP and ET) was 53.62 %. At the site level, it was in AH where the highest percentages were obtained, independently of the irradiation dose, which contrasts with TP (a site with better germination conditions). In Figure 1, it is observed that, for the three sites, the doses of 40 to 60 Gy are the ones that most influenced the survival of plants, which suggests that these doses give the plants a certain resistance to chocolate spot. The lowest value was in TP (20 %) at 250-350 Gy, while the highest was in AH (86 %) at 40 Gy. When using seed without

irradiating, the survival percentages were low, from 42 to 59 %, but they are even lower when the doses are increased above 160 and up to 350 Gy. This suggests changes in the genotype and instability in the phenotypic expression, not necessarily due to the chocolate spot. If the seed is previously treated before sowing, with doses of 40 to 60 Gy, there may be up to 29.83 % more survival than when treatment with the mutagenic agent is not applied.

Like in germination, high doses of irradiation also contribute to the decrease in survival rate, although it could be increased up to 29.83 % when low doses of 40 to 60 Gy were applied. In general, survival was low in the three producing sites of

Mexico (AH, TP and ET) with an average of 53.62 %. Ulukapi and Ozmen (2018) concluded that low doses of irradiation stimulate the dry weight of the root and the aerial part of *Phaseolus vulgaris*, but a high doses affects survival. In Mexico, the site with the driest and coldest climate was AH, and it was where the highest survival (>80 %) was obtained. Kora et al. (2017) recommended that the sowing should be done when there is no high humidity or temperature, to reduce the aggressive phase of *B. fabae* and survival of the plants is not affected.

Mutagenic effectiveness and efficiency. The mutagenic effectiveness (index of the response of a crop to the increase doses of irradiation) and efficiency (proportion of mutations in relation to undesirable effects) were affected by the production environment (site) and the irradiation dose of the mutagenic agent (Table 2). But, regardless of the site, effectiveness and efficiency decreased as the irradiation dose increased up to 350 Gy, because 250 and 350 Gy doses produced many dead plants and plants with sterile pods, leading to a decrease in mutagenic efficiency. The average effectiveness for Mexico, represented by the three sites was 0.53, and the average efficiency was 1.09. In general, the maximum effectiveness was recorded at 40 Gy with values from 0.791 to 0.814 and the maximum efficiency, also at 40 Gy with values from 1.21 to 1.29. The highest effectiveness was obtained in AH at 40 Gy with 0.814 while the highest efficiency was also obtained in AH at 40 Gy with 1.29. Gamma irradiation can induce useful or harmful mutations in plants (Ambavane et al., 2015). Its effectiveness and efficiency generally decrease when the irradiation doses increase, as happened in the present study in faba bean plants at 250 and 350 Gy. Similar results were obtained in *Glycine max* (Khan and Tyagi, 2010), *Lens culinaris* (Wani, 2017) and *Vigna radiata* (Wani et al., 2017). Because in Mexico the effectiveness average and efficiency shown before were low, these values may have been affected by the environmental condition of the sites.

Table 2. Mutagenic effectiveness and efficiency in the M_1 generation of faba bean irradiated with ^{60}Co, cultivated in three sites of Mexico: AH (Apan, Hgo.), TP (Tetela, Pue.) and ET (Españita, Tlx.)

Doses (Gy)	Mutagenic effectiveness			Mutagenic efficiency		
	AH	TP	ET	AH	TP	ET
0	---	---	---	---	---	---
20	0.708	0.660	0.679	1.20	1.15	1.18
40	0.814	0.791	0.802	1.29	1.21	1.25
60	0.790	0.753	0.775	1.23	1.18	1.20
80	0.665	0.629	0.654	1.05	1.04	1.04
160	0.430	0.400	0.415	1.02	1.01	1.02
250	0.237	0.208	0.223	1.00	1.00	1.00
350	0.211	0.195	0.200	1.00	1.00	1.00

Susceptibility and severity. The average susceptibility for Mexico, represented in three sites, was 27.97 %. However, it was in TP where higher values were obtained, attributed to the fact that in this region the average temperature (13.9 °C) and precipitation (971 mm) are higher, throughout the year, compared to ET and AH. Irradiation doses of 40 to 350 Gy increased the plant susceptibility (Figure 2), although very low doses may not have an effect on disease control and very high doses seem to have damaged the embryo.

