EXPERIMENTAL RESULTS

In this study, a combination of morphological approach, light microscopy, histochemistry, biochemical analysis and molecular analysis has revealed that:

- Induced changes (phenotypic reactions induced) on cellular and morphological levels in the tested cultivars,
    - No visible reactions
    - Macroscopically visible reactions
- Next, the induced reactions were investigated in more detail using light microscopy. Hypocotyls tissue of french bean and soybean were analyzed at 0.0, 6, 8, 12, 24, 36 and 48 hours after inoculation. The first morphorlogical changes were only observed at 6 hours at 30 °C and 12 hours at 20 °C, which further increased within 24h.

To further analyze the cellular changes induced by the isolates of fungal pathogens under study, histochemical analysis was also performed to inspect cell wall reactions in the form possible lignification. Figures 32, 34 and 57 give an overview of the lignified cell wall visualized within a plant cell using toluidine blue, cellular changes are accompanied by hypersensitivity. Cell death was observed (i.e. macro and microscopically visible cell death).

The microscopic analysis is an excellent technique to uncover potential triggering defense mechanisms that are operating early in the resistance induced in the studied pathosystems combinations of this study. Representative example photographs are shown.

I. Pathogenicity test

Pathogenicity test was performed with all the cultivars used of french bean and soybean Table (3), using the original isolates of each the french bean and soybean pathogenic isolates, Table (3), following Kotch postulates. The reisolated isolates from each of the studied pathogens were also tested for their pathogenicity on the specified cultivars. The list of tested cultivars for each of french bean and soybean and the tested fungal pathogens were indicated in Table (3). The reaction of all of the cultivars tested for french bean and soybean to inoculation with each of the pathogenic isolates (the original and the reisolated ones), at incubation temperature, 20 °C for french bean and 30 °C for soybean cultivars showed susceptibility except the bean cultivar Nebraska that showed intermediate reaction tendes towards resistance (Ir) whilest the soybean cultivar Crowford showed intermediate reaction towards susceptibility (Is) Table (4) and Figures (10, 12, 21 and 22).

This study was further performed using only four cultivars, two from each of the studied french bean and soybean; namely, cvs. Nebraska and Bronco; cvs. Crowford and Giza 22 respectively. This was based on the previous mentioned reaction types found in this work for cvs. Nebraska and Crowford but for the other two selected cultivars i.e. Bronco and Giza 22, they were selected because they had been previously studied by other researchers and were susceptible **(Abdallah, 1987).**

Table (3): The tested cultivars of each of french bean and soybean and the original isolates of the tested target fungal pathogens (refer to materials and methods).

Fungal pathogens	French bean (*Phaseolus vulgaris* L.) cultivars	Soybean (*Glycine max* L.) cultivars
F. solani f.sp. *phaseoli*	Nebraska	Crowford
F. solani f.sp. *glycine*	Bronco	Giza 22
C. lindemothianum	Giza 3	Giza 35
	Giza 4	Giza 111

The resulted reactions of the disease severity were devised based on the infection types that determined by evaluating each hypocotyls on arbitrary scale were according to the following:

Day	Score	
1	0	= no visible symptoms.
2	1	= light browning within the inoculation sites.
3	2	= moderate browning of small lesions < 5 mm long.
4	3	= dark browning accompanied with partial rotting.
5	4	= dark browning of large dark lesions > 5 mm long with rotting.
6	5	= complete rotting with dark browning a long the whole hypocotyls.

For the resistance reaction types, the following reactions were monitored:

Day	Score	
1	0	= no visible reactions localized within the inoculation sites (immune response).
2	1	= light brown small flecks within the inoculation site.
3	2	= numerous dark brown flecking.
4	3	= numerous dark brown streaks localized within the inoculation sites.

This score was monitored from the time of inoculation up to 10-12 days after inoculation.

Table (4): Cultivars reactions to inoculation with each of the pathogenic isolates (the original and the reisolated ones) at incubation temperature 20 °C for french bean and 30 °C for soybean.

Fungal isolate	Incubation temperature (°C)	French bean cultivars		Soybean cultivars	
		Nebraska	Bronco	Crowford	Giza 22
A	20	Ir	S	-	-
A	30	-	-	Is	S
B	20	Ir	S	-	-
B	30	-	-	Is	S

A = the original isolate

B = the reisolated isolate

Ir = intermediate reaction tending towards resistance

Is = intermediate reaction tending towards susceptibility

S = susceptible, longitudinal spreading streak, accompanied with collapse and rotting of hypocotyl tissue

The following results represented the pathogenicity test for:

1- Whole plants (french bean and soybean)

2- Excised organs

2.1- Intact hypocotyls

2.2- Pods

An experiment was carried out to check the susceptibility and resistance in french bean (*Phaseolus vulgaris* L.*)* and soybean (*Glycine max* L.) towards *Fusarium solani* f.sp. *phaseoli* at room temperature (25-30°C) using "Growing plant inoculation technique".

The reaction was done using *Fusarium solani* f.sp. *phaseoli* (as a target pathogen). Four cultivars were used, for french bean cvs., Nebraska and Bronco, and for soybean cvs. Crowford and Giza 22.

Fig. 1. Reaction of french bean (*Phaseolus vulgaris* L.) cv. Nebraska, 25 days after inoculation with *F. solani* f.sp. *phaseoli* at room temperature (25-30°C). Note the very localized fungal mycelium (arrowed) and the normal growth of cv. Nebraska with very slightly general weakness in very few number of individuals indicating the resistance of cv. Nebraska.

Fig. 2. Uninoculated (control) cv. Nebraska, 10 days after the seedling in sandy soil at room temperature.

Fig. 3. Uninoculated (control) cv. Nebraska, 15 days after the seedling in sandy soil at room temperature.

Fig. 4. Uninoculated (control) cv. Nebraska , 25days after the seedling in sandy soil at room temperature.

Fig. 5. Uninoculated (control) cv. Nebraska, 50 days after the seedling in sandy soil at room temperature. Note the shape of the pods.

(A)

(B)

Fig. 6. (A), reaction of cv. Bronco , 15 days after inoculation with *F. solani* f.sp. *phaseoli* at room temperature (25-30^{o}C). (B), reaction of cv. Bronco, 25 days after inoculation with the same fungus at room temperature. Note the severity of disease, where grade 1 involved yellowing of the leaves and dryness of leaf edges (R_1), grade 2 with distortion of secondary leaves and stunting of some individuals (R_2) and grade 3 that contained the more sever disease where some cotyledenary leaves are not fully opened and some secondary leaves became dried (R_3), note also there was no further progress in plant growth (B).

Fig. 7. Uninoculated (control) cv. Bronco, 15 days after the seedling in sandy soil at room temperature.

Fig. 8. Uninoculated (control) cv. Bronco, 50 days after the seeding in sandy soil at room temperature.

Fig. 9. Uninoculated (control) cultivars , the black arrow shows the pods of cv. Nebraska, While the white arrow refers to the pods of cv. Bronco (Control), 55 days after the seedling in sandy soil at the room temperature.

Fig. 10. Reaction of hyppcotyls of cv. Nebraska intermediate resistant (Ir), 10 days after inoculation with *F. solani* f.sp. *phaseoli* at 20 °C. Note the light brown small flecks within the inoculation site accompanied with partial rotting.

Fig. 11. Reaction of hypocotyls of cv. Bronco (susceptible), 2 days after inoculation with *F. solani* f.sp. *phaseoli* at 20 °C. Note the light brown flecks with partial rotting.

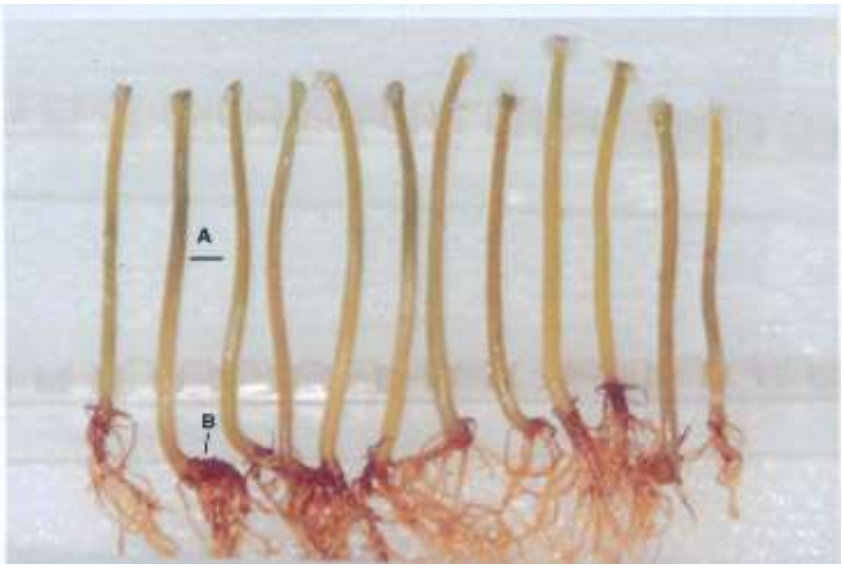
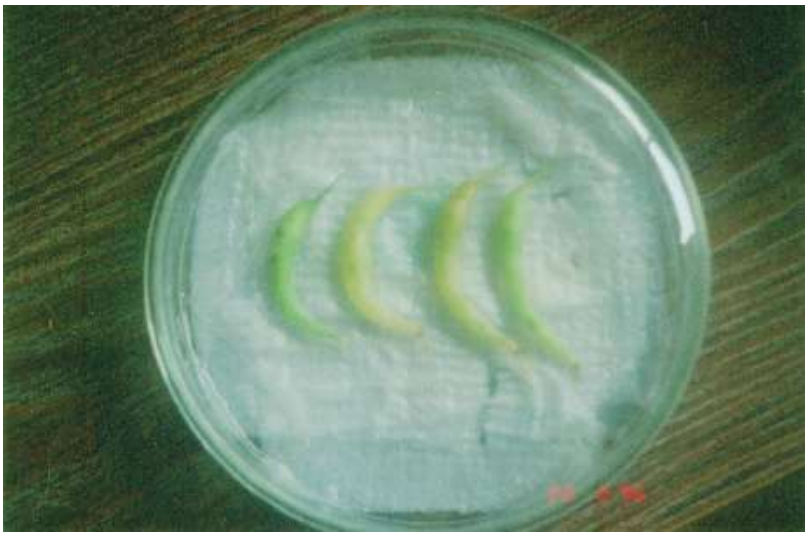


Fig. 12. Reaction of hypocotyls of cv. Bronco (susceptible), 10 days after inoculation with *F. solani* f.sp. *phaseoli* at 20 °C. Note the complete rotting with dark browning along the whole hypocotyls.

(A)



(B)

Fig. 13. (A), Reaction of pods of cv. Nebraska intermediate resistant (Ir) , 1 day after inoculation with *F. solani* f.sp. *phaseoli* at room temperature (25-30°C) B, Reaction of pods of cv. Bronco (susceptible), 1 day after inoculation with *F. solani* f.sp. *phaseoli* at the same temperature. Note the more resistance of cv. Nebraska than that of cv. Bronco where in (A) one pod only (the young one in age) that affected by the infection and lost its green pigment (chlorophyll) with brown regions but in (B) two pods from four was affected by the infection .

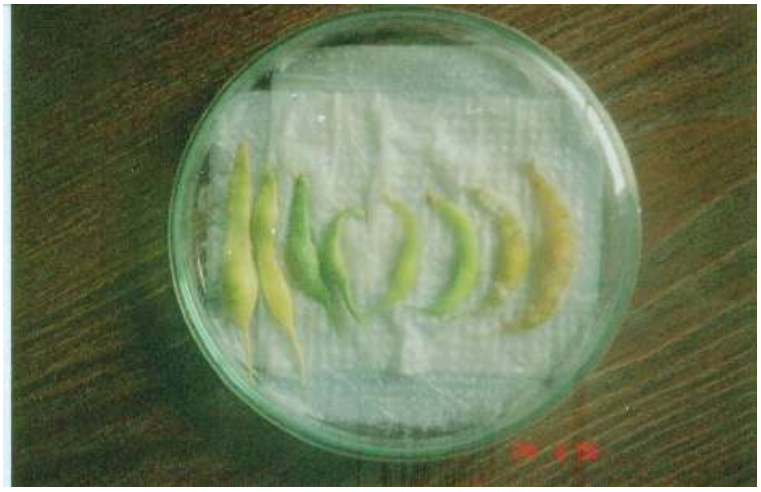


Fig. 14. Reaction of pods of cv. Nebraska intermediate resistant (Ir) on the left, and cv. Bronco (susceptible) on the right, 2 days after inoculatin with *F. solani* f.sp. *phaseoli* at room temperature (25-30^{o}C). Note the susceptibility of pods of cv. Bronco where the fungus grow on the pod surface with appearance of brown rotting , on the other hand , the pods of cv. Nebraska appeard with more resistant with losing of chlorophyll without any rotting.

Fig. 15. Reaction of soybean (*Glycine max* L.) cv. Crowford, 10 days after inoculation with *F. solani* f.sp. *phaseoli* at room temperature (25-30^{o}C). Note the presence of fungal mycelium and light browning of hypocotyls. Note also the reaction grade (R) of the cotyledenary leaves, where they appeared with partial necrosis, yellowing, chlorosis and they are not fully opened (R_3) and note that became fully opened (R_2) also note the opened cotyledenary leaves that followed by secondary leaves (R_1).

Fig. 16. Reaction of soybean cv. Crowford , 15 days after inoculation with *F. solani* f.sp. *phaseoli* at room temperature (25-30°C). Note the hypocotyls browned and roted as plants developed further (arrowed).

Fig. 17. Uninoculated (Control) cv. Crowford, 20 days after the seedling in sandy soil at room temperature.

Fig. 18. Reaction of soybean cv. Giza 22 (as a whole plant), 10 days after inoculation with *F. solani* f.sp. *phaseoli* at room temperature (25-30^{o}C). Note the presence of fungal mycelium on the hypocotyls (arrowed) Note also the brown rot of the hypocotyls (arrowed) and the cotyledenary leaves are not fully opened.

(A)

Fig. 19. Uninoculated (control) cv. Giza 22, 10 days after the seedling in sandy soil at room temperature.

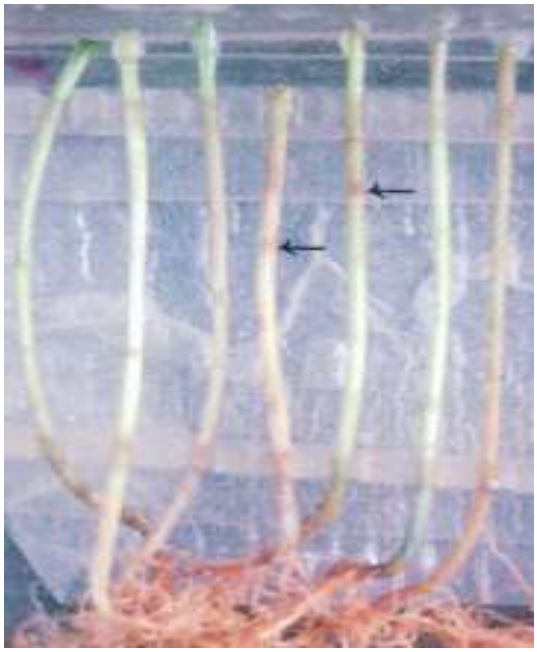


Fig. 20. Reaction of hypocotyls of cv. Crowford intermediate susceptible (Is), 2 days after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the light brown large flecks with partial rotting.

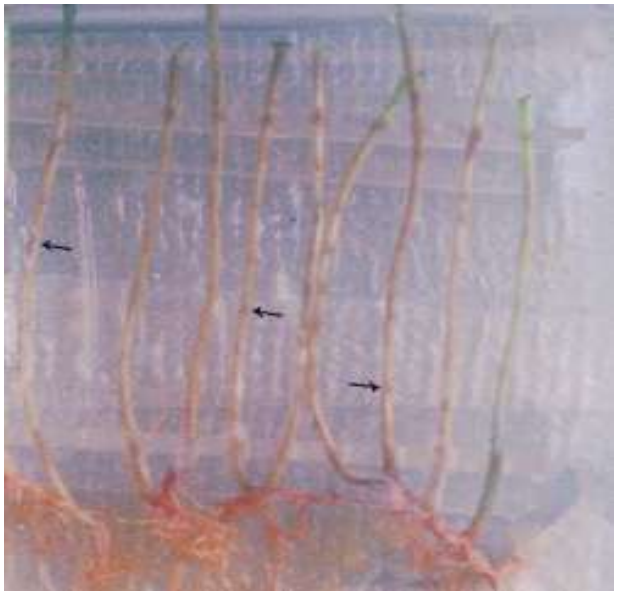


Fig. 21. Reaction of hypocotyls of cv. Crowford intermediate susceptible (Is), 5 days after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the numerous dark brown flecking and dark browning of large dark leasions with rotting accompanied with appearance of fungal mycelium.

Fig. 22. Reaction of hypocotyls of cv. Giza 22 (susceptible), 5 days after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the complete rotting with dark browning along the whole hypocotyls and note the appearance of fungal hyphase that spreaded along the hypocotyls and the root system.

Conclusion:

From these results it is apparent that at room temperature cv. Nebraska was on a degree of resistance (Ir) Fig. (1), also the reaction results of the four cultivars tested to inoculation with *F. solani* f.sp. *phaseoli* at incubation temperature 20 °C for french bean and 30 °C for soybean were similar that appeared at room temperature where cv. Nebraska behaved intermediate resistant (Ir) Fig. (10) and showed that cv. Crowford was intermediate susceptible (Is) Fig. (20 & 21), while cv. Bronco and Giza 22 were susceptible (s) Fig. (12 & 22 respectively).

II. Induced resistance of french bean and soybean against the target pathogens *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *Glycine* and *C. lindemothianum*.

The following reaction types, induced as a result of inoculating the selected cvs. of each of french bean and soybean hypocotyls (intact) with the non pathogenic Fusarium potato isolate, that is served as a biotic elicitor in this study, and 4 hours later inoculated with each of the pathogenic isolates of the three pathogens i.e. *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum.* Both of the tested isolates (the original and the reisolated one) for all the three target pathogens behaved similarly, Table (5).

Table 5

Cultivars reactions to each of the tested isolates *F. solani* f.sp. *phaseoli* , *F. solani* f.sp. *glycine* and *C. lindenothianum* (The original and the reisolated one) at four different incubation temperatures

Fungal isolate	Incubation temperatue (°C)	Cultivars * of *Phaseolus vulgaris* L.		Cultivars of *Glycine max.* L.	
		Nebraska	Bronco	Crowford	Giza 22
A	20	R	R	R	Is
A	25	R	Is	R	Is
A	30	Ir	S	Ir	S
A	35	S	S	S	S
B	20	R	R	R	Is
B	25	R	Is	R	Is
B	30	Ir	S	Ir	S
B	35	S	S	S	S

*= intact hypocotyls (excised) of 10 days old seedlings of different cultivars of bean and soybean .

A = The original isolate.

B = The reisolated isolate.

R = resistant , hypersensitive flecks only .

S = Susceptible , longitudinal spreading streak , accompanied by collapse and rotting of hypocotyl tissue.

Ir = intermediate reaction tending towards resistance .

Is = intermediate reaction tending towards susceptibility.

From the above results it is apparent that the two isolates behaved the same.

It was clear as revealed by the size of necrotic lesions was reduced visibly on the induced hypocotyls (Fig.27) indicating that the non pathogenic isolate (Fusarium potato isolate) could be effectively used in protecting the Fusarium diseases of french bean and soybean of this study. Also has the ability to induce resistance against further infection by the pathogenic *Colletotrichum lindemothianum*.

II.1. Infection of french bean and soybean intact hypocotyl by *F. solani* f.sp. *phaseoli* (the causal pathogen of root and hypocotyl rot of french bean and soybean):

Experiments were carried out to check the resistance and susceptibility in bean and soybean cultivars at four different temperatures, i-e. (20, 25, 30 and 35°C). The obtained culture of the pathogen as well as the reisolated one was each inoculated onto hypocotyls when plants were 9-10 days old.

Infection sites were closed resistant when hypersensitive flecks were seen 1-2 days after inoculation and infection progressed no further. They were classed susceptible when longitudinal spreading streaks accompanied by collapse and rotting of hypocotyl tissue were observed.

More details can be deduced as follow:

1) At 20°C:

a- Macroscopic observations carried out 2 days after inoculation demonstrated the development of light brown necrotic flecking below the position of inoculation in all cultivars. In cultivars of both french bean and soybean, light brown necrotic flecks appeared 2 days after inoculation, and remained unchanged for 10 days after inoculation.

Fig. 23. Reaction of hypocotyls of cv. Nebraska, 10 days after inoculation with *F. solani* f.sp. *phaseoli* at 20°C . Note light brown necrotic flecks in infection sites on the hypocotyls.

Fig. 24. Uninoculated (control) hypocotyls of cv. Nebraska

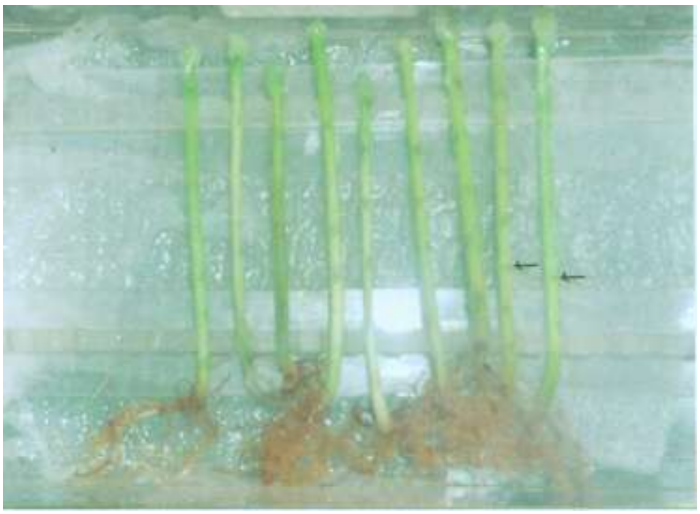


Fig. 25. Reaction of hypocotyls of cv. Bronco , 10days after inoculation with *F. solani* f.sp. *phaseoli* at 20°C Note the localized light brown necrotic flecks on the hypocotyls

Fig. 26. Uninoculated (Control) hypocotyls of cv. Bronco

Fig. 27. Reaction of hypocotyls of cv. Crowford, 10 days after inoculation with *F. solani* f.sp. *phaseoli* at 20°C Note the limited light brown necrotic flecks on the hypocotyls.

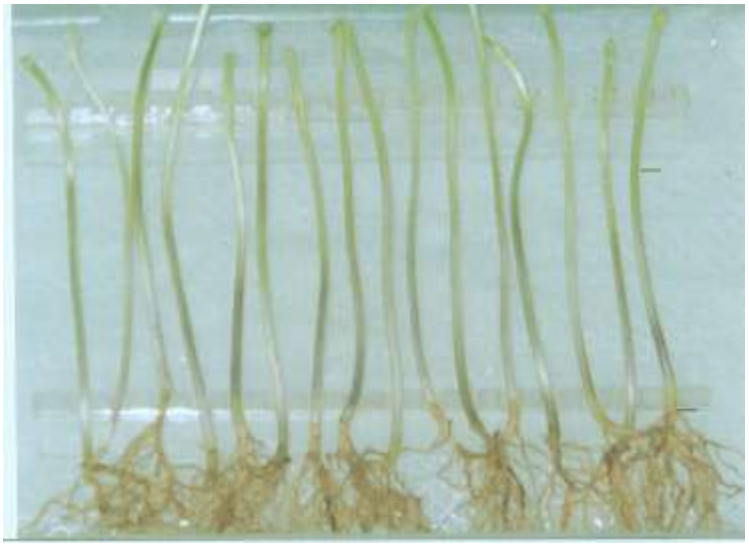


Fig. 28. Uninoculated hypocotyls (control) of cv. Crowford.

Fig. 29. Reaction of hypocotyls of cv. Giza 22, 10 days after inoculation with *F. solani* f.sp.*phaseoli* at 20°C. Note the presence of localized light brown necrotic flecks on the hypocotyls with general weakness in these hypocotyls tissues tending to rotting as compared with hypocotyls of cv. Crowford i.e cv. Giza 22 with hypocotyls that behaved intermediate susceptibility at 20°C.

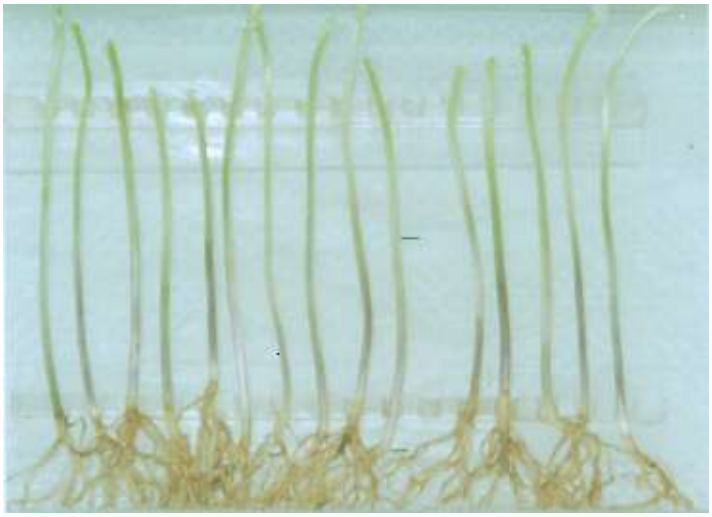


Fig. 30. Uninoculated hypocotyls (Control) of cv. Giza 22.

Table 6

Reaction of different cultivars of french bean and soybean, inoculated with *F. solani* f.sp *phaseoli* at 20°C.

Cultivar	Symptoms								Final reaction type
	Time (hours) after inoculation								
	0	4	6	8	12	24	36	48	
Nebraska	-ve	F^{+}	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	$F^{++}/B^{+}/rot^{-}$	R
Bronco	-ve	F^{+}	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	$F^{+++}/B^{+}/rot^{-}$	R
Crowford	-ve	F^{+}	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	$F^{++}/B^{+}/rot^{-}$	R
Giza22	-ve	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	F^{+++}	$F^{+++}/B^{++}/rot^{++}$	Is

F^{+} = very few brown flecks are observed at the inoculation sites.

F^{++} = few brown flecks are observed at the inoculation sites.

F^{+++}= numerous brown flecks are observed at the inoculation sites.

B = browning of hypocotyl tissue; represent grades of browning intensity as follows:

B^{+} = light brown , B^{++} = moderate brown .

rot^{-} = not rotted hypocotyls; rot^{++} = moderately rotted hypocotyls.

R = resistant, the presence of flecks and slight browning of hypocotyls without rotting.

Is = intermediate reaction , the presence of flecks , and moderate browning of the hypocotyl with moderate rotting (i.e. intermediate reaction tending towards susceptibility).

-ve = there was no any reaction (negative result).

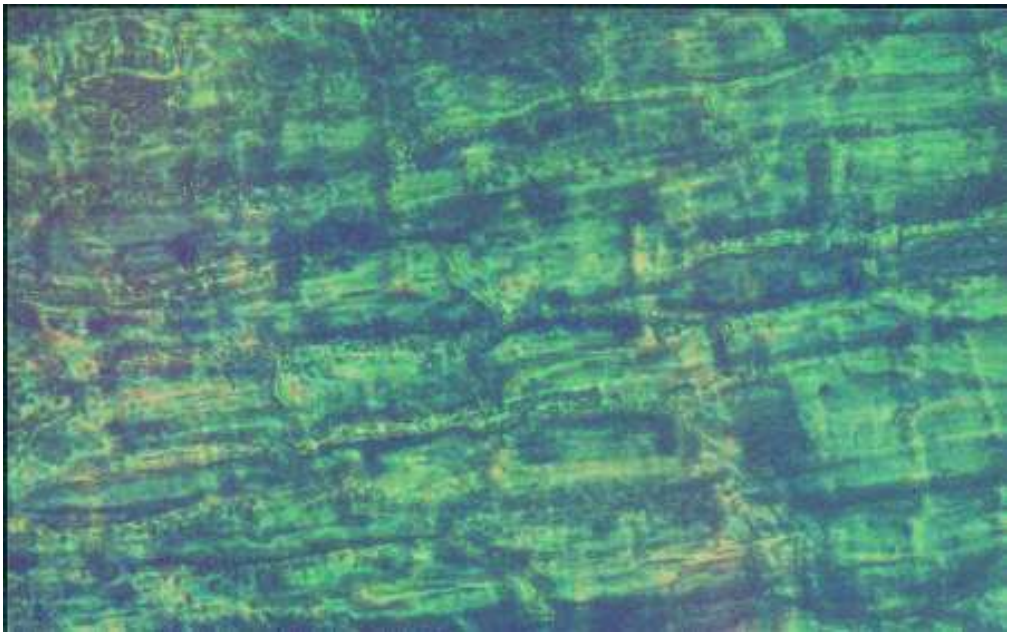


Fig. 31. Surface view of the hypocotyl tissue of cv. Bronco, 4 hours after inoculation with the non pathogenic Fusarium potato isolate at 20 °C. Note the non germination of the fungal spores (x40 magnification).

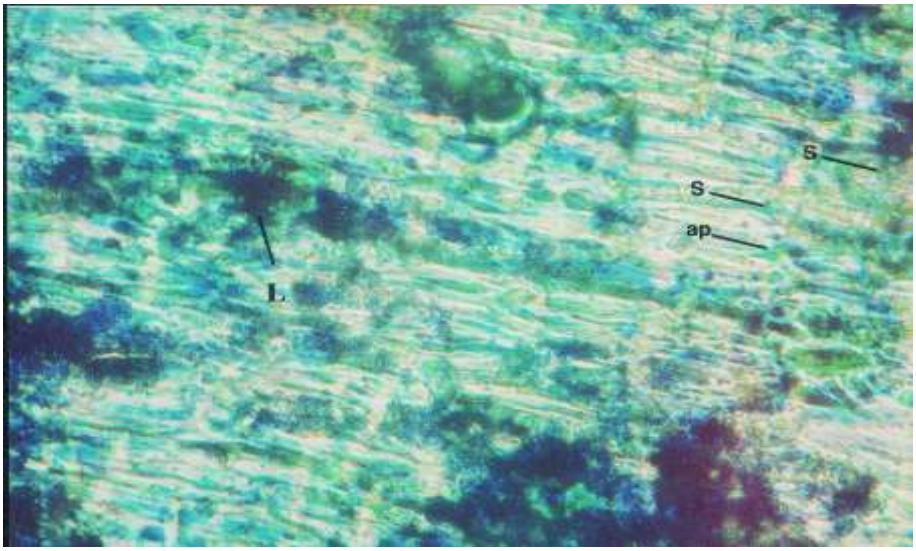


Fig. 32. Surface view of cv. Nebraska (resistant) hypocotyl, 4 hours after inoculation with *F. solani* f.sp. *phaseoli* at 20°C. Note the non germination of the spores (s) and the presence of appressoria (ap). Also note the beginning of accumulation of Lignin (L). (x40 magnification).

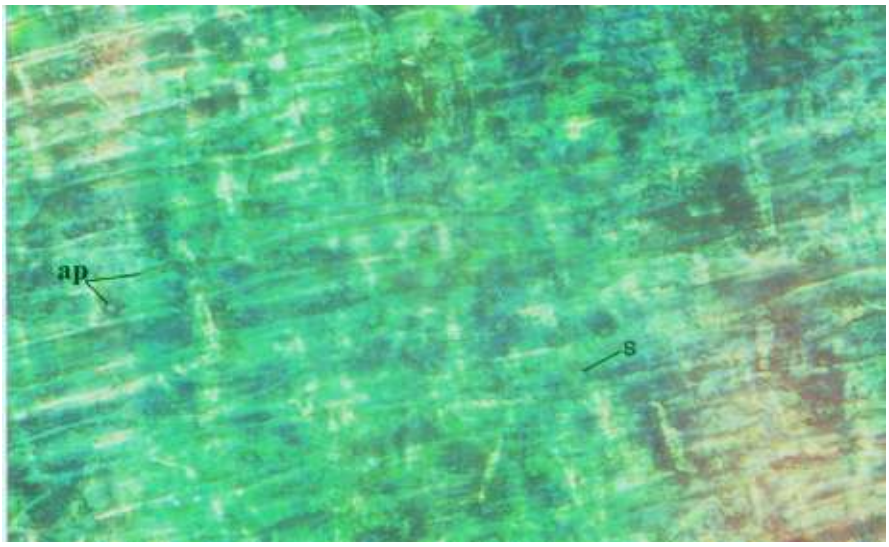


Fig. 33. Surface view of cv. Bronco (resistant) hypocotyl, 4 hours after inoculation with *F. solani* f.sp. *phaseoli* at 20°C. Note the non germinating spores (s) and non germinating appressoria (ap) (x40 magnification).

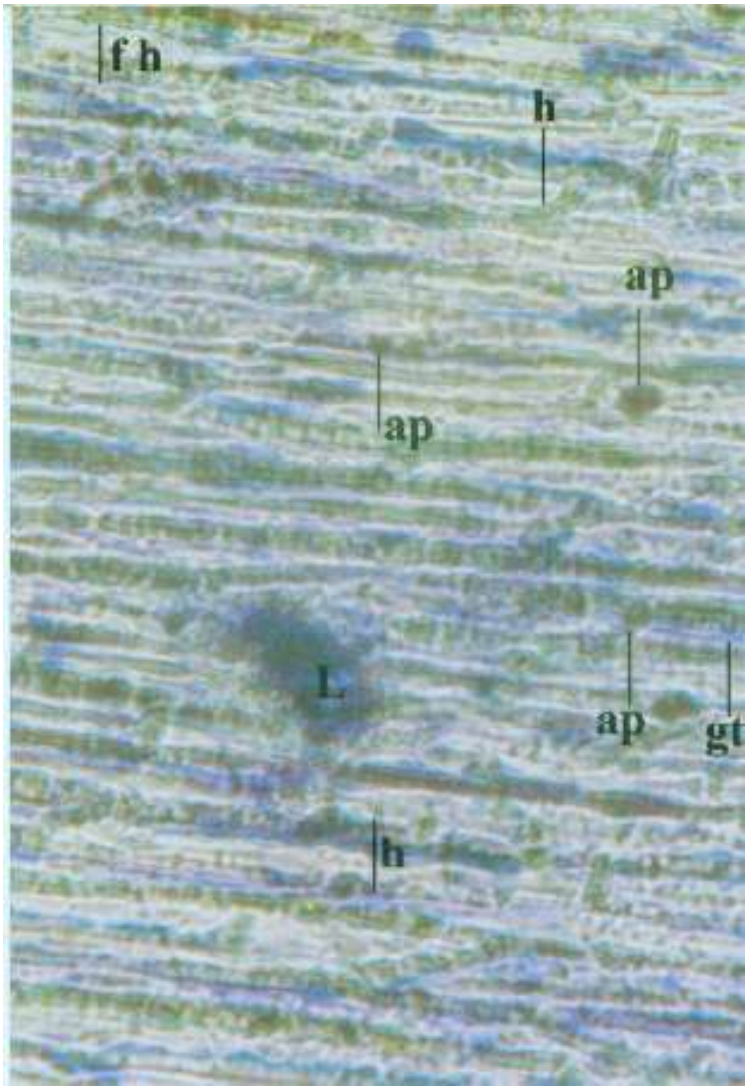


Fig. 34. Surface view of cv. Bronco (resistant) hypocotyl, 12 hours after inoculation with *F. solani* f.sp.*phaseoli* at 20°C. Note the transformation of macroconidia into swelling appressoria (ap) that give germe tube (gt) , and limited fungal hyphae (fh) inside the cells (intracellularly) and fungal hyphae (h) along the cell walls (intercellularly) . Note also the localized liginfication (L) (x40 magnification).

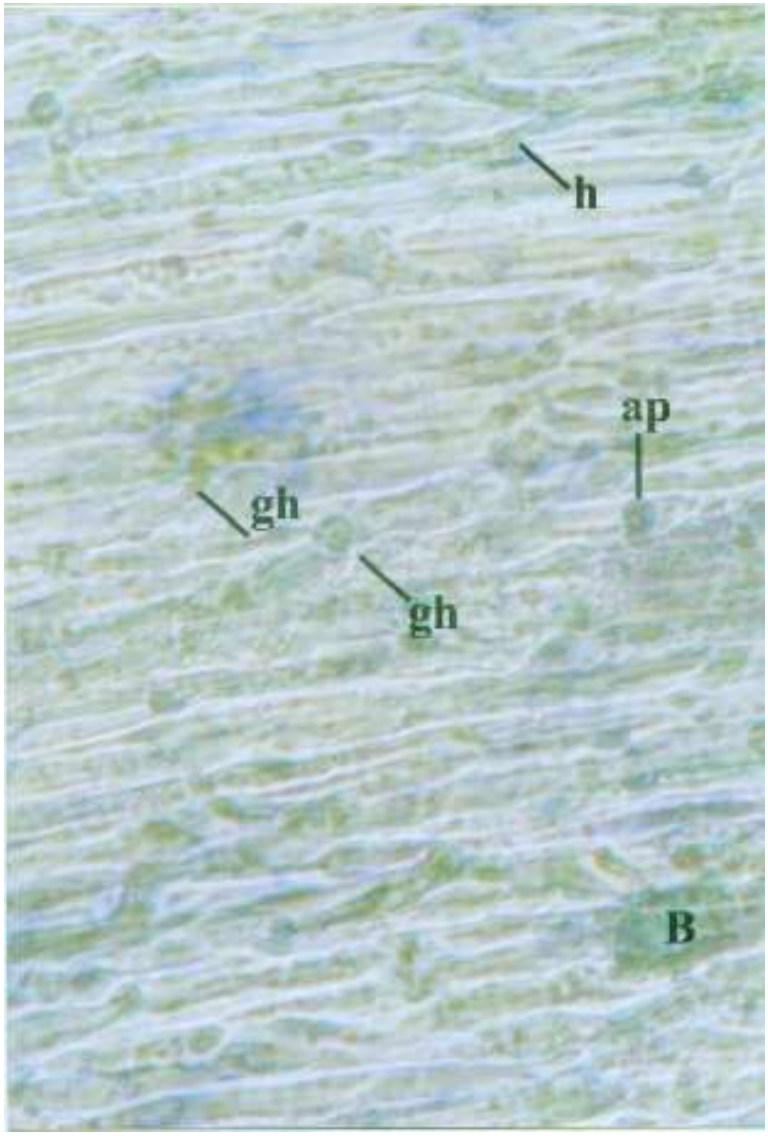


Fig. 35. Surface view of cv. Bronco (resistant) hypocotyl, 1 day after inoculation with *F. solani* f.sp. *phaseoli* at 20 ^{o}C. Note the limited germination of swelling appressoria and the non development of germinating hyphae (gh) also Note the formation of very light browning region (B) (x40 magnification).