However, this percentage did not decrease as the dose increased to 350 Gy, since in the three sites low doses (from 40 to 60 Gy) offered a better response to decrease the plant susceptibility to the fungus. At 20 Gy, there was no change in the plants that favored the lowest percentage; the same happened in doses from 160 to 350 Gy, where this percentage gradually increased. The site with the lowest percentage of susceptibility was AH with 10.60 % at 40 Gy, being 5.6 times lower, compared to the site with the highest values (TP at 0 Gy) with 60.06 %.

Regardless of the site where the faba bean is grown, when the seed is not treated before sowing with gamma irradiation, the percentage of susceptibility is high, from 39.63 to 60.06 %

and always higher than at any other dose. The lesions caused by the chocolate spot in the faba bean crop, were more frequent and severe in the leaves than in other plant organ. The average severity for Mexico, represented by three sites was 17.65 %.
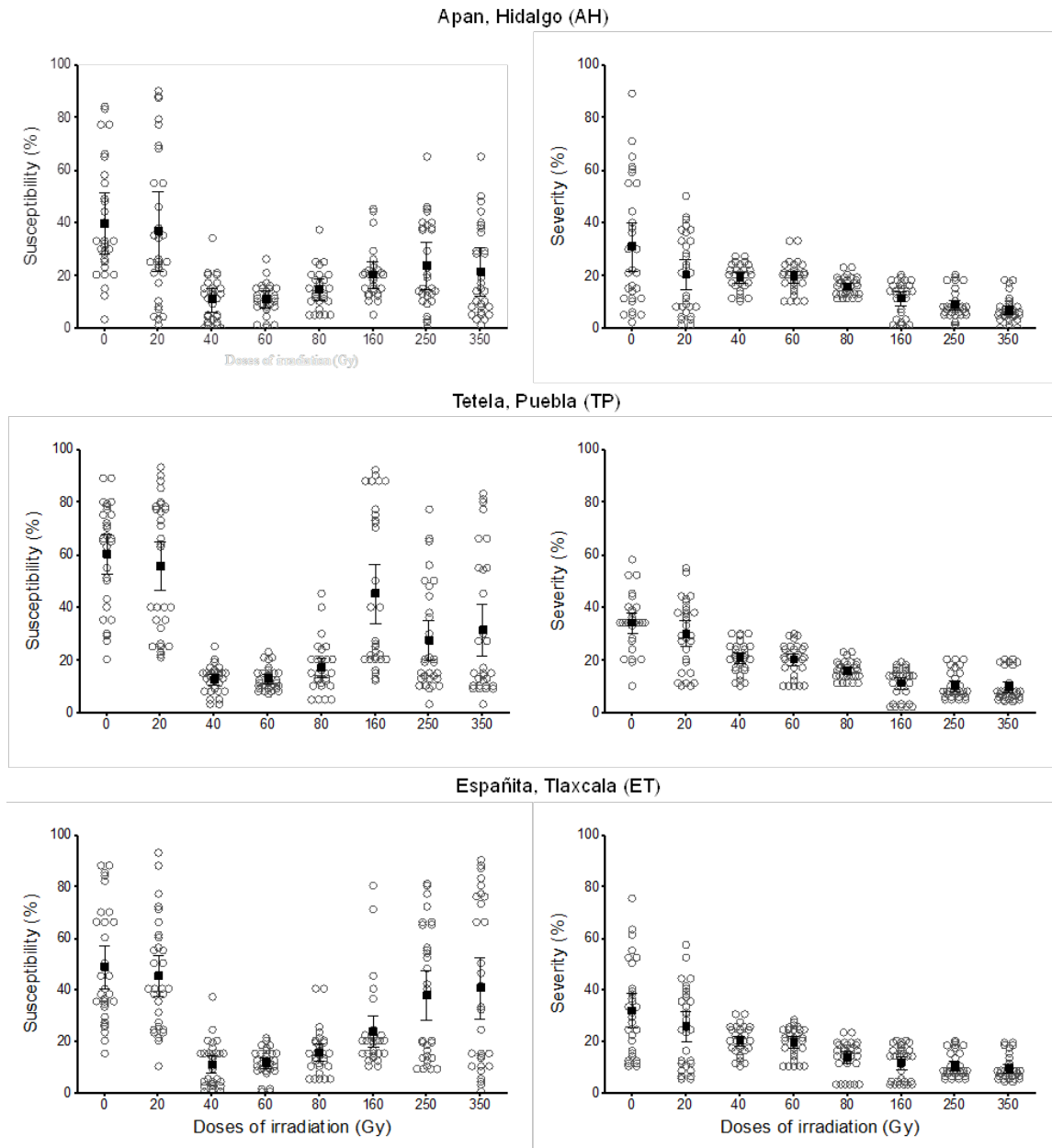