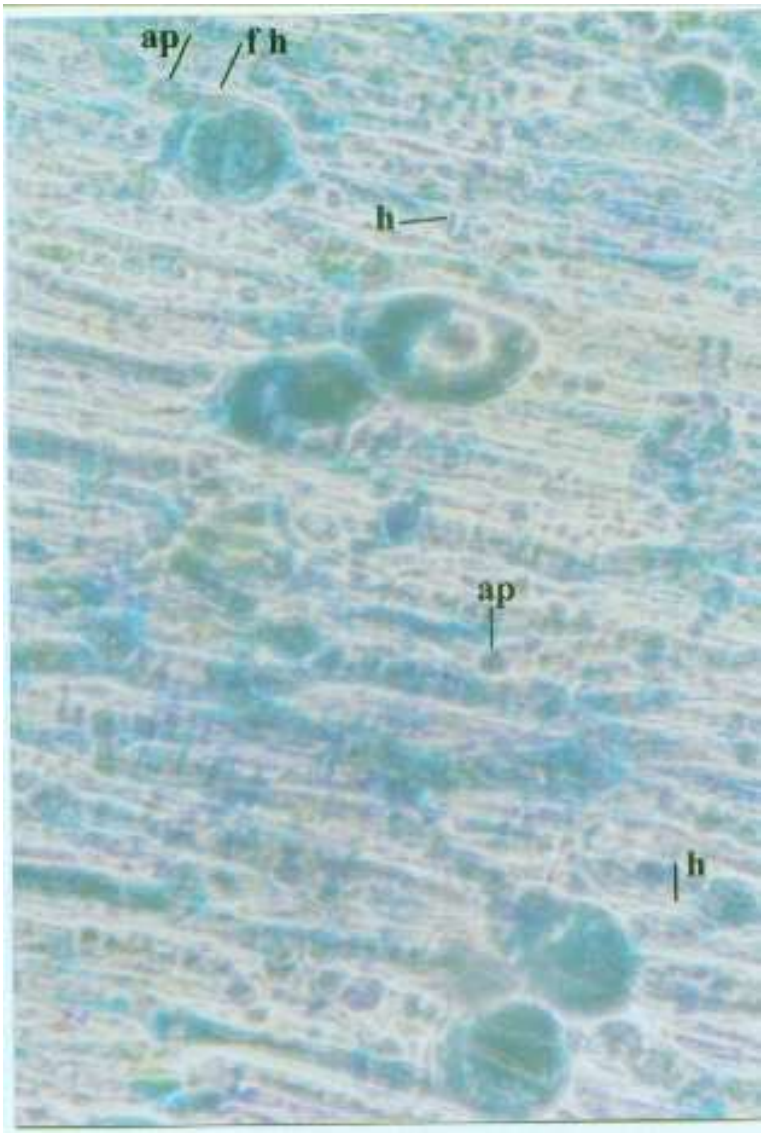


Fig. 36. Surface view of cv. Bronco (resistant) hypocotyl, 2 days after inoculation with *F.solani* f.sp. *phaseoli* at 20°C. Note the ungerminating appresoria and that give limited growth of interacellularly hyphae (h) and intercellularly fungal hyphae (fh). i.e. there was no further hyphal extention within epidermal and cortical cells after 48 hours of inoculation indicating to the cultivar behaves resistant at 20°C (x40 magnification).

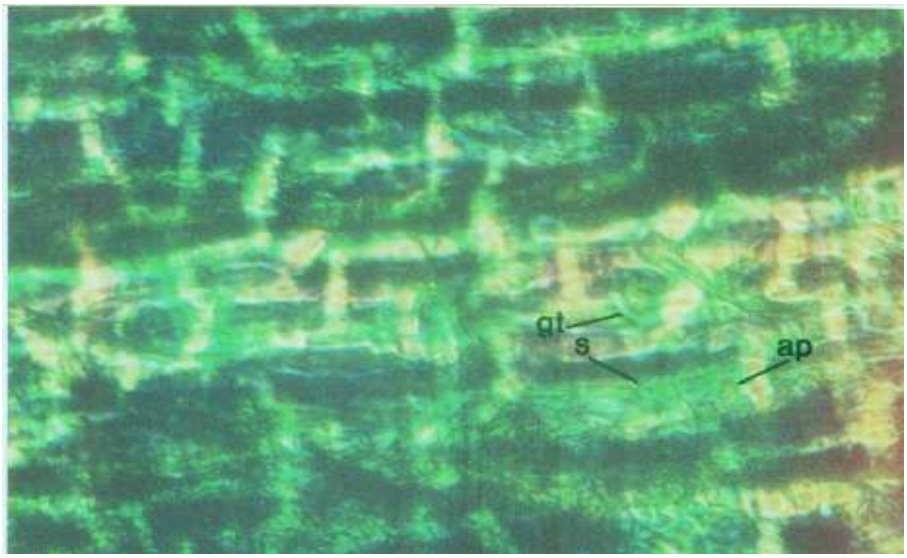


Fig. 37. Surface view of cv. Crowford (resistant) infected hypocotyl, 6 hours after inoculation with *F. solani* f.sp. *phaseoli* at 20°C. Note the non germinating spores (s) and very few number of germinating appressoria (ap) (x40 magnification).

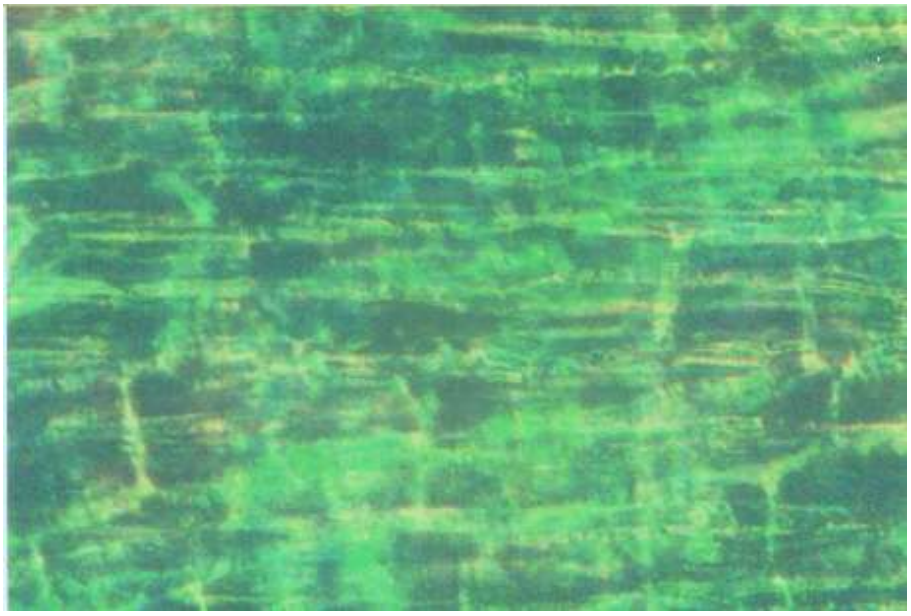


Fig. 38. Surface view of cv. Giza 22 (intermediate susceptible) infected hypocotyl, 4 hours after inoculation with *F.solani* f.sp.*phaseoli* at 20°C. Note there was no any clearly germination of the spores (x40 magnification).

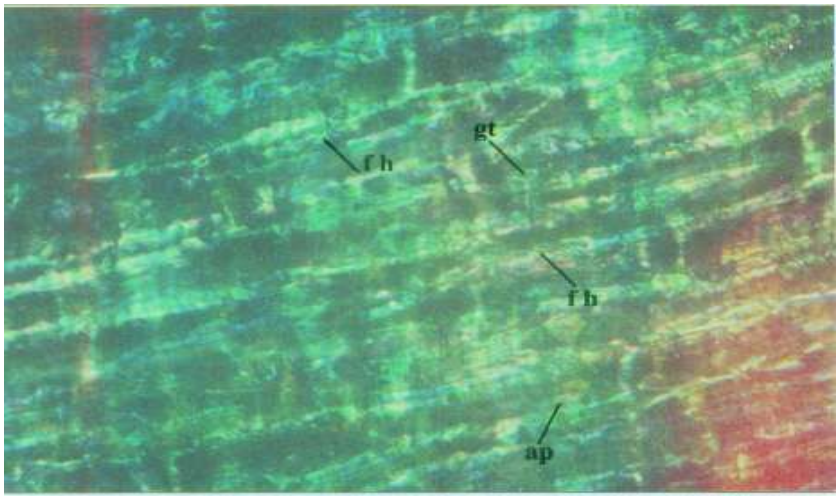


Fig. 39. Surface view of cv. Giza 22 (intermediate susceptible) hypocotyl, 6 hours after inoculation with *F. solani* f.sp. *phaseoli* at 20°C. Note the start of germination of appressoria (ap) that given germ tubes (gt) also note the germination of spores that given germ tubes and thin fungal hyphae (fh) (x40 magnification).

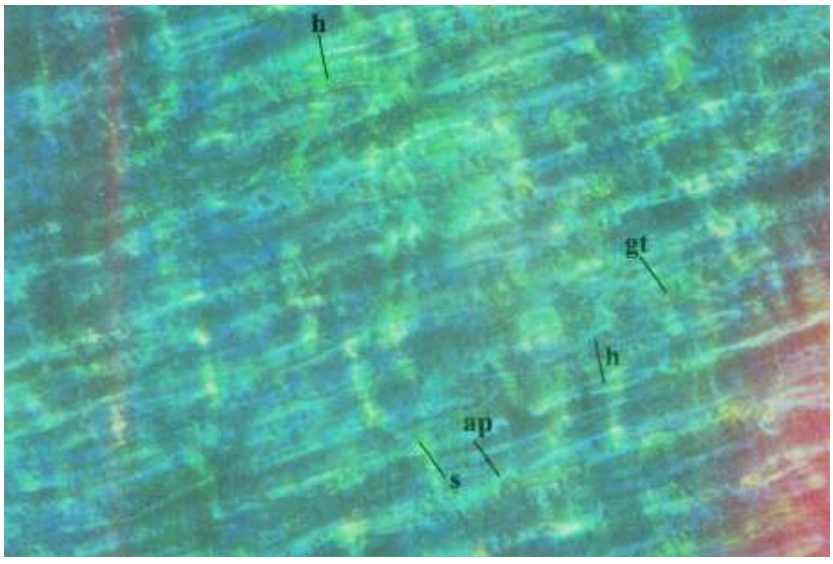


Fig. 40. Tangential section of cv. Giza 22 (intermediate susceptible) hypocotyl, 1 day after inoculation with *F. solani* f.sp. *phaseoli* at 20°C. Note the germinating and non germinating appressoria also note the localized extention of the hyphae (h) within the epidermal and cortical cells and note the presence of non germinating spores (s) i.e there was no more developing fungal growth after one day infection (x40 magnification).

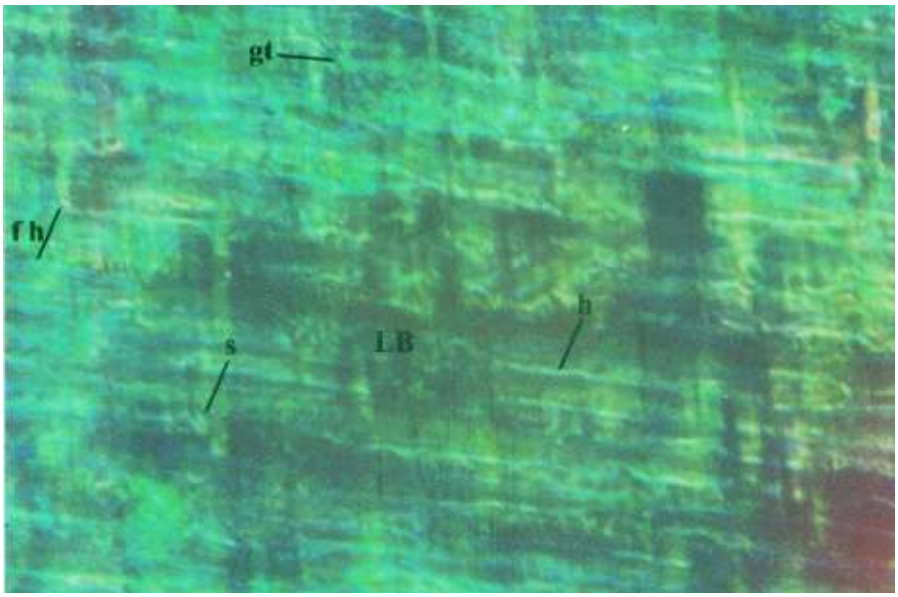


Fig. 41. Reaction of epidermal cells of cv. Giza 22 (intermediate susceptible) hypocotyl to the penetration of *F. solani* f.sp. *phaseoli*, 2 days after inoculation at 20°C. Note the penetrating hyphae(h) that appeared embeded with dark deposits within a region of hypersensitive cells that appeared as light browning (LB) area, also note the presence of non germinating spores (s) and the presence of penetrating thin hyphae in localized region indicating that no more progress of the infection after 2 days of inoculation (x40 magnification).

Conclusion

a- From these results it is apparent that at 20 °C cv. Giza 22 showed intermediate degree of susceptibility (Is). Other tested cultivars was high degree of resistance (R).

b- Light microscopic observations were carried out to investigate the histological features characteristic for french bean and soybean-Fusarium interactions. After zero, 4, 6 and 8 hours there was no germination of macroconidia (Figs. 32 & 33). The macroconidia germinated producing germ tubes was occurred after 12 h of inoculation in case of french bean (Fig. 34) but that was occurred after 6 hours of inoculation in case of soybean (Figs. 37 & 39). Also, there was no more progressing of macroconidial germination for 2 days of inoculation (Figs. 35 & 36), the case which was observed for all french bean and soybean cultivars.

2) At 25°C:

Macroscopic observations that recorded in Table (7), showed the presence of few brown necrotic flecks on the hypocotyls of cvs. Nebraska and Crowford, while in cv. Bronco newly formed flecks more than that in the cvs. Nebraska and Crowford were observed 2 days after inoculation. These flecks remained unchanged in case of cvs. Nebraska and Crowford. On the other hand, hypocotyls of cvs. Bronco and Giza 22 showed the presence of numerous brown flecks, 2 days after inoculation here some of theses flecks extended to short streaks without further progress. Slight browning without rotting of the inoculated hypocotyls of all cultivars, except those hypocotyls of cvs. Bronco and Giza 22 that rotted and browned moderately.

From the above results it can be concluded that at 25^{o}C,cvs. Bronco and Giza 22 showed an intermediate reaction tending slightly towards susceptibility (Is). The cvs. Nebraska and Crowford were the only cultivars that exhibited a greater resistance (R) at 20^{o}C and 25^{o}C.

Table 7

Reactions of different cultivars of french bean , and soybean to inoculation with *F. solani* f.sp *phaseoli* at 25°C.

Cultivar	Symptoms								Final reaction type
	Time (hours) after inoculation								
	0	4	6	8	12	24	36	48	
Nebraska	N	F^{+}	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	$F^{++}/B^{+}/rot^{-}$	R
Bronco	N	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	st	$st/B^{++}/rot^{+}$	Is
Crowford	N	F^{+}	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	$F^{++}/B^{+}/rot^{-}$	R
Giza22	N	F^{++}	F^{++}	F^{+++}	F^{+++}	st	st	$st/B^{++}/rot^{++}$	Is

N = no reaction.

F^{+} = very few brown flecks are observed at the inoculation sites.

F^{++} = few brown flecks are observed at the inoculation sites.

F^{+++} = numerous brown flecks are observed at the inoculation sites.

st = brown streaks are observed at the inoculation sites.

B = browning of hypocotyls tissue; represent grades of browning intensity as follows:

B^{+} = Light brown, B^{++} = Moderate brown.

rot^{-} = hypocotyls are not rotted.

rot^{+} = hypocotyls are slightly rotted.

rot^{++} = hypocotyls are moderately rotted.

R = resistant, flecks and slight browning of hypocotyls without rotting.

Is = intermediate reaction tending towards susceptibility, the presence of limited streaks accompanied by moderate browning and moderate rotting of hypocotyls.

Accordingly to observations of the infection type monitoring 10-15 days after inoculation with *F. solani* f.sp. *phaseoli.*

3) At 30°C:

a- Macroscopic observations showed a few brown necrotic flecks in the inoculation sites,1 day after inoculation of hypocotyls of cvs. Crowford and Nebraska, these flecks remained unchanged in case of cv. Crowford through 1 day later and very few number of hypocotyls became moderately rotted also the root system became rotted with brown exudates (Fig. 46A). While in case of cv. Nebraska, some of the observed necrotic flecks became elongated, also very few number of hypocotyls became moderately rotted (Fig. 44A).

On the other hand , the inoculated hypocotyls of cvs. Giza 22 and Bronco developed numerous brown flecks within 1 day after inoculation. One day later, these flecks elongated forming streaks then these hypocotyls became rotted and brown (Figs. 45 & 47) and the intact hypocotyls of these two cultivars (Bronco and Giza 22) appeared without any symptoms after 5 days of inoculation with the non pathogenic Fusarium potato isolate (the biotic elicitor in this study) at 30 °C, Figs. (42 & 43), also the microscopic examination of the hypocotyls tissues of cv. Giza 22 did not showed any germination of the non related fungal pathogen spores after 4 hours of inoculation at 30 °C, Fig. (59).

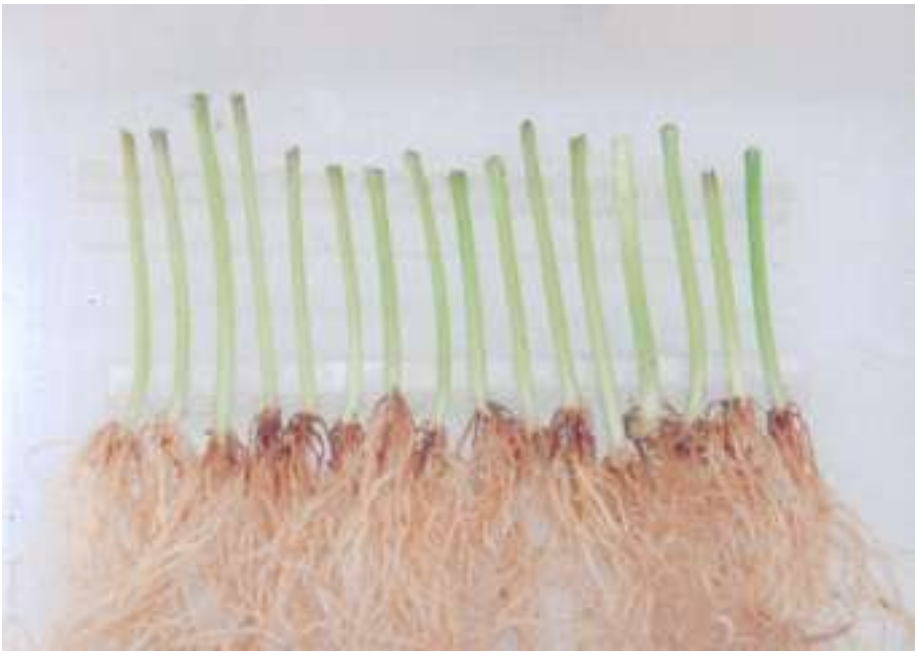


Fig. 42. The intact hypocotyls of cv. Bronco, 5 days after inoculation with the non pathogenic Fusarium potato isolate (biotic elicitor) at 30 °C.