Figure 2. Susceptibility to fungi and disease severity in faba bean cultivation from irradiated seeds in three sites of Mexico. Black squares: the mean values. Vertical bars: the standard error

However, AH was the site with the lowest mean temperature (12.7 °C) and the lowest rainfall (610 mm), where the attack by fungi was less severe, with only 16.47 %. For the three sites, as the irradiation dose was higher (up to 350 Gy), the percentage of severity in the leaves was lower, with only 6.76 %. It is worth mentioning that when the seed for sowing is not irradiated, the severity of the disease can be high and varied from 30.76 to 34 %, but this value decreases up to five times if it is irradiated with 350 Gy. This suggests that the irradiation doses of 20 to 350 Gy

contribute in reducing the percentage of the disease severity (Figure 2).

In response to invasions by necrotrophic fungi, like *B. fabae*, plants develop different defense mechanisms, like lignification of the cell wall, production of phytoalexin, and acquisition of systemic resistance (Shetty et al., 2008). However, the effects of tissue age on the expression of defense-associated compounds have not been studied in detail since the production of defense compounds may assist necrotrophic pathogens to colonize healthy host tissues (El-Komy, 2015). The lesions of the chocolate spot were greater in lower leaves (older), than in the upper ones (younger) and the susceptibility did not decrease as the irradiation dose increased to 350 Gy. There was a positive effect using doses of 40 to 60 Gy since the susceptibility decreased, compared to the control treatment (0 Gy), which showed a value of 60.06 %. Villegas-Fernández et al. (2012) concluded that the resistance-susceptibility of *V. faba* against *B. fabae* appears to be more genotype specific than tissue age. The severity also decreased when the irradiation dose increased; the average value for the Mexican sites was 17.65 %, a much lower value than the 47.9 % reported by Villegas-Fernández et al. (2012). In the same way, Sahile et al. (2010) found an incidence of the disease of 47 to 100 % and a severity of 17 to 49 %, when precipitation and planting density were high, confirming that the susceptibility and severity of the chocolate spot varied between sites, due to altitude, temperature and precipitation.

Area under disease progress curve (AUDPC). The irradiation doses influenced the progress of the disease in the field, with statistical differences ($P \le 0.05$) in each site where *B. fabae* is present (Table 3). As the irradiation doses were higher the AUDPC decreased; the maximum AUDPC occurred in TP with 1842 % in days, when no irradiation was applied. The use of gamma irradiation prevented the progress of the disease up to 68.7 % at 350 Gy, regardless of the site. In AH, the average of the AUDPC was lower with 950.2 % in days, compared to TP or ET. This represents a reduction of the disease progress of 21.7 % in days in AH (less humid and colder) compared to TP (more humid and warmer site).