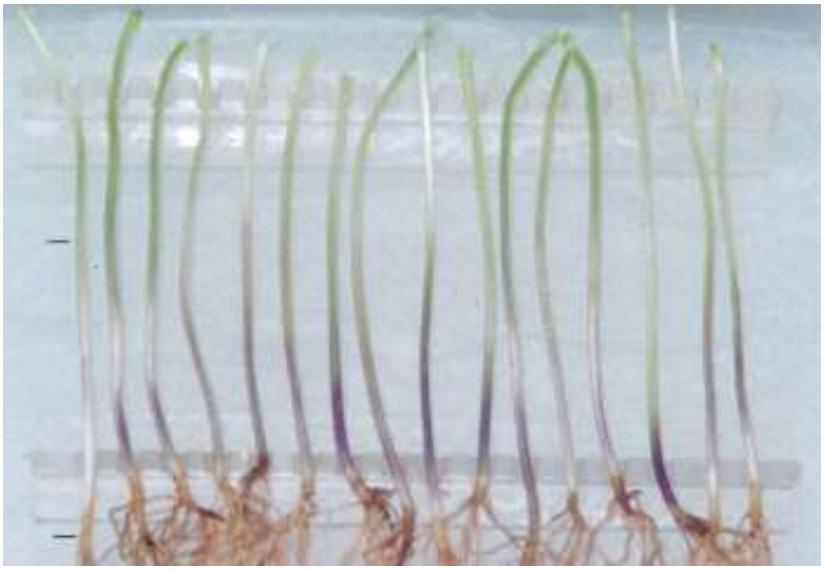
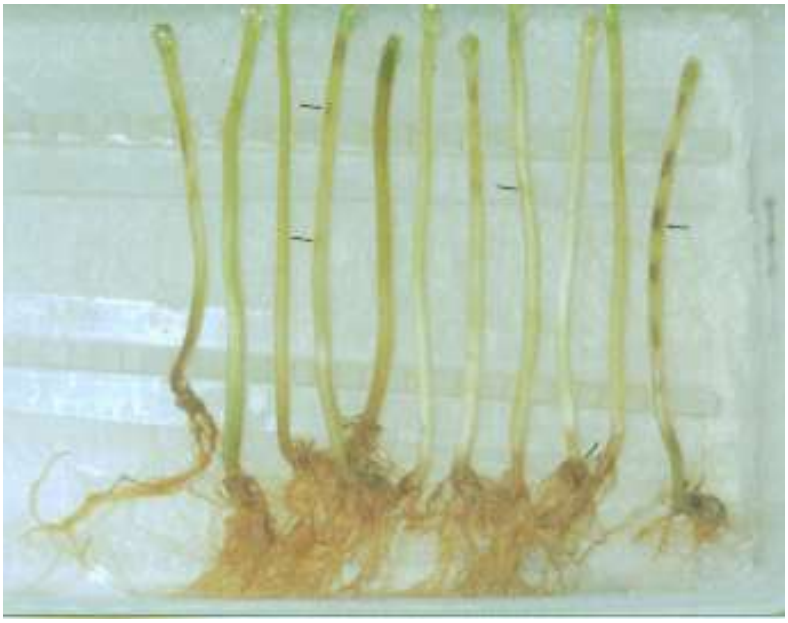
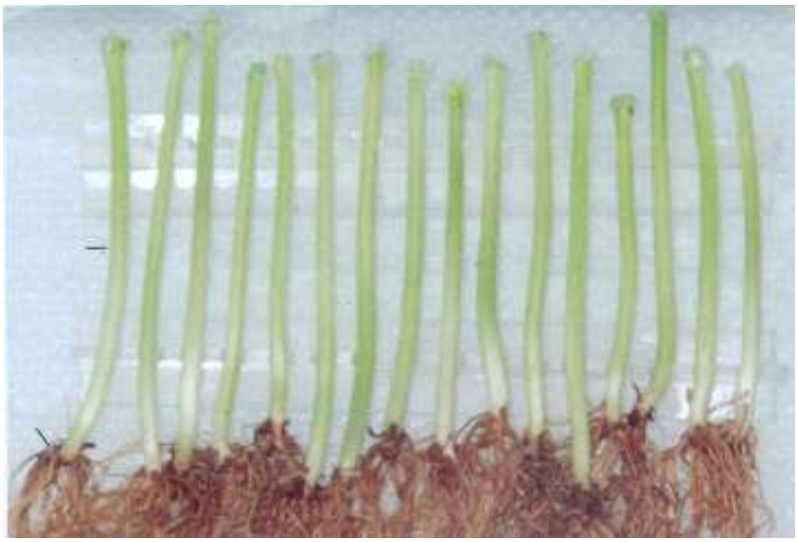


Fig. 43. The intact hypocotyls of cv. Giza 22, 5 days after inoculation with the non related pathogen Fusarium potato (initiator of induced resistance) at 30 °C.



(A)

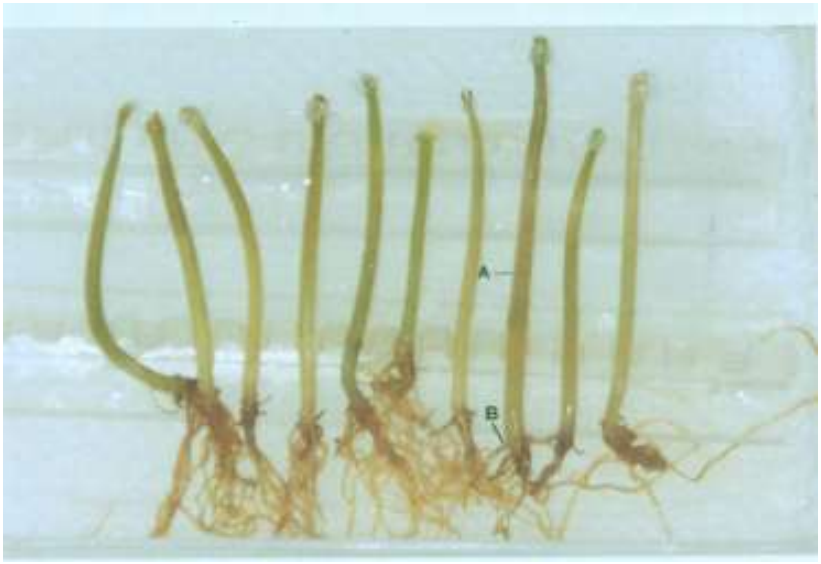


(B)

Fig. 44. A, reaction of hypocotyls of cv. Nebraska partially resistant (Ir), 2 days after inoculation with *F. solani* f.sp. *phaseoli* at 30°C. Note few hypersensitive brown flecks that became elongated at the inoculation sites. Note also the moderately rotting and light browning of very few number of hypocotyls. and Note the moderately brown rotting of the root system that became dark in colour than that at 20^{o}C.

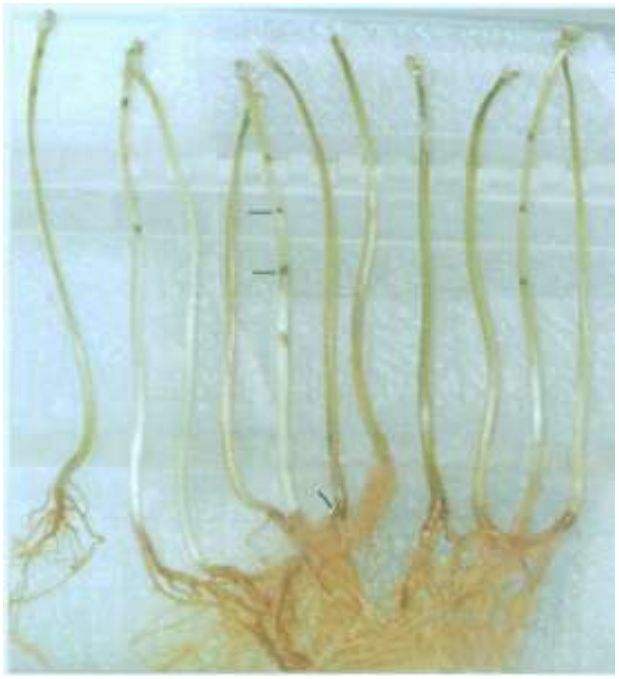
B, Uninoculated (control) hypocotyls of cv. Nebraska.

(A)

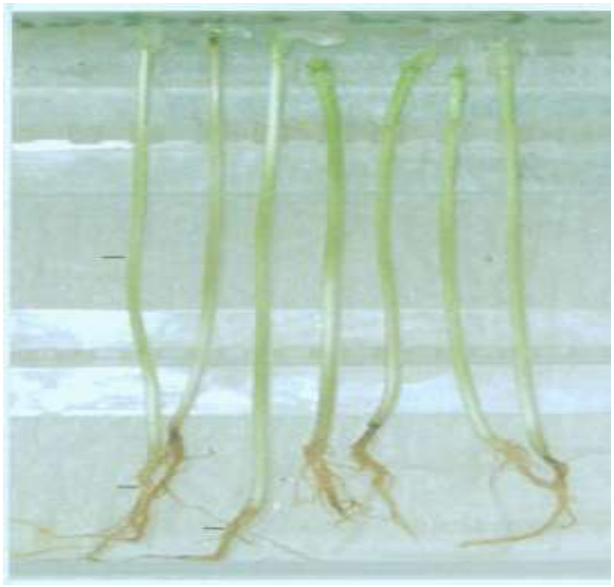


(B)

Fig.45. A, reaction of hypocotyls of cv. Bronco (susceptible), 1 day after inoculation with *F. solani* f.sp. *phaseoli* at 30^{o}C. Note the appearance of rotting and browning of the hypocotyls and root system in (A) that are lesser than that appearance on the hypocotyls and root system in (B) after 2 days of inoculation.



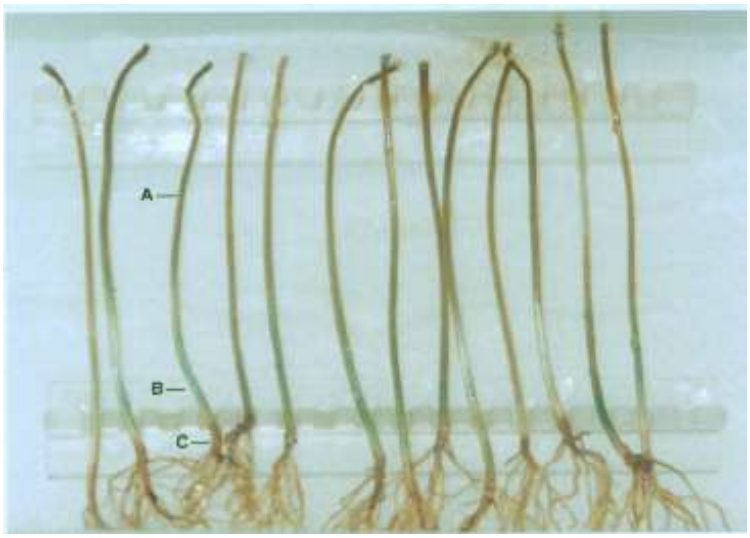
(A)



(B)

Fig. 46. A, Reaction of hypocotyls of cv. Crowford, partially resistant (Ir), 2 days after inoculation with *F. solani* f.sp. *phaseoli* at 30°C. Note the few brown necrotic flecks in the inoculation sites. Note also the moderately rotting and light browning of few number of hypocotyls. And note the slightly brown rotting of the root system and the flecks on the hypocotyls were more dark than that at 20°C. B, uninoculated (control) hypocotyls of cv. Crowford.

(A)



(B)

Fig.47. Reaction of hypocotyls of cv. Giza 22 (susceptible). A, 1 day after inoculation with *F. solani* f.sp. *phaseoli* at 30^{o}C while B, 2 days after inoculation with the same fungal pathogen. Note the rotting and browning of the hypocotyls, this rotting and browning increase with the time i.e. in (B) was more than that in (A). Note the appearance of blue green colour, indicating the presence of glyceollin (as a result of host-pathogen interaction) that decrease with the time (B).

Table 8

Reaction of different cultivars of french bean and soybean to inoculation with *F. solani* f.sp. *phaseoli* at 30°C.

Cultivar	Symptoms								Final reaction type
	Time (hours) after inoculation								
	0	4	6	8	12	24	36	48	
Nebraska	N	F^{+}	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	$F^{++}/B^{++}/rot^{-}$	Ir
Bronco	N	F^{++}	F^{++}	F^{+++}	F^{+++}	st	st	$st/B^{++}/rot^{+++}$	S
Crowford	N	F^{+}	F^{+}	F^{+}	F^{++}	F^{++}	F^{++}	$F^{++}/B^{++}/rot^{-(+)}$	Ir
Giza22	N	F^{++}	F^{++}	F^{+++}	F^{+++}	st	st	$st/B^{++}/rot^{+++}$	S

N = no reaction.

F^{+} = very few brown flecks are observed at the inoculation sites.

F^{++} = few brown flecks are observed at the inoculation sites.

F^{+++} = numerous brown flecks are observed at the inoculation sites.

B = browning of hypocotyl tissues; represent grades of browning intensity as follows:

B^{+} = Light brown, B^{++} = Moderate brown.

rot^{-} = hypocotyls are not rotted.

rot^{+} = hypocotyls are slightly rotted.

rot^{++} = hypocotyls are moderately rotted.

$rot^{-(+)}$ = hypocotyls are not rotted but there was few number of slightly rotted hypocotyls (the reaction tending towards susceptibility).

st = brown streaks are observed in the inoculation sites.

rot^{+++} = hypocotyls are rotted.

Ir= intermediate reaction, few streaks and moderate browning of the hypocotyls without rotting.

S= susceptible, elongated flecks developing streaks and moderate browning of the hypocotyls with rotting .

According to observations of the infection type monitoring 15 days after inoculation with each of the fungal isolate tested in this work.

b. Microscopic observations of the inoculation sites of cvs. Giza 22 and Bronco (susceptible) hypocotyls showed that 1 day after inoculation, fungal hyphae penetrated and extended longitudinally, both interacellularly and intercellularly (Fig.63). The germ tubes of macroconidia formed a hyphal network on the surface of the hypocotyls. After penetration, hyphae extended in all directions. They are observed clearly within the epidermal and cortical cells, 1 day after inoculation (Fig.63) and 2 days after inoculation (Fig.55). The browning of the infected cells (Fig.63) and aggregation of fungal hyphae that closed the stomata (Fig.55) were also observed.

On the other hand in cvs. Crowford and Nebraska intermediate resistant (Ir) hypocotyls, after 1 day of inoculation there was no more development in germinating of the fungal hyphae within the hypocotyl tissue especially in case of cv. Crowford (Fig. 58), while in case of cv. Nebraska, the reaction was characterized by a restriction of the majority of fungal hyphae within the stomata leading to closing them. These regions were stained with blue colour (Fig. 48 & 49) then dark blue (Fig. 50). 2 days after inoculation, no more progressing in germination of penetrating hyphae was observed (Fig. 50).

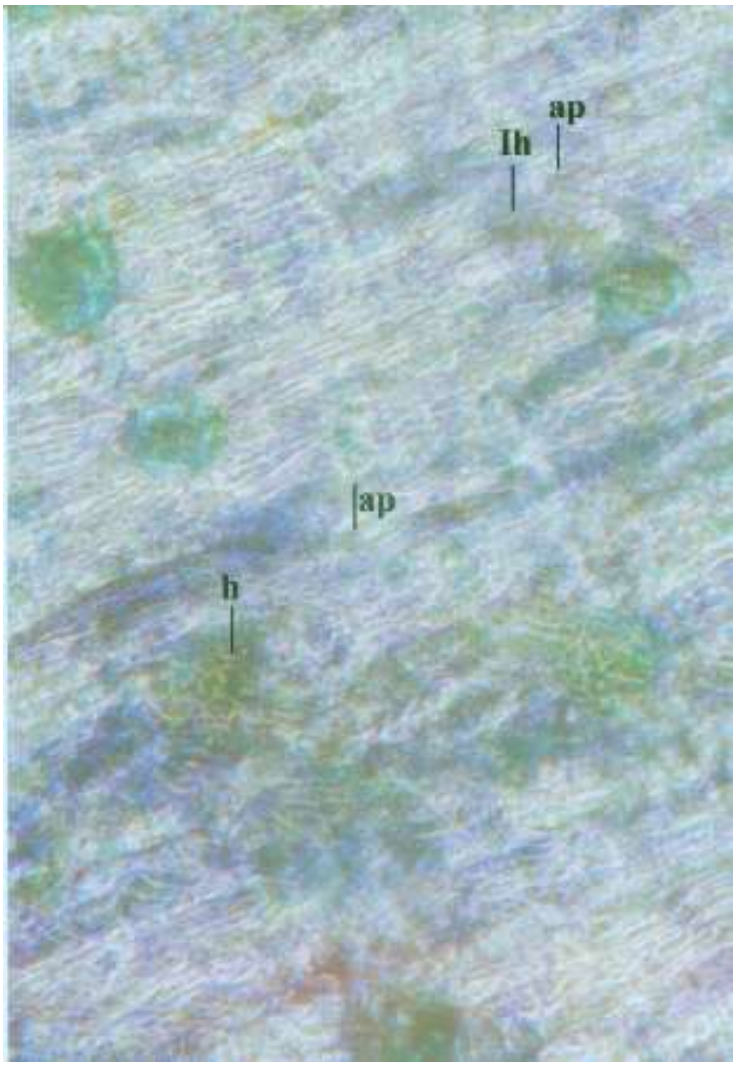


Fig. 48. Reaction of epidermal cells of cv. Nebraska intermediate resistant (Ir) hypocotyl to the penetration of *F. solani* f.sp. *phaseoli*, 12 hours after inoculation at 30 °C. Note swollen infection hyphae (Ih) that observed in discoloured and non discoulered cells, also appearance of germinating and non germinating appressoria (ap). Note also the stomata were stained blue colour (x40 magnification).