Table 3. Area under disease progress curve (AUDPC) for fungi, mainly chocolate spot, in three sites of Mexico: AH (Apan, Hgo.), TP (Tetela, Pue.) and ET (Españita, Tlx.) at different irradiation doses

Sites	(% days)								
	0 Gy	20 Gy	40 Gy	60 Gy	80 Gy	160 Gy	250 Gy	350 Gy	Mean
AH	1417 a	1313 b	1200 c	1054 d	979 d	582 e	571 e	486 e	950.2
TP	1842 a	1706 b	1551 c	1382 d	1218 e	773 f	688 f	550 g	1213.7
ET	1650 a	1548 a	1400 b	1278 c	1094 d	669 e	635 e	500 f	1096.7
Mean	1636.3	1522.3	1383.6	1238	1097	674.6	631.3	512	

Means with the same letter in each file are not significantly different according to Tukey test ($P \le 0.05$)

A regression equation based on the logistic model was used to describe the disease progress rate (DPR) of the chocolate spot in faba bean plants at different irradiation doses. The determination coefficient (R^2) varied from 75.40 to 83.68 in non-irradiated seeds, and from 29.66 to 55.45 % in seeds irradiated with 350 Gy (highest dose), taking the severity a linear form, with decrements as the irradiation increased (Table 4). The analysis showed differences ($P \le 0.05$) from 20 Gy onwards.

In the AH, TP and ET sites, where unirradiated seeds were used, the chocolate spot increased at a rate of 0.033 to 0.037 units·day^{-1}. This rate was exceeded approximately seven times due to the application of gamma irradiation at different doses (from 20 to 350 Gy); the 350 Gy dose being the one that significantly reduced the DPR in the three sites. The highest DPR was in TP with an average of 0.006 units·day^{-1}. In this site, the highest AUDPC was recorded, reaching up to 1482 % in days, at a dose of 0 Gy, attributed to the fact that in this site, with humidity and temperature higher than in AH and ET, the conditions could have favored the onset of infection and a more accelerated DPR.

Table 4. Disease progress rate (DPR) by fungi, mainly chocolate spot, in three sites of Mexico: AH (Apan, Hgo.), TP (Tetela, Pue.) and ET (Españita, Tlx.), at different irradiation doses

Sites	Doses (Gy)	DPR (units·day⁻¹)	R^2 (%)[b]	Significance (P)
AH	0	0.033	77.12	0.05
	20	0.002	55.40	0.02
	40	0.003	70.27	0.00
	60	0.004	68.52	0.00
	80	0.005	62.91	0.03
	160	0.008	63.93	0.02
	250	-0.005	54.78	0.00
	350	-0.006	55.45	0.03
	Mean	0.005	63.54	
TP	0	0.037	75.40	0.04
	20	0.003	50.08	0.05
	40	0.004	48.71	0.03
	60	0.005	47.93	0.00
	80	0.006	53.12	0.01
	160	0.009	43.74	0.01
	250	-0.007	28.94	0.02
	350	-0.008	29.66	0.05
	Mean	0.006	47.19	
ET	0	0.034	83.68	0.02
	20	0.002	58.95	0.05
	40	0.003	47.87	0.03
	60	0.004	50.16	0.00
	80	0.005	65.98	0.04
	160	0.008	66.87	0.02
	250	-0.006	27.91	0.04
	350	-0.007	37.60	0.05
	Mean	0.005	54.87	

[a]Standar error of main factor. [b]Coefficient of determination or proportion explained by the model

Kora et al. (2017) found similar results with 1282 % in days, in Ethiopia, and Terefe et al. (2015) concluded that the chocolate spot can reach epidemic levels in a few days. Therefore, irradiation of the seeds before sowing helps to decrease AUDPC when the doses increased. However, low AUDPC values were not necessarily related to a higher yield, since at 350 Gy the SY was only 240 kg·ha⁻¹ (Table 5) and the highest yields were obtained with 40 Gy. This finding contrasts with the values obtained by El-Sayed et al. (2011) in five faba bean cultivars. Irradiation at 40 Gy increased the yield and decreased DPR, while in the non-irradiated seeds the DPR increased from 0.033 to 0.037

units·day⁻¹, that is, seven times higher.

As in the other variables, AUDPC and DPR are favored by conditions of low relative humidity and low temperature (El-Komy et al., 2015; Haile et al., 2016).

Yield and its components. The interaction between irradiation dose and sites showed statistical differences (P≤0.05) in yield of faba bean (Table 5). In AH, the highest SY was 4,442 kg·ha⁻¹ obtained with 40 Gy. At the site level, the highest SY occurred in AH with an average of 2,508.87 kg·ha⁻¹. The lowest relative yield loses (RYL) of SY were obtained with 40 Gy (no losses) in the three sites, while the highest RYL of SY was found at a dose of 350 Gy in TP.