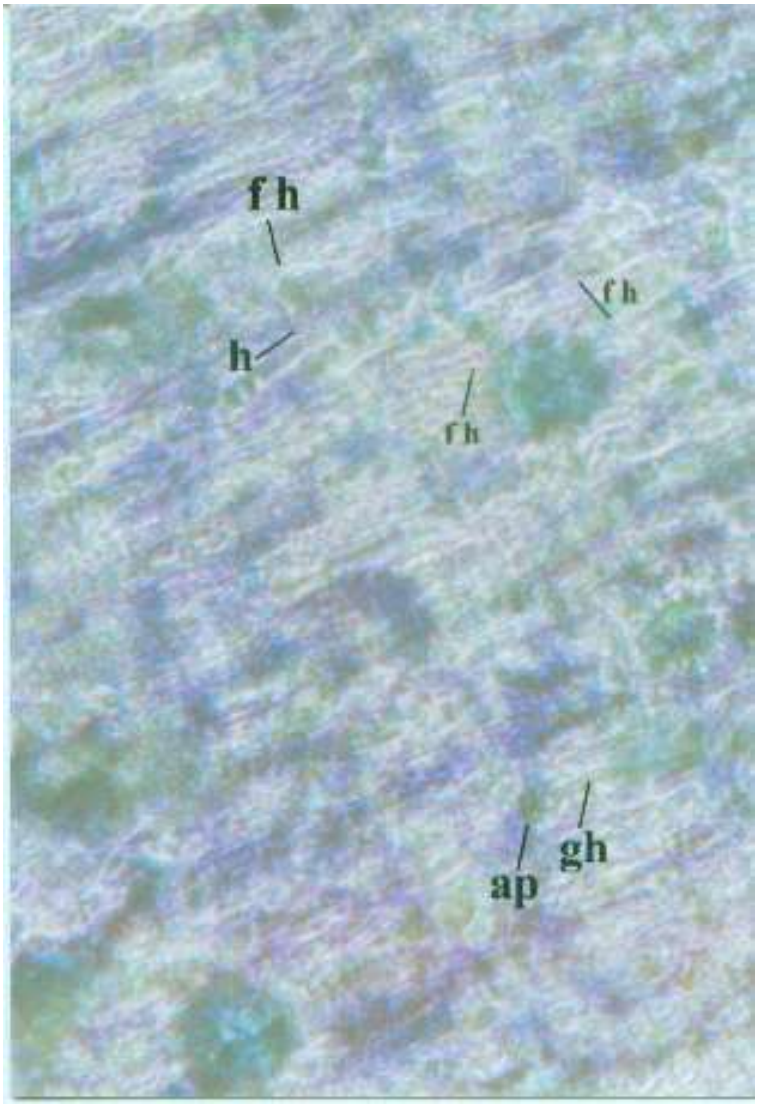


Fig. 49. Reaction of epidermal cells of cv. Nebraska intermediate resistant (Ir) hypocotyl to the penetration of *F. solani* f.sp. *phaseoli*, 24 hours after inoculation at 30 °C. Note the swollen germinating hyphae (gh) and penetrating fungal hyphae (fh) are observed in discoloured and non-discoloured cells; also fungal hyphae growing longitudinally both interacellulary (fh) and intercellularly (h) (x40 magnification).

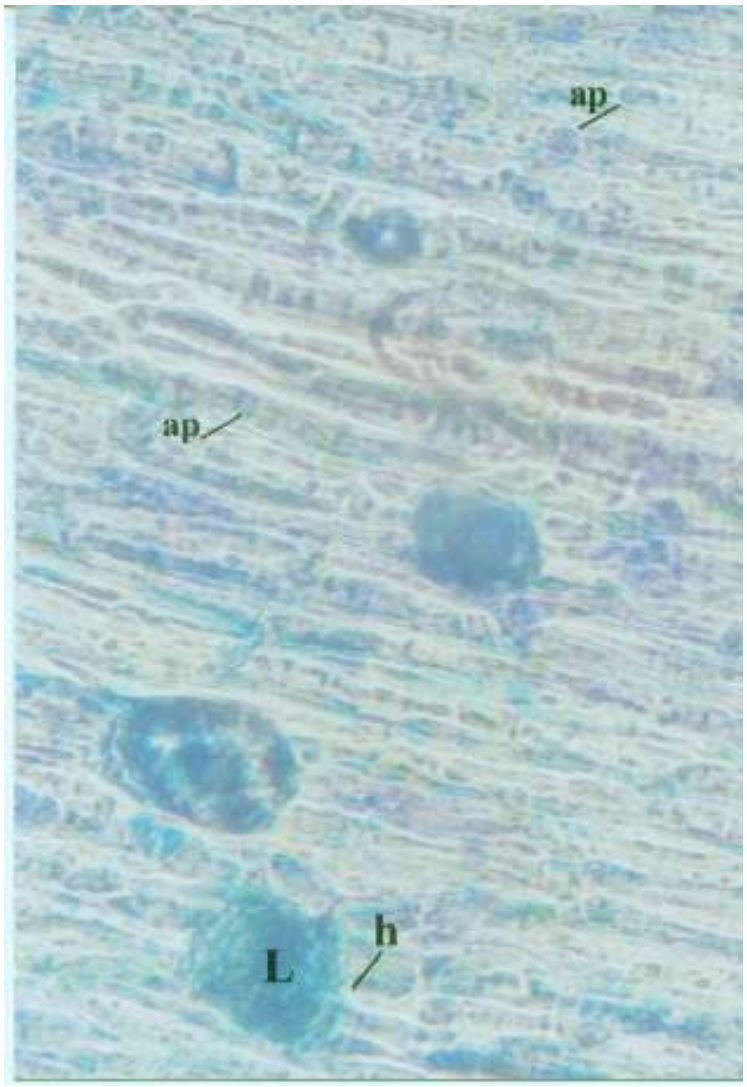


Fig. 50. Reaction of epidermal cells of cv. Nebraska intermediate resistant (Ir) hypocotyl to the penetration of *F. solani* f.sp. *phaseoli*, 48 hours after inoculation at 30 °C. Note the presence of non-germinated appressoria (ap) and thin hyphae were observed close to the stomata that stained dark blue. Note also the development of the germination of fungal hyphae within these hypocotyl tissues was limited as compared that of cv. Bronco after 48 hours of inoculation (x40 magnification).

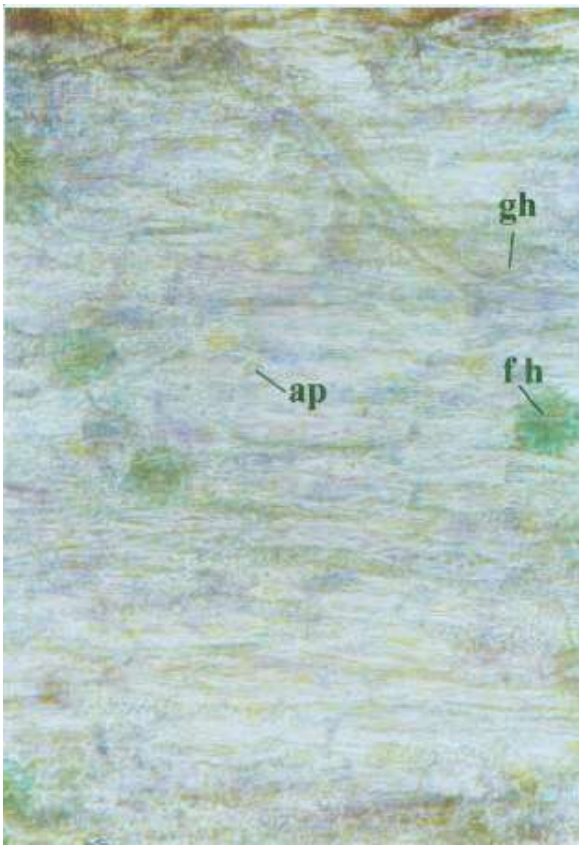


Fig. 51. Surface view of cv. Bronco (susceptible) hypocotyl, 6 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the appearance of few number of appressoria (ap) and the beginning of germination (gh) (very limited number of germination). Also note very thin fungal hyphae (x40 magnification).

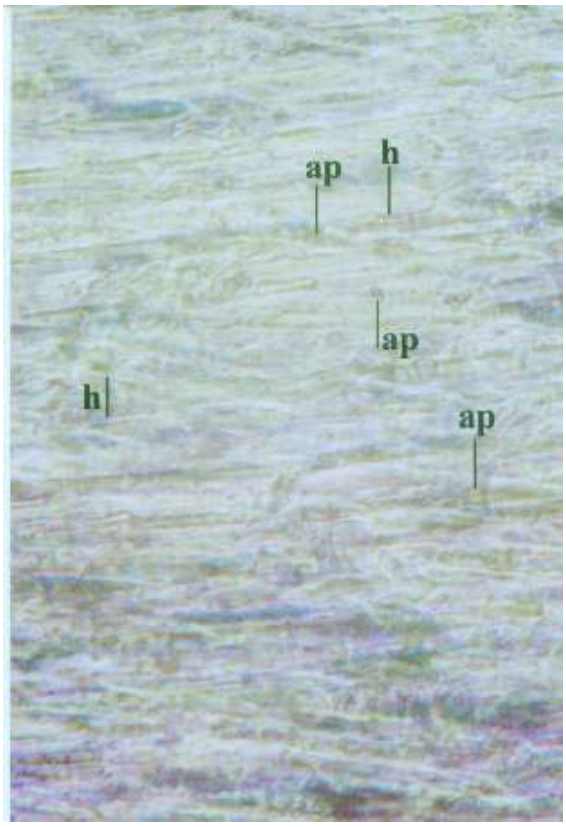


Fig. 52. Surface view of cv. Bronco (susceptible) hypocotyl, 12 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the appearance of large number of appressoria (ap) and germinating spores. Note also the rapid development of hyphal germination (h) and extending longitudinally both interacellularly and intercellularly (x40 magnification).

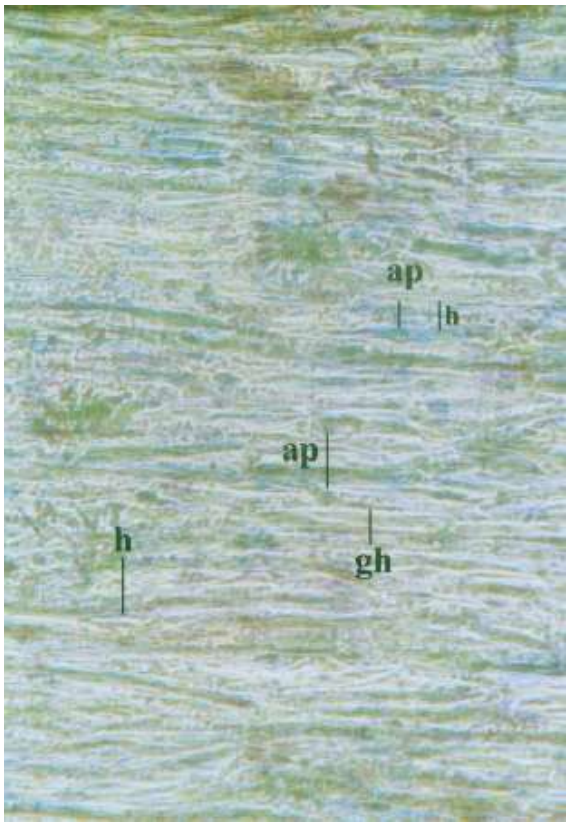


Fig. 53. Surface view of cv. Bronco (susceptible) infected hypocotyl, 24 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the germination of high number of appressoria (ap) giving germinating hyphae (gh) that spreaded superficially. Note also the distribution of penetrating hyphae (h) in the epidermal cells (x40 magnification).

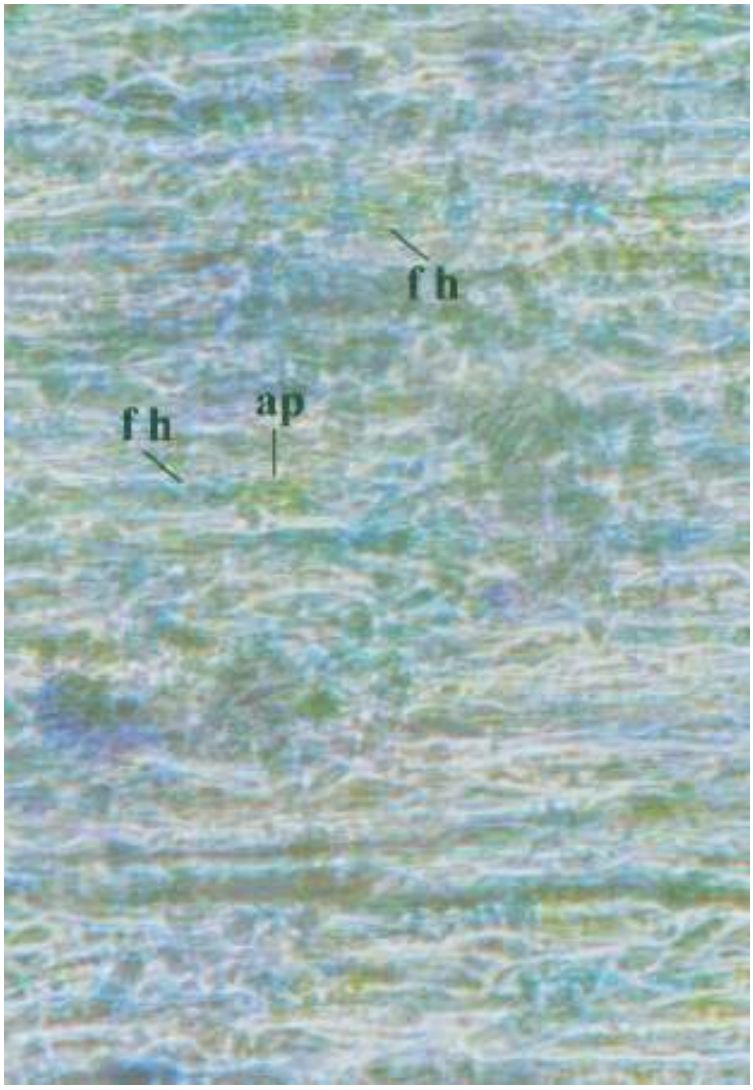


Fig. 54. Surface view of cv. Bronco (susceptible) infected hypocotyl, 36 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the epidermal cells accompanied by spreading of fungal hyphae (fh) in all directions (x40 magnification).

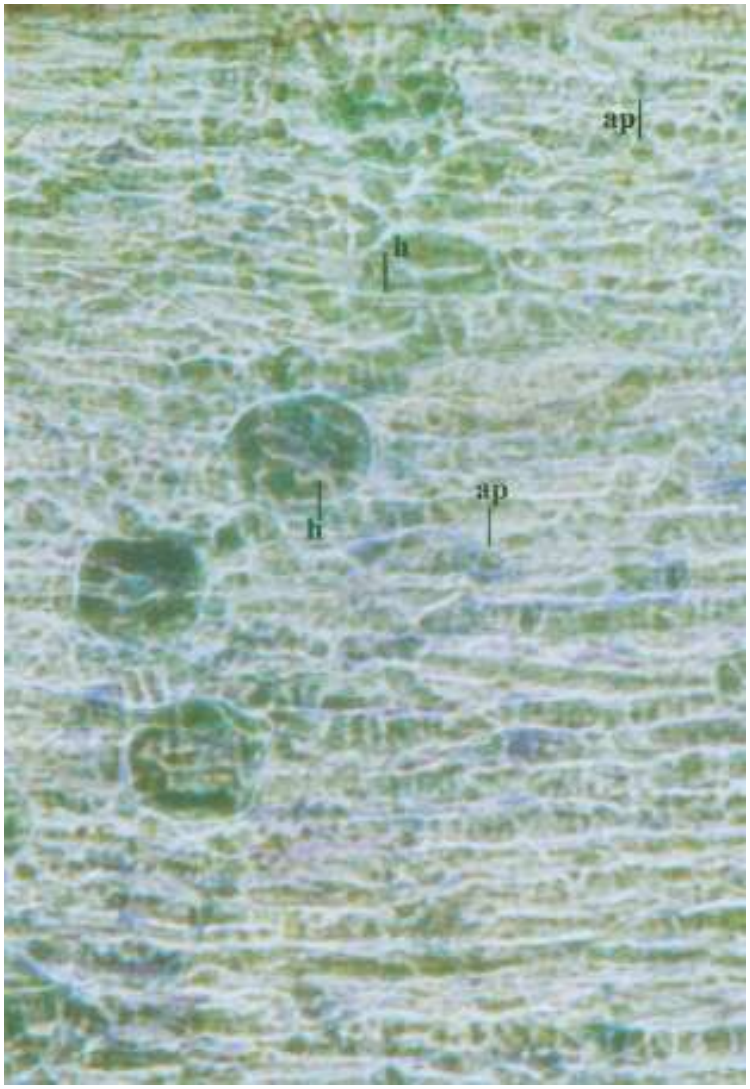


Fig. 55. Surface view of cv. Bronco (susceptible) infected hypocotyl, 2 days after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the appearance of large number of appressoria (ap) that became ready for germination. Note also fungal hyphae (h) penetrated and extended longidudinally, both intracellularly and intercellularly, where the germ tubes of microconidia formed a hyphal network on the surface of the hypocotyl i.e. the hyphae extended in all directions. Also aggregation of fungal hyphae that appeared as dark brown regions were observed close to the stomata and extended in all directions (x40 magnification).

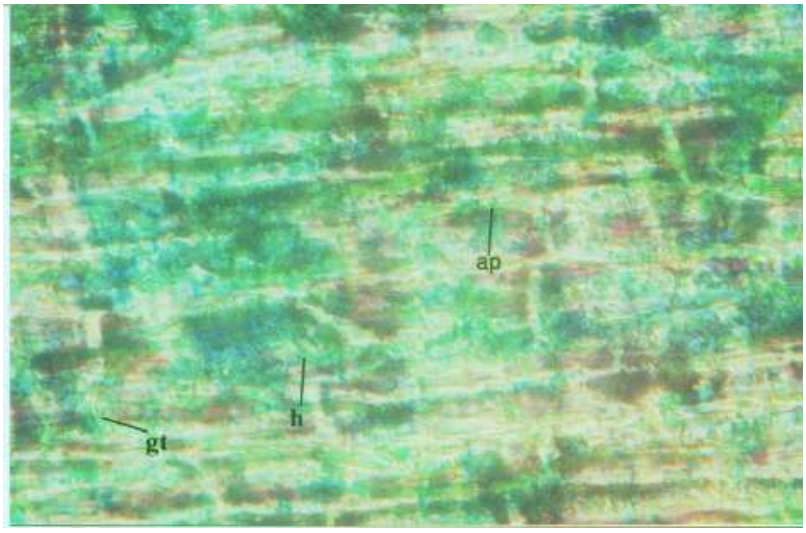


Fig.56. Surface view of cv. Crowford intermediate resistant (Ir) hypocotyl, 4 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the appearance of appressoria (ap) and the beginning of germination of macroconidia giving germ tubes (gt) (x40 magnification).

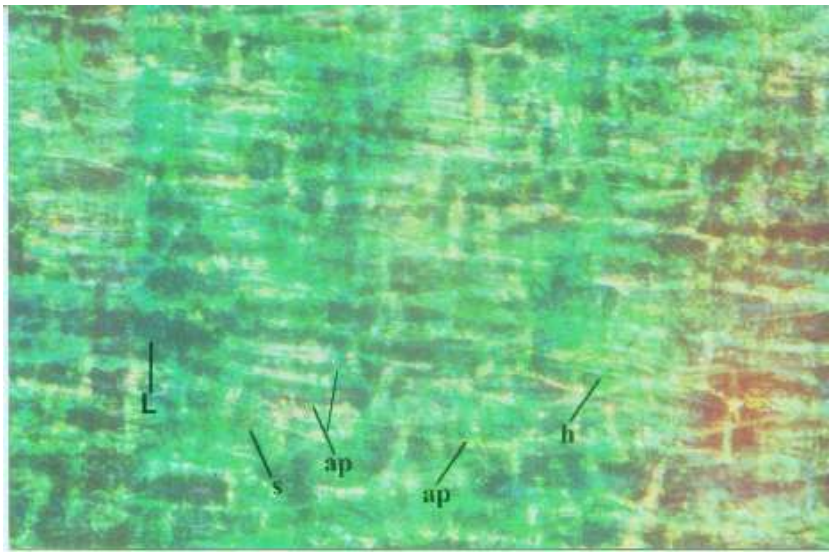


Fig. 57. Surface view of cv. Crowford intermediate resistant (Ir) hypocotyl, 12 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the presence of high number of non germinating spores also germinated spores with short germ tubes. Note also the embedded infection hyphae (h) and note the aggregation of lignin along the cell wall and within the hypersensitive cell (L) (x40 magnification).