Important increases of RYL were recorded with doses below 40 Gy and above 60 Gy in the three sites. The doses of 40 to 60 Gy were the ones that offered the best response to the RYL of SY.

Table 5. Yield components of faba bean and losses due to fungi, mainly chocolate spot in three sites of Mexico: AH (Apan, Hgo.), TP (Tetela, Pue.) and ET (Españita, Tlx.), at different irradiation doses

Sites	Doses (Gy)	SY (kg·ha⁻¹)	RYL (%)	HSW (g)	RYL (%)
AH	0	1280 de	31.94	46.68 cd	12.30
	20	3881 b	12.28	50.71 ab	2.50
	40	4442 a	0.00	58.80 a	0.00
	60	4009 b	4.55	53.91 a	1.34
	80	3550 bc	24.36	47.82 bc	2.93
	160	2396 cd	36.11	43.90 cd	13.05
	250	273 f	54.73	40.82 e	18.39
	350	240 f	59.41	40.71 e	18.44
	Mean	2508.87		47.91	
	CV (%)	11.86		5.35	
TP	0	1055 de	35.84	44.57 cd	14.61
	20	3122 c	19.13	47.83 bc	2.71
	40	3527 bc	0.00	54.52 a	0.00
	60	3495 bc	7.96	49.99 ab	1.84
	80	2400 cd	26.39	45.44 cd	3.50
	160	1301 de	39.14	42.55 de	13.93
	250	264 f	56.01	40.18 e	18.80
	350	243 f	61.63	40.21 e	18.81
	Mean	1925.87		45.66	
	CV (%)	19.27		3.71	
ET	0	1263 de	33.78	45.66 cd	12.87
	20	3517 bc	15.82	48.32 bc	2.58
	40	4082 b	0.00	54.05 a	0.00
	60	4000 b	7.77	51.08 ab	1.70
	80	3384 c	25.58	47.55 bc	3.50
	160	2020 cd	36.85	42.16 de	13.52
	250	269 f	54.83	41.03 de	18.78
	350	256 f	61.22	41.00 de	19.00
	Mean	2348.87		46.35	
	CV (%)	12.60		5.94	
CV (%)		14.57		5.00	

SY: seed yield. RYL: relative loss. HSW: hundred seed weight. Means with the same letter in a column are not significantly different according to Tukey test ($P \leq 0.05$). CV: coefficient of variation

The effect of irradiation doses was also seen in HSW in the three sites (Table 5). At the site level, the highest mean was 47.91 g, obtained in AH, which was the least susceptible site for the disease progression. The highest HSW was 58.80 g, obtained in AH, with a dose of 40 Gy. The lowest was 40.18 g, obtained in TP with 250 Gy. The highest RYL of HSW were from 250 to 350 Gy (high doses), with 18.39 to 19.00 %, while from 40 to 60 Gy (low doses) the losses were minimal (0 to 1.84 %) in any site.

The average yield obtained in this investigation (2508.87 kg·ha⁻¹) are lower than that reported by Kora et al. (2017), within the range of those obtained by Etemadi et al. (2018), and higher than the average yield of small producers (CSA, 2014), as well as the yield of improved cultivars in Ethiopia (Sahile et al., 2010). Yield variations may be attributed to genetic variability, seed size, planting density, seed purpose (fresh or dry consumption) and chocolate spot incidence (Etemadi et al., 2019).