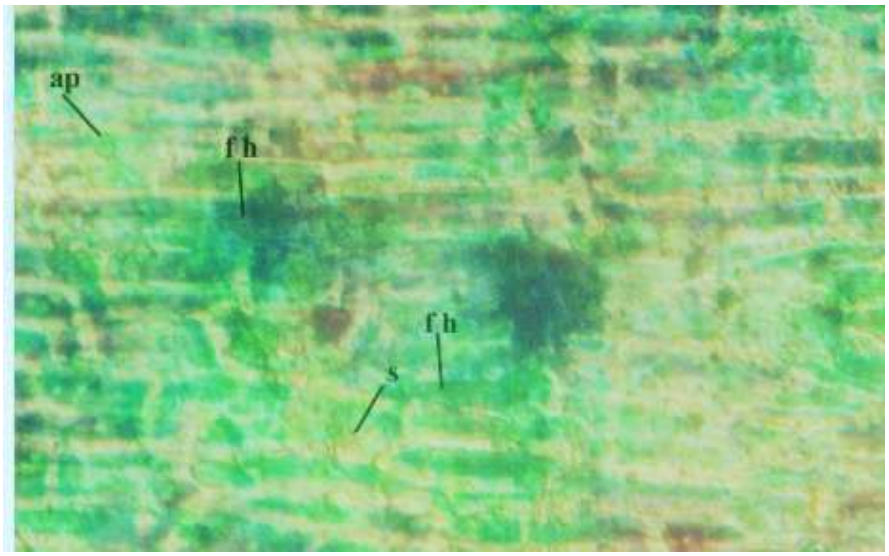


Fig. 58. Surface view of cv. Crowford intermediate resistant (Ir) hypocotyl, 24 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30 °C. Note the presence of non germinating spores (s) and appressoria (ap). Note also fungal hyphae (fh) embedded in hypersensitive reacting cells (dark discoloured regions) and in undiscoloured cells. i.e. there was no more development of germination of fungal hyphae as compared to cv. Giza 22 (x40 magnification).

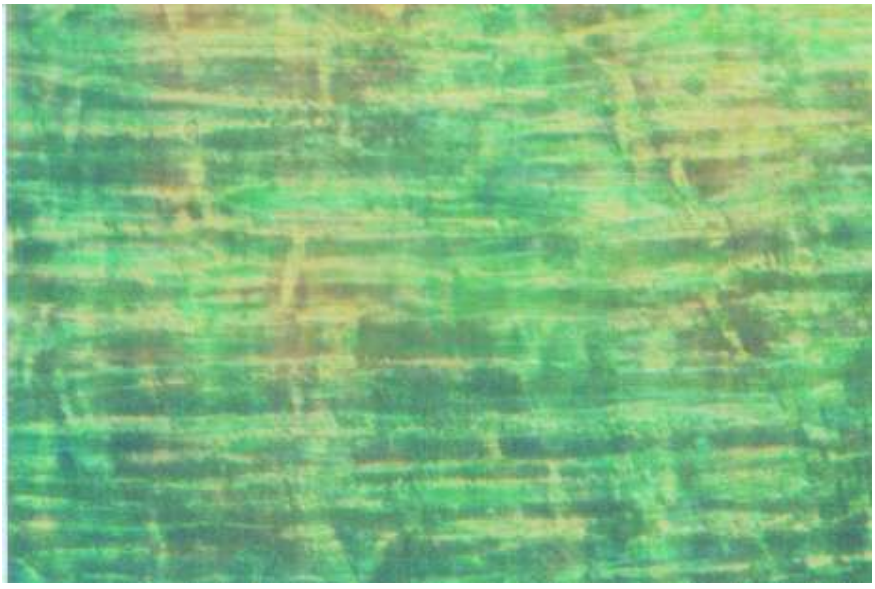


Fig.59. Tangential section of cv. Giza 22 (susceptible) hypocotyl, 4 hours after inoculation with Fusarium potato isolate (initiator) at 30^{o}C. Note there was no appearance reaction (x40 magnification).

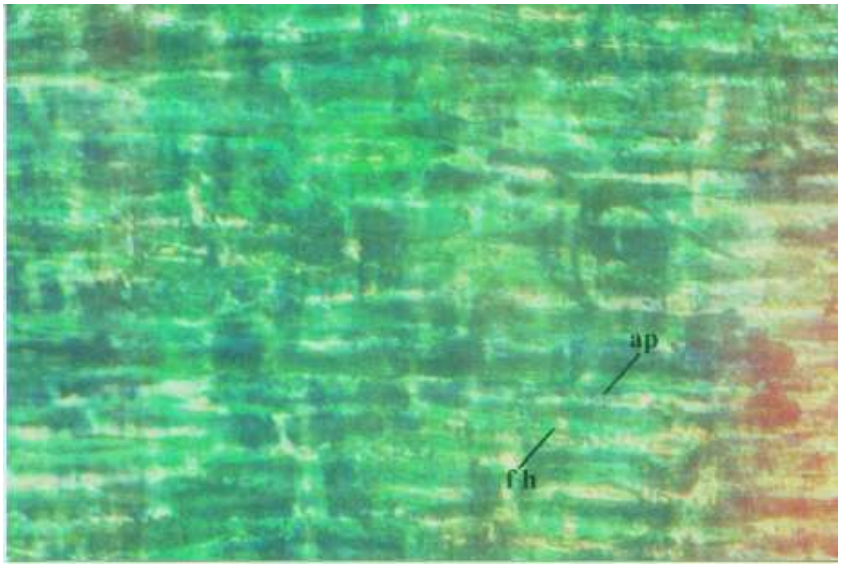


Fig. 60. Surface view of cv. Giza 22 (susceptible) hypocotyl, 4 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30°C. Note appearance of very few number of appressoria and germinating spores (x40 magnification).

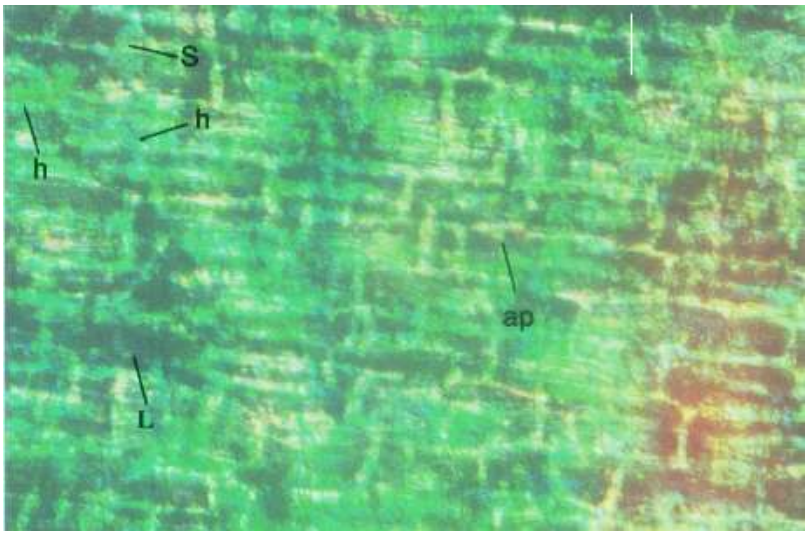


Fig. 61. Surface view of cv. Giza 22 (susceptible) hypocotyl, 6 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30°C. Note germinating macroconidia (s) (the real germination occurs after 6 hrs of inoculation) and the appearance of appressoria (ap). Note also the presence of hyphae (h) that appread espically in the lignin accumulation regions (L) (x40 magnification).

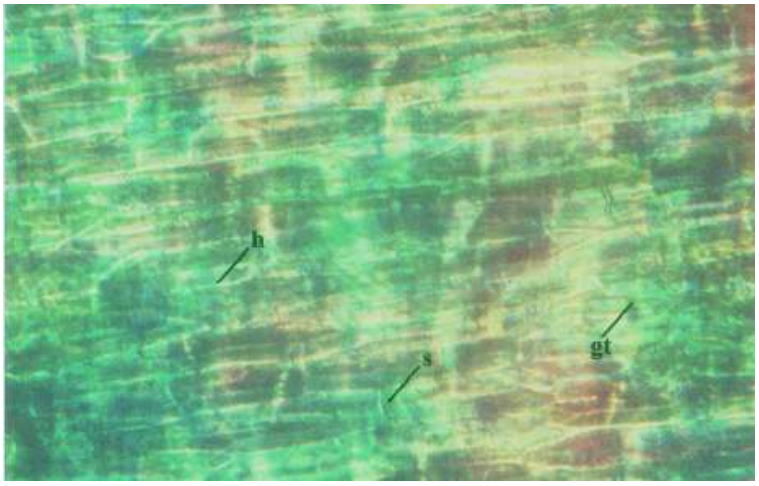


Fig.62. Surface view of cv. Giza 22 (susceptible) hypocotyl, 12 hours after inoculation with *F. solani* f.sp. *phaseoli* at 30°C. Note germinating macroconidia (s) and germ tubes (gt) on the surface of hypocotyl epidermal cells. Note the large number of penetrating hyphae (h) that embedded in dark regions of hypersensitive reaction. Also note the development of the host-pathogen interaction as compared with that occurred after 6 hours of infection (x40 magnification).

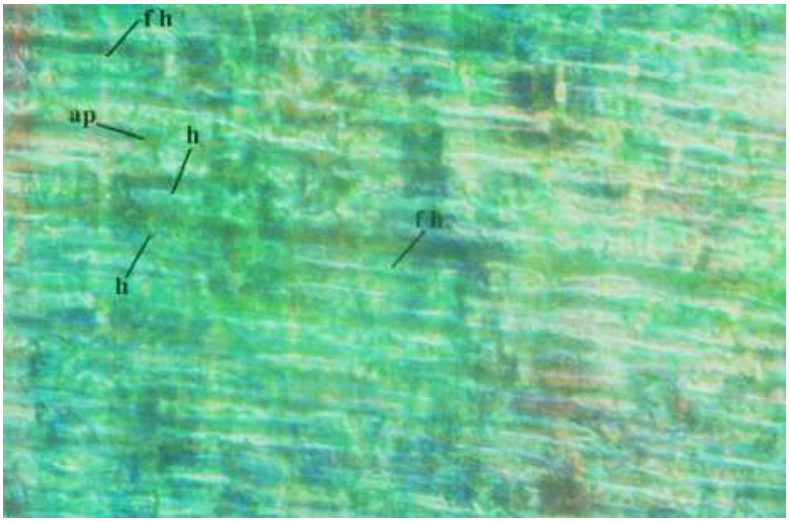


Fig. 63. Surface view of cv. Giza 22 (susceptible) infected hypocotyl, 1 day after inoculation with *F. solani* f.sp. *phaseoli* at 30°C. Note penetrating hyphae (fh) appear in discoloured and undiscoloured epidermal cells and fungal mycelium spreading longitudinally both interacellularly (fh) and intercellularly (h) (x40 magnification).

Conclusion:

From the previous results, we found that at 20°C, the germination of the spores was occurred in case of soybean after 6 hours of inoculation (Figs. 37 & 39) and in case of french bean, the germination was occurred after 12 hours of inoculation (Fig. 34).

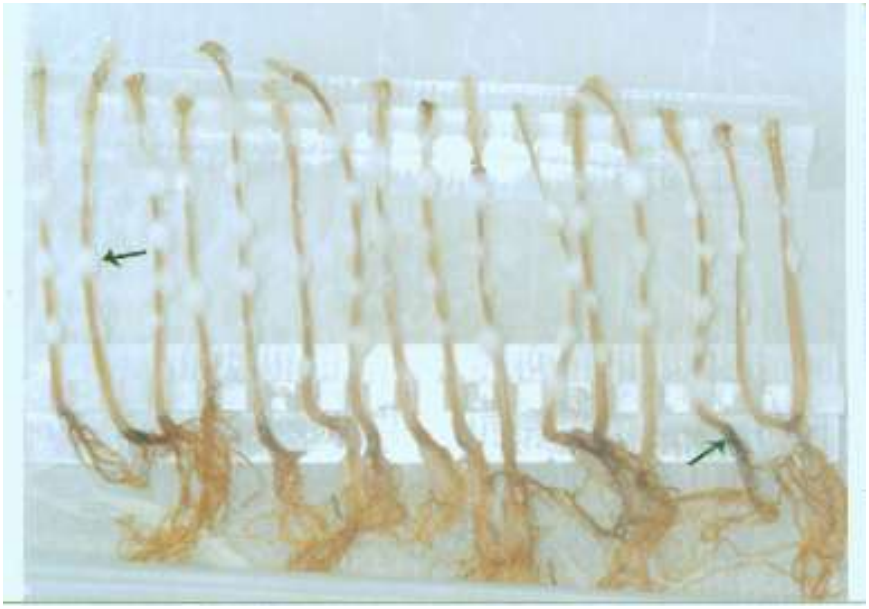
But at 30°C, the germination of spores was occurred in soybean after 4 hours of inoculation (Fig. 60), while in french bean, the germination was occurred after 6 hours of inoculation (Fig. 51). So the temperature has effective role in determination the time at which the germination will occur, hence, the temperature play an important role in the developing of infection.

4) At 35°C:

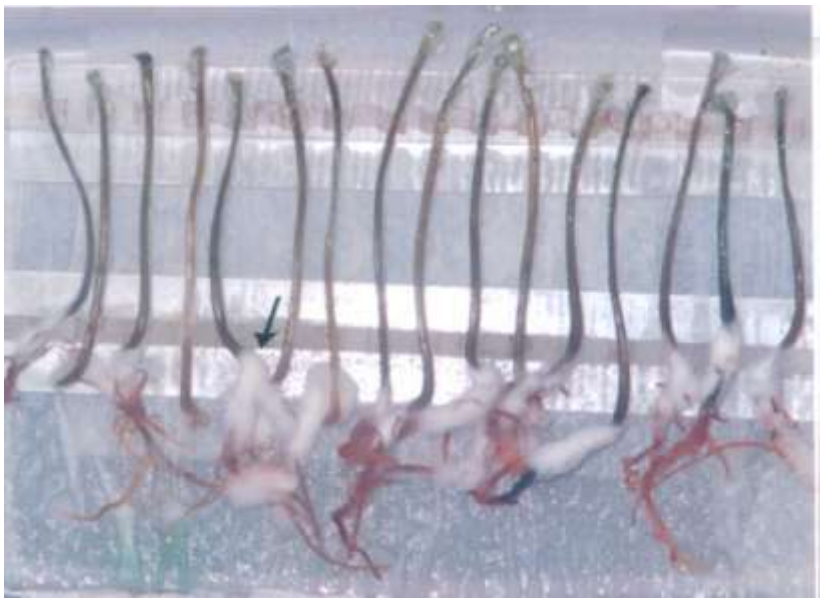
Macroscopic observations showed a growth of the fungus in the inoculation sites, 3 days after inoculation of the hypocotyls of all cultivars.

From this result it can be concluded that at 35°C, cultivars showed full susceptibility (s) to inoculation of $1x10^6$ spore /ml of *F. solani* f.sp. *phaseoli*.

Fig.64. Reaction of hypocotyls of cv. Nebraska (susceptible), 3 days after inoculation with *F. solani* f.sp. *phaseoli* at 35°C. Note the mycelium of the fungus firstly in the site of inoculation then spreaded along the hypocotyls and the root system (arrows).



(A)



(B)

Fig. 65. A, reaction of hypocotyls of cv. Nebraska (susceptible), 3 days after inoculation with *F. solani* f.sp. *phaseoli* at 35°C. Note the appearance of fungal hyphae in the inoculation sites. B, reaction of the same cultivar, 3 days after only inoculation of the root system with the same pathogen. Note the high mass of fungal hyphae on the roots indicating the high sensitivity of the roots than that of the hypocotyls.

Conclusion

From the previous results it is evident that hypocotyls of cv. Giza 22 showed full susceptibility (s) to inoculation of $1x10^6$ spores ml^{-1} of *F.solani* f.sp. *phaseoli* at 30° and 35° C but lesser susceptibility (Is) at 20° and 25 ℃. On the other hand, cv. Crowford was full susceptible (s) at 35 ℃ and was partially resistant (Ir) at 30 ℃, but was resistant (R) at 20 ℃ and 25 ℃. Where cv. Bronco was full susceptible (s) at 35° and 30 ℃, but partially susceptible (Is) at 25 ℃ and was resistant (R) at 20 ℃. Also cv. Nebraska was full susceptible (s) at 35 ℃, but showed an intermediate reaction (Ir) at 30 ℃, and was resistant at 20 ℃ and 25 ℃. So we can conclude that, all cvs. Showed full susceptibility (s) at 35°C, but were resistant at 20 ℃ except cv. Giza 22 that showed an intermediate reaction (Is) at 20 ℃. Hence, the incubation temperature of the inoculated hypocotyls has an important effect in host resistance to *F. solani* f.sp. *phaseoli* infection.

II.2. Infection of french bean and soybean intact hypocotyl by *F. solani* f.sp. *glycine* and *C. lindemothianum* (the causal pathogen of bean anthranose) separately:

Experiments, were carried out to check the resistance and susceptibility of all the previously tested cultivars of French bean and soybean, to the two fungal pathogens (*F. solani* f.sp. *glycine* and *C. lindemuthianum*), at the same previous incubation temperatures. Excised hypocotyls (intact hypocotyls) from 10 days old seedlings were inoculated by the droplet technique and macroscopic examination also microscopical observations on symptom formation were carried out at intervals for 2 days. Infection sites were classified as resistant when hypersensitive flecks appeared 2 days after inoculation with no further progress of infection. Susceptibility was recognized when dark brown spreading lesions and rotting of hypocotyls were observed. The results show that at 20 ℃, all cultivars showed resistant except cv.Giza 22 showed an intermediate reaction of susceptibility (Is). In contrary, all cultivars showed full susceptibility (s) at 35 ℃. But at 25 ℃, cvs. Nebraska and Crowford were resistant (R), whereas cvs. Bronco and Giza 22 were partially susceptible (Is). At 30 ℃, cvs. Nebraska and Crowford showed an intermediate reaction of resistance (Ir), while cvs. Bronco and Giza 22 were susceptible (s).

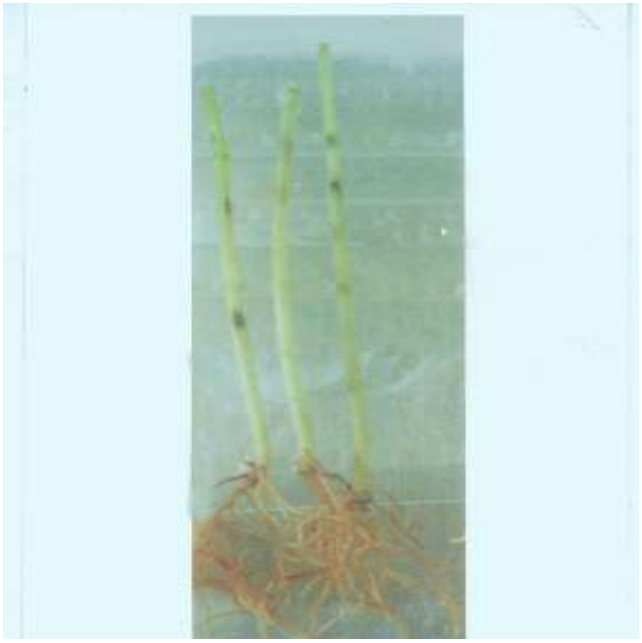


Fig. 66. Reaction of hypocotyls of cv. Nebraska partially resistant (Ir), 2 days after inoculation with *F.solani* f. sp. *glycine* at 30°C. Note few hypersensitive brown flecks that became elongated at the inoculation sites. Note also the very light browning of the hypocotyls and moderately browning of the roots.

Fig.67. Reaction of hypocotyls of cv. Nebraska resistant (R), 10 days after inoculation with *C. lindemuthianum* at 20°C. Note the localized light brown necrotic flecks in infection sites on the hypocotyls.

Fig. 68. Reaction of hypocotyls of cv. Nebraska partially resistant (Ir), 2 days after inoculation with *C. lindemuthianum* at 30°C. Note few hypersensitive brown flecks that became elongated at the site of infection and note the moderately rotting and light browning of few number of hypocotyls also note the moderately brown rotting of the roots.

Conclusion:

From the above results, we found that, the reaction of french bean and soybean hypocotyls against *F. solani* f.sp. *phaseoli* was the same reaction that obtained against *F. solani* f.sp *glycine* and *C. lindemuthianum* with the tested cultivars at the same conditions.

III. Phytoalexins production

Phytoalexin production, namely phaseollin was monitored (measured) at 24 h after inoculation of french bean cultivars (cvs. Nebraska and Bronco) with the pathogenic *Fusarium solani* f.sp. *phaseoli*, which was challenged with the non pathogenic fungal pathogen i.e. non related Fusarium pathogen, namely *Fusarium solani* (potato derived isolate) for 4h. Healthy cultivars were also subjected to similar analysis. Total content of phaseollin was determined. The concentration of phaeollin was 118219 and 103731 μgg^{-1} biomass dry wt, as evaluated by the area peak of phaseollin at retention time in the range of 20.83-20.94, in healthy cv. Nebraska and cv. Bronco respectively. But with the induced systems, the concentration of phaseollin was 227049 and 260422 μgg^{-1} biomass dry wt respectively.

Also, phytoalexin production, namely glyceollin was monitored at 24h after inoculation of soybean cultivars (cvs. Giza 22 and Crowford) with the pathogenic *F. solani* f.sp. *phaseoli*, then challenged with the non pathogenic fungal pathogen i.e. non related Fusarium pathogen, namely Fusarium potato isolate. Healthy cultivars were also subjected to similar analysis. Total content of glyceollin was determined. The concentration of glyceollin was 135740 and 37886 μgg^{-1} biomass dry wt, as evaluated by the area peak of glyceollin at retention time in the range of 20.56-20.72, in healthy cv. Giza 22 and cv. Crowfoird respectively. But with the induced systems, the concentration of glyceollin was 294814 and 482243 μgg^{-1} biomass dry wt respectively.

Table (9) wide table

Fig. 69. HPLC-chromatograms of phytoalexin phaseollin extracted from healthy hypocotyls of french bean cv. Nebraska (a) with absorbance area 118219 $\mu g g^{-1}$ biomass dry wt and retention time (RT) 20.83, and phaseollin extracted from cv. Nebraska / *F. solani* f.sp. *phaseoli* resistant (induced) combination, 1 day after inoculation at 20 °C (b) with absorbance area 277049 $\mu g g^{-1}$ biomass dry wt. Absorbance at 283 nm and retention time (RT) 20.83.

Fig. 70. HPLC-chromatograms of phytoalexin phaseollin extracted from healthy hypocotyls of french bean cv. Bronco (a) with absorbance area 103731 μgg^{-1} biomass dry wt and retention time (RT) 20.94, and phaseollin extracted from cv. Bronco / *F. solani* f.sp. *phaseoli* induced combination, 1 day after inoculation at 20 °C (b) with absorbance area 260422 μgg^{-1} biomass dry wt. Absorbance at 283 nm and retention time (RT) 20.88.

Fig. 71. HPLC-chromatograms of phytoalexin glyceollin extracted from healthy hypocotyls of soybean cv. Crowford (a) with absorbance area 378860 $\mu g g^{-1}$ biomass dry wt and retention time (RT) 20.72, and glyceollin extracted from cv. Crowford / *F. solani* f.sp. *phaseoli* induced combination, 1 day after inoculation at 30 °C (b) with absorbance area 482243 $\mu g g^{-1}$ biomass dry wt. Absorbance at 286 nm and retention time (RT) 21.04.

Fig. 72. HPLC-chromatograms of phytoalexin glyceollin extracted from healthy hypocotyls of soybean cv. Giza 22 (a) with absorbance area 135740 $\mu g g^{-1}$ biomass dry wt and retention time (RT) 20.56, and glyceollin extracted from cv. Giza 22 / *F. solani* f.sp. *phaseoli* induced combination, 1 day after inoculation at 30 °C (b) with absorbance area 294814 $\mu g g^{-1}$ biomass dry wt. Absorbance at 286 nm and retention time (RT) 21.63.

Conclusion:

In case of french bean, the HPLC analysis showed production of phaseollin in both healthy and the induced systems. However, a remarkable accumulation was shown with the induced systems as compared with healthy ones (Figs. 69 and 70). This indicates that both the pathogen and the challenge non pathogen used in these systems have a role as inducers for phytoalexins production.

In soybean, similar conclusion can be deduced from the above mentioned results, i.e. Fusarium pathogen and the non pathogen, both have the ability to induce phytoalexin (glyceollin) production in soybean systems (cvs. Crowford and Giza 22) studied. Further, the soybean cultivar Crowford was proved to exhibit a potential source for phytoalexin production (with respect to glyceollin) among the other tested cultivars of soybean versus to the other legume plant tested (i.e. french bean cvs. Nebraska and Bronco), Table (9).

IV. Total soluble protein analysis (SDS-PAGE of proteins electrophoresis)

Banding patterns of total soluble proteins were detected using coomassi Brilliant blue-R250 for two cultivars of french bean (cvs. Nebraska and Bronco) and two cultivars of soybean (cvs. Crowford and Giza 22). The four cultivars were presented in (Figures 73 & 74 and Tables 10 & 11). Also, the banding pattern of total soluble proteins extracted from the mycelia of original isolates of the causal pathogens (*F. Solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. Lindemothianum*) and their reisolated isolates were detected. The banding patherns characterized with molecular weights (MW) that ranged from 212-605 KDa. The results are summarized in Tables (13 & 14).

IV.1. French bean (*Phaseolus vulgaris* L.) cultivars

Figures (73) and Table (10) showed the SDS-PAGE of protein banding pattern and the presence versus absence of Electrophoretic bands for two induced french bean genotype compared to healthy ones. Two bands with molecular weights of 42.7 and 27 KDa were found in healthy plants while absent in induced ones. Also, a band with molecular weight 55.6 KDa was present in healthy two cultivars while absent in the rest of induced ones except cv. Bronco that induced with *C. Lindemothianum*. The bands of 49.8 and 23.6 KDa were absent in healthy plants while present in induced ones. These bands can be considered as a potential markers associated with the infection of two cultivars except in case of cv. Bronco that inoculated with *C. lindemothianum* where these bands were absent.

The band of 66.4 KDa was found only in cv. Nebraska that inoculated with *F. solani* f.sp. *glycine* and that *C. lindemothianum*. This band can be considered as a potential marker associated with the inoculation of cv. Nebraska with these two fungal pathogens. The band of 33.6 KDa was observed in cv. Nebraska that inoculated with *F. solani* f.sp. *glycine* While absent in the rest of healthy and induced cultivars. Also, the band of 31.6 KDa was present only in cv. Bronco that inoculated with *C. lindemothianum* and the band with molecular weight 29.6 KDa was found in cv. Nebraska that inoculated with *C. lindemothianum* while absent with the rest of healthy and induced cultivars.

Some bands (Table 10) such as 66.4 KDa (lanes 5 and 7), 33.6 KDa (lane 3) and 29.6 KDa (lane 7) were observed in resistant cv. Nebraska with different causal pathogens while absent with susceptible cv. Bronco indicating these bands can be considered as a potential markers associated with resistant Nebraska against different invading pathogens.

IV.2. Soybean (*Glycine max* L.)cultivars:

Figure (74) and Table (11) showed the SDS-PAGE of protein banding pattern and the presence versus absence of Electrophoretic bands for two induced soybean genotype compared to healthy ones. Two bands with molecular weight of 57.18 and 29.2 KDa were found in each of healthy and induced plants. The bands of 55.6, 31.5 and 27 KDa were present in healthy plants and in all induced cv. Crowford but were absent in all induced cv. Giza 22. These

bands can be considered as a potential markers associated with resistance of cv. Crowford against different causal fungal pathogens. One band with molecular weight 85.6 KDa was found in healthy cultivars and was found in cv. Crowford that inoculated with *F. Solani* f.sp. *phaseoli* while absent in the rest of induced cultivars. Also, a band of 33.6 KDa was found in cv. Crowford that inoculated with *F. Solani* f.sp. *phaseoli* while absent in the rest of healthy and induced two cultivars. Only one band of 42.7 KDa was found in healthy cv. Giza 22 but this band was not obsrved in the other healthy and induced cultivars. This band can be considered as a potential marker associated with healthy cv. Giza 22.

IV.3. The original and reisolated isolates of the target pathogens:

Figure (75) and Table (12) showed the SDS-PAGE of protein banding patterns and the presence versus absence of Elecrophoretic bands of the representative isolates of fungal pathogens (*F. Solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* that used for inoculation of french bean and soybean) and their reisolated isolates. Two bands with molecular weights of 42.6 and 8.8 KDa were found with the original and the reisolated ones. These bands can be considered as a potential markers associated with both the original and the reisolated isolates of the target pathogens i.e. there is no effect of the interaction between the host and the pathogen on these bands. Another two bands of 66.4 and 21.6 KDa were observed with the original isolates, and they were observed with the reisolated isolate of *F. Solani* f.sp. *phaseoli*. One band with

molecular weight of 47.7 KDa was found with original isolate of *F. Solani* f.sp. *phaseoli* while it was not present with the rest of the original and the reisoalted ones. This band can be considered as a potential marker associated with the original isolate of *F. Solani* f.sp. *phaseoli*. Two bands of 33.6 and 32.4 KDa were observed in the reisolated isolate of *F. solani* f.sp. *phaseoli* while it was absent with the rest of the original and the reisolated ones. These bands can be considered as a potential markers associated with the reisolated isolates of *F. solani* f.sp. *phaseoli*. These bands have a relationship with the interaction betwen the host with *F. solani* f.sp. *phaseoli*.. A band of 20 KDa was present with both the original and the reisolated isolates of *C. lindemothianum* while it was absent with the other original and the reisolated isolates. Only one band with molecular weight of 18.1 KDa was found with the reisolated isolate of *C. Lindemothianum*. This band could be considered as a potential marker associated with the reisolated isolate of *C. lindemothianum*.

The bands with molecular weights 33.6, 32.4 and 18.1 KDa could be considered as a potential markers associated with interaction of each of the french bean and soybean plants with their target pathogens tested separately in this study.

Fig. (73): SDS-PAGE protein banding pattern of representative samples of french bean (*Phaseolus vulgaris* L.) inoculated with Fusarium potato isolate for 4 h, then inoculated with different target fungal pathogens i.e *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and C. *lindemothianum* separately (i.e. cultivar-pathogen/resistant (induced) combination), for 24 h at 20 °C.

1 = *Phaseolus vulgaris* L. cv. Nebraska (healthy)

2 = *Phaseolus vulgaris* L. cv. Bronco (healthy)

3 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with Fusarium potato isolates for 4 h, then inoculated with *F. solani* f.sp. *Phaseoli*

4 = *Phaseolus vulgaris* L. cv. Bronco inoculated with Fusarium potato isolates for 4 h, then inoculated with *F. solani* f.sp. *Phaseoli*

5 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with Fusarium potato isolates for 4 h, then inoculated with *F. solani* f.sp. *glycine*

6 = *Phaseolus vulgaris* L. cv. Bronco inoculated with Fusarium potato isolates for 4 h, then inoculated with *F. solani* f.sp. *glycine*..

7 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with Fusarium potato isolates for 4 h, then inoculated with *Colletoterichum lindemothianum*

8 = *Phaseolus vulgaris* L. cv. Bronco inoculated with Fusarium potato isolates for 4 h, then inoculated with *Colletoterichum lindemothianum*

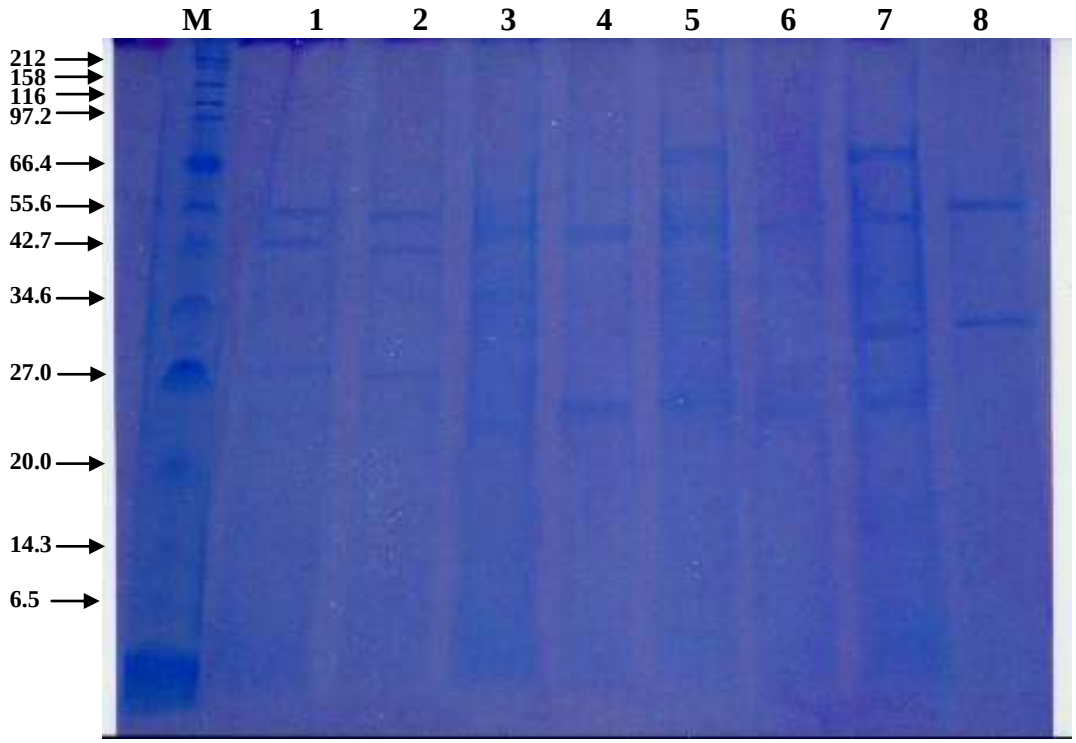


Table 10: (1) and (0), presence and absence of SDS-PAGE protein bands from soluble protein extracted from french bean (*Phaseolus vulgaris* L.) cvs. Nebraska and Bronco inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* separately (i.e. cultivar-pathogen induced combination) for 24 h at 20 °C.

Number of row	MW (KDa)	French bean (*Phaseolus vulgaris* L.)							
		Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
1	212	0	0	0	0	0	0	0	0
2	158	0	0	0	0	0	0	0	0
3	116	0	0	0	0	0	0	0	0
4	97.2	0	0	0	0	0	0	0	0
5	66.4	0	0	0	0	1	0	1	0
6	55.6	1	1	0	0	0	0	0	1
7	49.8	0	0	1	1	1	1	1	0
8	42.7	1	1	0	0	0	0	0	0
9	33.6	0	0	1	0	0	0	0	0
10	31.6	0	0	0	0	0	0	0	1
11	29.6	0	0	0	0	0	0	1	0
12	27.0	1	1	0	0	0	0	0	0
13	23.6	0	0	1	1	1	1	1	0
14	20.0	0	0	0	0	0	0	0	0

Fig. (74): SDS-PAGE protein banding pattern of representative samples of soybean (*Glycine max* L.) cvs Giza 22 and Crowford inoculated with Fusarium potato isolate for 4 h, then inoculated with different target fungal pathogens i.e *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and C. *lindemothianum* separately (i.e. cultivar-pathogen/resistant (induced) combination), for 24 h at 30 °C.

1 = *Glycine max* L. cv. Giza22 (healthy)

2 = *Glycine max* L. cv. Crowford (healthy)

3 = *Glycine max* L. cv. Giza22 inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *Phaseoli*

4 = *Glycine max* L. cv. Crowford inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *Phaseoli*

5 = *Glycine max* L. cv. Giza22 inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *glycine*

6 = *Glycine max* L. cv. Crowford inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *glycine*.

7 = *Glycine max* L. cv. Giza22 inoculated with Fusarium potato isolate for 4 h, then inoculated with *Colletoterichum lindemothianum*

8 = *Glycine max* L. cv. Crowford inoculated with Fusarium potato isolate for 4 h, then inoculated with *Colletoterichum lindemothianum*

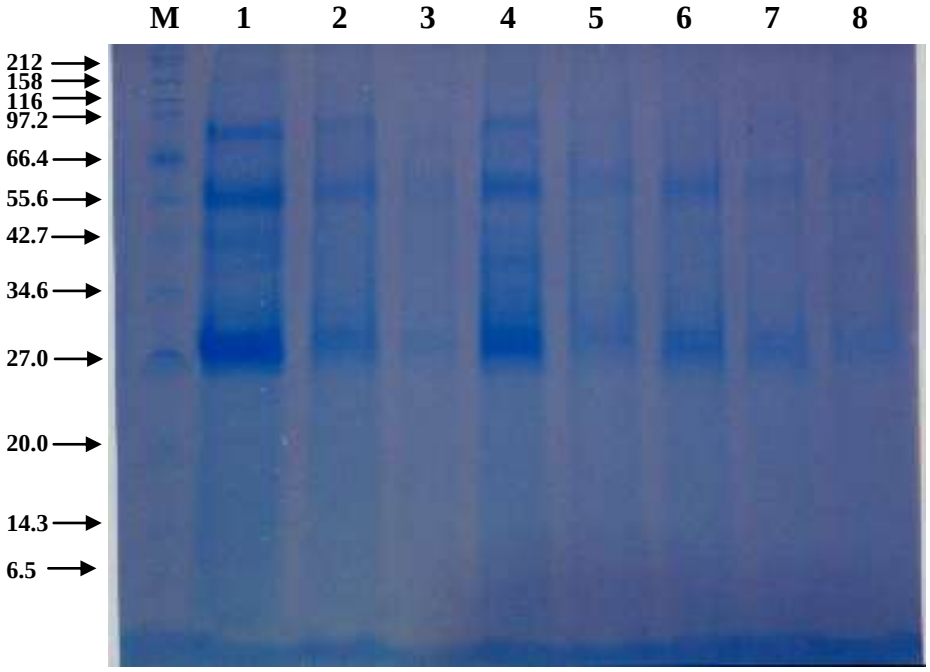


Table 11: (1) and (0), presence and absence of SDS-PAGE protein bands from soluble protein extracted from soybean *(Glycine max.* L.) inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* separately (i.e. cultivar-pathogen induced combination) for 24 h at 30 °C.