The relationship between yield components is compensatory and mutually interdependent; because a significant change in a yield component does not necessarily affect the final yield in SY. However, it is possible to obtain a higher SY and HSW when all yield components are maximized (Mirshekari et al., 2013). The linear regression of the severity index due to fungi in the yield of faba bean and its components, revealed statistical differences between sites and irradiation doses (Figure 3). In AH, the slope of the regression line was (-75.381) indicates that for each unit increase in the percentage severity index, there was a loss of SY of 75.381 kg·ha^{-1} at 118 DAS. And based on the coefficient of determination (R^2), the equation explains that approximately 77 % of the variation in yield was due to the severity of the disease. Similar considerations can be made for the other two sites, and confirm that the plant yield is highly influenced by the severity of the disease and indicates that the linear regression of the percentage of severity can be used to predict the loss of yield in faba beans with a high coefficient of determination.
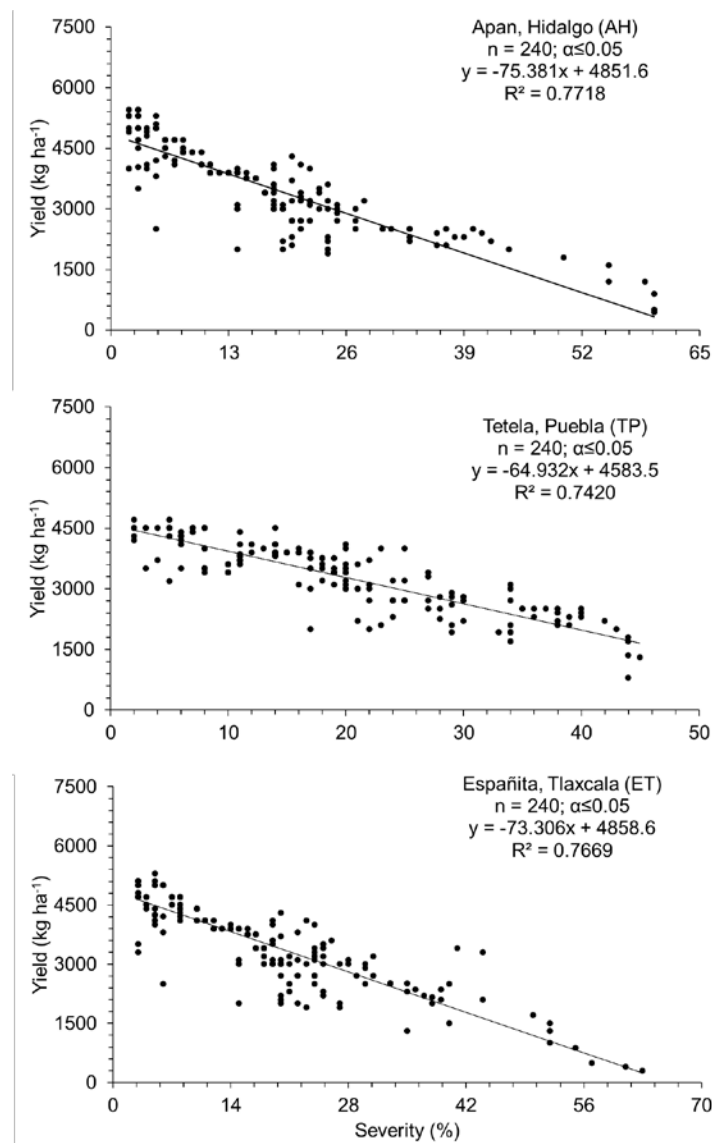

Figure 3. Relationship between losses in faba bean seed yield and severity index of fungi, mainly chocolate spot, at 118 DAS in three sites of Mexico

According to the averages of the regression coefficients of the lines and their corresponding R^2 shown in Figure 3, it was obtained that up to 118 DAS, the RYL of SY average in the Mexican studied sites were 71.20 kg·ha^{-1} due to the presence of the disease, and of these RYL, 76.02 % was attributed to the severity of the disease. This result confirms that of Sahile et al. (2010) who concluded that the severity of the disease is negatively related to yield and its components.

CONCLUSIONS

The irradiation doses of 20 to 350 Gy of gamma rays to seeds influenced germination, survival, susceptibility and crop yield and they reduce the severity and progress of the diseases, mainly the chocolate spot. A low dosage of 40 Gy offered the best response for the control of the disease since high doses of 250 and 350 Gy, reduced germination, survival and produce many sterile pods, leading to a decrease in mutagenic effectiveness and efficiency. It was determined that the coldest and least humid producing regions are related to a lower susceptibility and severity of fungal diseases as well as with a higher faba bean yield.

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