Number of row	MW (KDa)	Soybean (*Glycine max*. L.)							
		Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
1	212.0	0	0	0	0	0	0	0	0
2	158.0	0	0	0	0	0	0	0	0
3	116.0	0	0	0	0	0	0	0	0
4	85.6	1	1	0	1	0	0	0	0
5	66.4	0	0	0	0	0	0	0	0
6	57.18	1	1	1	1	1	1	1	1
7	55.6	1	1	0	1	0	1	0	1
8	42.7	1	0	0	0	0	0	0	0
9	33.6	0	0	0	1	0	0	0	0
10	31.5	1	1	0	1	0	1	0	1
11	29.2	1	1	1	1	1	1	1	1
12	27.0	1	1	0	1	0	1	0	1
13	20.0	0	0	0	0	0	0	0	0

Fig. (75): SDS-PAGE protein banding pattern of representative samples of the causal pathogens (i.e. *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and C. *lindemothianum*) and their reisolated isolates that used for inoculation of french bean and soybean in this study.

1 = Sample of *F. solani* f.sp. *phaseoli*

2 = Sample of reisolated isolate of *F. solani* f.sp. *phaseoli* from cv. Nebraska.

3 = Sample of *F. solani* f.sp. *glycine* that isolated from soybean cv. Giza 22.

4 = Sample of reisolated isolates of *F. solani* f.sp. *glycine* from cv. Giza 22.

5 = Sample of *Colletotrichum lindemothianum*.

6 = Sample of reisolated isolate of *C. lindemothianum* from french bean cv. Nebraska.

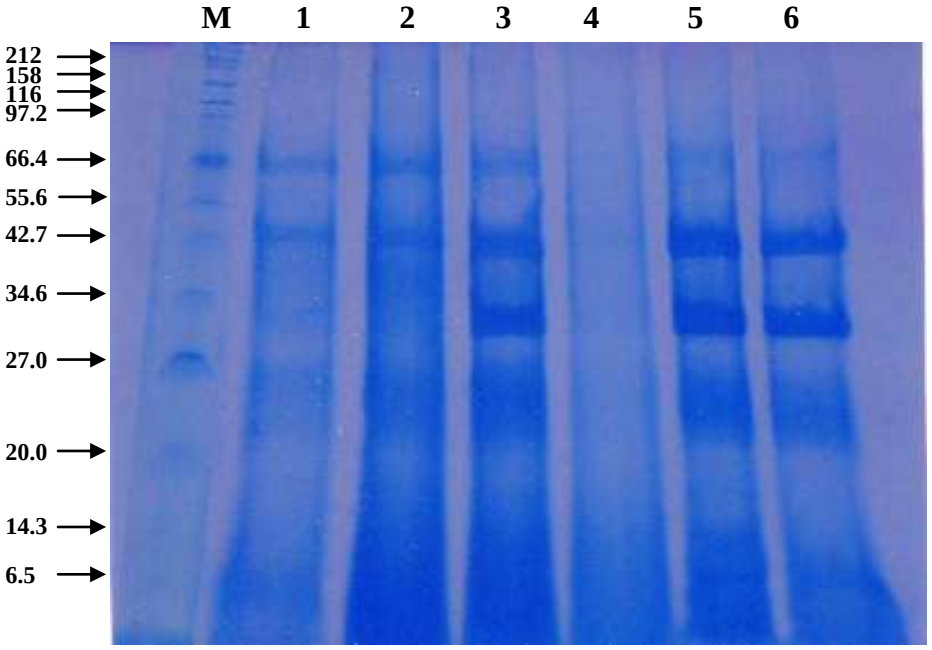


Table 12: (1) and (0) presence and absence of SDS-PAGE protein bands from soluble protein extracted from the mycelia of *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* and their reisolated isolates that used for inoculation of french bean and soybean.

Number of row	MW (KDa)	The original and reisolated isolates of the target pathogens					
		Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
1	212	0	0	0	0	0	0
2	158	0	0	0	0	0	0
3	116	0	0	0	0	0	0
4	97.2	0	0	0	0	0	0
5	66.4	1	1	1	0	1	0
6	55.6	0	0	0	0	0	0
7	47.7	1	0	0	0	0	0
8	42.6	1	1	1	1	1	1
9	33.6	0	1	0	0	0	0
10	32.4	0	1	0	0	0	0
11	29.6	1	0	1	0	1	1
12	27.0	0	0	0	0	0	0
13	21.6	1	1	1	0	1	0
14	20.0	0	0	0	0	1	1
15	18.1	0	0	0	0	0	1
16	10.1	0	1	1	0	0	0
17	8.8	1	1	1	1	1	1

Fig. (13) Wide Table

Table (14): Presence (+) versus absence (-) of SDS-PAGE protein bands from soluble protein extracted from the mycelia of *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* and their reisolated isolates that used for inoculation of *Phaseolus vulgaris* L. and *Glycine max* L.

No. of Row	MW (KDa)	*F. solani* f.sp. *phaseoli*		*F. solani* f.sp. *glycine*		*C. lindemothianum*	
		Original isolate (lane 1)	Reisolated isolate (lane 2)	Original isolate (lane 3)	Reisolated isolate (lane 4)	Original isolate (lane 5)	Reisolated isolate (lane 6)
1	212	-	-	-	-	-	-
2	158	-	-	-	-	-	-
3	116	-	-	-	-	-	-
4	97.2	-	-	-	-	-	-
5	85.6	-	-	-	-	-	-
6	66.4	+	+	+	-	+	+
7	57.18	-	-	-	-	-	-
8	55.6	-	-	-	-	-	-
9	49.8	-	-	-	-	-	-
10	47.7	(+)	-	-	-	-	-
11	42.6	+	+	+	+	+	+
12	33.6	-	+	-	-	-	-
13	32.4	-	+	-	-	-	-
14	31.6	-	-	-	-	-	-
15	29.6	+	-	+	-	+	+
16	27.0	-	-	-	-	-	-
17	21.6	+	+	+	-	+	-
18	20.0	-	-	-	-	+	+
19	18.1	-	-	-	-	-	+
20	10.1	-	+	+	-	-	-
21	8.8	+	+	+	+	+	+

+ within circle, indicates the specificity of this band of 47.7 KDa for *F. solani* f.sp. *phaseoli* (original isolate)

Conclusion

Bands with molecular weights 49.8 KDa and 23.6 KDa were commonly present in all of the induced hypocotyls tissue and were not detected in healthy (control) ones (Table 13). This indicates that they are associated with pathogenesis. The bands with molecular weights 55.6, 42.7 and 27.0 KD, were only present in the control. The bands with molecular weights 33.6 KDa and 29.6 KDa were only present in cv. Nebraska induced tissue, inoculated with *F. solani* f.sp. *phaseoli* and *C. lindemothianum* respectively. Whereas, only one band with MW 31.6 KDa was detected in Bronco induced tissue / *Colletotrichum* combination. But with cv. Nebraska/ *F. solani* f.sp. *glycine* combination a unique band was observed with molecular weight 66.4 KDa. Also, the same band was detected with the induced cv. Nebraska/ *Colletotrimchum* combination.

This results concludes the existence of two common bands in the tested cultivars (control). These bands did not present in the induced combinations of similar cultivars, indicating that they are not associated with pathogensis.

Nevertheless, the presence of specific band with the molecular weight 66.4 KDa seems to be related to cultivar specific (cv. Nebraska) with only the close related pathogen (*F. solani* f.sp. *glycine*) and to the non related pathogen (*C. lindemothianum*). Each pathogen even within a closely related genus might exhibit its own defence protein machinery system mechanism. Further, this implies

to the non related pathogen (i.e. of different genus such as *C. lindemathianum* that used in this study).

Also, each cultivar seems to contribute to the induction of specific protein related to the pathogenic fungal isolate, involved in the studied induced combinations. This assumption is based on clearly the presence of only of the three observed bands as mentioned before, with molecular weights 33.6, 31.6 and 29.6 KDa.

The profile pattern of total protein differs among the two host plants studied. Common bands at 57.18 KDa and 29.6 KDa were abundant in control (healthy) and induced combinations in soybean. These bands specifically characterize soybean only because they were not detected in french bean.

Two cvs. of soybean behaved differently i.e. indicated from the presence of three bands with molecular weights 55.6, 31.6 and 27.0 KDa. They were associated with the pathogensis in the cv. Crowford induced combinations and they were also present in the healthy (control) of similar cultivar. The band with molecular weight 85.6 KDa was also commonly present in the tested healthy cultivars and was detected only with the induced cv. Crowford/ *F. solani* f.sp. *phaseoli* combination. It is interesting that one band with molecular weight 33.6 KDa that was induced in cv. Crowford/ *F. solani* f.sp. *phaseoli* combination.

To determine whether the observed protein bands in the induced systems is related to fungal involvement or and to the interaction between the fungal pathogens and their hosts, the protein

profile of the target pathogen (original and reisolated isolates) were detected. This was clearly shown in Table (13) & (14).

The inducible band of 66.4 KDa is related to the fungal pathogen protein (*F. solani* f.sp. *glycine* & *C. lindemothianum*) only in cv. Nebraska / *F. solani* f.sp. *glycine* & *C. lindemothianum* pathosystems. This band is possibly contributed to PR (pathogensis) of defence type. However complete analysis should include the susceptible combination to finalize this assumption. Further, this band characterized the isolates of *F. solani* f.sp. *glycine* and *C. lindemothianum* (the original ones only but it was not associated with the reisolated isolates respectively).

The band at 57.18 KDa specifically characterizing the tested soybean cvs. (healthy) and the studied induced pathosystems. It is not detected in french bean whether the healthy or the induced pathogens. Similarly, the bands of 55.6 KDa represents the plant origin contribution in pathogensis with soybean cv. Crowford and french bean cv. Bronco / *C. lindemothianum* induced system.

The bands of 49.8 and 23.6 KDa are of importance in the studied induced systems in french bean only and not with soybean system. It is induced as a result of all of the inoculating fungal pathogens after the challenge with the non pathogenic Fusarium isolate, i.e. important in the interaction of the induced system.

The band of 85.6 KDa was observed in both of the soybean cvs. Crowford and Giza 22 (healthy), but it was detected in the cv. Crowford/ *F. solani* f.sp. *phaseoli* induced system. Its absence

from the tested pathogens, pointed to its possible involvement in the induced resistant interaction mentioned above.

Note: Whether this band is representing a proteins related pathogensis of defense types, is not clear since the susceptible contributions were not subjected in this study for this type of analysis.

The band of 42.7 KDa, observed in healthy french bean cvs. Nebraska ad Bronco, and soybean cv. Giza 22 indicating that is involved in the normal metabolic activities of each of the host plant specied her with, and the fungal pathogens (i.e. has no role in the resulted induced resistance systems).

The induced band of 33.6 KDa for only the reisoalted *F. solani* f.sp. *phaseoli*, was detected with cv. Nebraska/ *F. solani* f.sp. *phaseoli* induced resistant system and cv. Crowford/ *F. solani* f.sp. *phaseoli* induced resistant system, indicating that the host plant (french bean or soybean) reactivated the pathogensis of this isolate regardless its origin.

Similar band of 31.6 KDa was present in healthy soybean cvs. e.g. Crowford and Giza 22 and in all of the induced resistant system(s) but of cv. Crowford only. And not detected with the tested fungi, however, it was also observed in cv. Bronco/ *C. lindemothianum* induced system. Indicating its contribution from the host plant involved in the induced system. Therefore it is possibly has a relation in the pathogensis of this interacting system(s).

The band of 27.0 KDa seems to be contribute in a specific manner, based on the tested host plant. It is observed in healthy french bean cvs. and soybean cvs as well as but associated only with the induced resistant systems of the studied soybean pathosystems. It is expressed only with the interaction between the soybean cv. Crowford/ with all pathogens induced systems.

The band of 20.0 KDa was only detected with *C. lidemothianum* (the original and the reisolated isolates), but the band of 18.1 KDa was only detected with the reisolated isolate of *C. lindemothianum*. Since it is not observed in either the tested healthy cvs. of both french bean and soybean or in the studied induced pathosystems, this band seems largely to contribute to the pathogensis of this *Colletotrichum* pathogen and not the Fusarium pathogen.

The bands observed at 10.1 KDa and 8.8 KDa characterized the genus Fusarium whether f.sp. *phaseoli* or f.sp. *glycine* but the one at 8.8 KDa is further characterizing the other target tested pathogen (i.e. *Colletotrichum lindemothianum*). It is regarded as a fungal shared band regardless the genus of the tested fungi.

V. Peroxidase induction in french bean (*Phaseolus vulgaris* L.) and soybean (*Glycine max* L.) in response to microbial challenge:

V.1. French bean (*Phaseolus vulgaris* L.) cultivers:

Figure (76) and Table (15) showed the zymogram of peroxidase isozyme banding pattern and the presence versus absence of Electrophoretic bands for two french bean genotype after 24 hours of inoculation with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* (i.e. cultivar-pathogen / susceptible (non induced) combination) at 20 °C compared to healthy ones. The band No.r6 was found in each of healthy and infected cultivars. The band No.r1 was present in all infected and healthy cultivars of *Phaseolus vulgaris* L. except the healthy cv. Nebraska where was absent. The three bands No.r3, r9 and r10 were found in both cvs. Nebraska and Bronco that infected with *F. solani* f.sp. *glycine* and *C. lindemothianum* while absent with the rest of infected and healthy cultivars except the band No. r10 that was found in healthy cv. Bronco. These bands No. r3 and r9 can be considered as a potential marker associated with the two cultivars that inoculated with the same two fungal pathogens. The two bands No. r4 and r8 were observed only in cv. Nebraska that inoculated with *F. solani* f.sp. *glycine* and *C. lindemothianum* while absent with the rest of infected and healthy cultivars. these bands can be considered as a potential marker associated with cv. Nebraska when inoculated with the same pathogens. So the bands No. r_3, r_4, r_8, r_9 and r_{10} can be considered as a potential markers associated with susceptible (non-

induced) combination. A band No. r_5 was found in cv. Bronco that inoculated with *C. lindemothianum* while absent with the rest of healthy and infected cultivars. Also, a band No r_{11} was found in cv. Nebraska that inoculated with *F. solani* f.sp. *glycine* while absent with the rest of healthy and infected cultivars.

Figure (77) and Table (17) showed the zymogram of peroxidase isozyme banding pattern and the presence versus absence of Electrophoretic bands for two french bean genotype inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* for 24h at 20 °C compared to healthy ones. The band No. r_1 was found in each of healthy and induced two cultivars. Two bands No. r_3 and r_6 were observed in healthy cultivars while absent with the rest of induced ones. These two bands can be considered as a potential marker associated with healthy cultivars. the two bands No. r_7 and r_8 were found in each of healthy and induced two cultivars except cv. Nebraska that induced with *F. solani* f.sp. *phaseoli* and cv. Bronco that inoculated with *C. lindemothianum*. The band No. r_2 was found in all induced cultivars except cv. Nebraska that inoculated with *F. solani*, f.sp. *phaseoli* while absent with healthy cultivars. This band can be considered as a potential marker associted with host resistance through induction of peroxidase production. One band No. r_4 was found in each of healthy and induced cv. Bronco that inoculated with *F. solani* f.sp. *phaseoli* and *F. solani* f.sp. *glycine* also was found in cv. Nebraska that inoculated with *F. solani* f.sp. *glycine* while absent with the rest of induced and healthy cultivars. The band No. r_5 was observed in healthy

cv. Nebraska and in the two inoculation cultivars that inoculated with *C. lindemothianum* while this band was not observed in the rest of healthy and induced cultivars. This band can be considered as a potential marker associated with the infection of two cultivars with *C. lindemothianum*. Also the band No. r_9 was found in healthy cv. Nebraska and in the two cultivars that inoculated with *F. solani* f.sp. *glycine* while absent with the rest of healthy and induced cultivars. This band can be considered as a potential marker associated with the interaction between the cvs. Nebraska and Bronco with *F. solani* f.sp. *glycine*.

V.2. Soybean (*Glycine max* L.) cultivars

Figure (76) and Table (15) showed the zymogram of peroxidase isozyme banding pattern and the presence versus absence of Electrophoretic bands for two soybean (*Glycine max* L.) genotype after 24 hours of inoculation with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* (i.e. cultivar pathogen / susceptible (non-induced) combination) at 30°C compared to healthy ones. The band No. r_1 was found in each of healthy and infected two cultivars. Two bands No. r_2 and r_4 were observed in both cvs. Giza 22 and Crowford that inoculated with *C. lindemothianum* while absent with in the rest of healthy and infected cultivars. These two bands can be considered as a potential marker associated with the interaction betwen both cvs. Giza 22 and Crowford with *C. lindemothianum*. The two bands No. r_5 and r_7 were found only in cv. Crowford that infected with *C. lindemothianum* while absent with of the rest of healthy and infected cultivars. So these bands can be considered as a potential markers associated with inoculation of

cv. Crowford with *C. lindemothianum*. Two bands No. r_8 and r_9 were found in each of healthy and infected cv. Crowford while absent with healthy and infected cv.Giza 22. So these two bands can be considered as a potential marker associated only with healthy and inoculated cv. Crowford. The band No. r_3 was found in each of healthy and inoculated two cultivars except the cultivars that inoculated with *C. lindemothianum*.

Figure (78) and Table (18) showed the zymogram of peroxidase isozyme banding pattern and the presence versus absence of Electrophoretic bands for two soybean genotype inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* (i.e. cultivar-pathogen / resistant (induced) combination) for 24 hours at 30 °C compared to healthy cultivars. Three bands No. r_1, r_2 and r_7 were found in each of healthy and induced cultivars. Two bands No. r_4 and r_5 were found in cv.Giza 22 that inoculated with *F. solani* f.sp. *phaeoli* and *F. solani* f.sp. *glycine* while absent with the rest of healthy and induced cultivars. These bands can be considered as a potential marker associated with cv. Giza 22 only when that inoculated with *F. solani* f.sp. *phaseoli* and *F. solani* f.sp. *glycine* The two bands No. r_3 and r_6 were observed in each of healthy and induced cv. Crowford while absent with cv. Giza 22. One band No. r8 was found in both healthy cultivars while absent with of the rest of induced ones. This band can be considered as a potential marker associated with healthy plants. Two bands No. r_9 and r_{10} were found in healthy cv. Crowford also were found in cv. Crowford that inoculated with *F. solani* f.sp. *glycine* while were absent with the

rest of healthy and induced cultivars. The results were summarized in Table (16 & 19).

Table 15 Wide Table

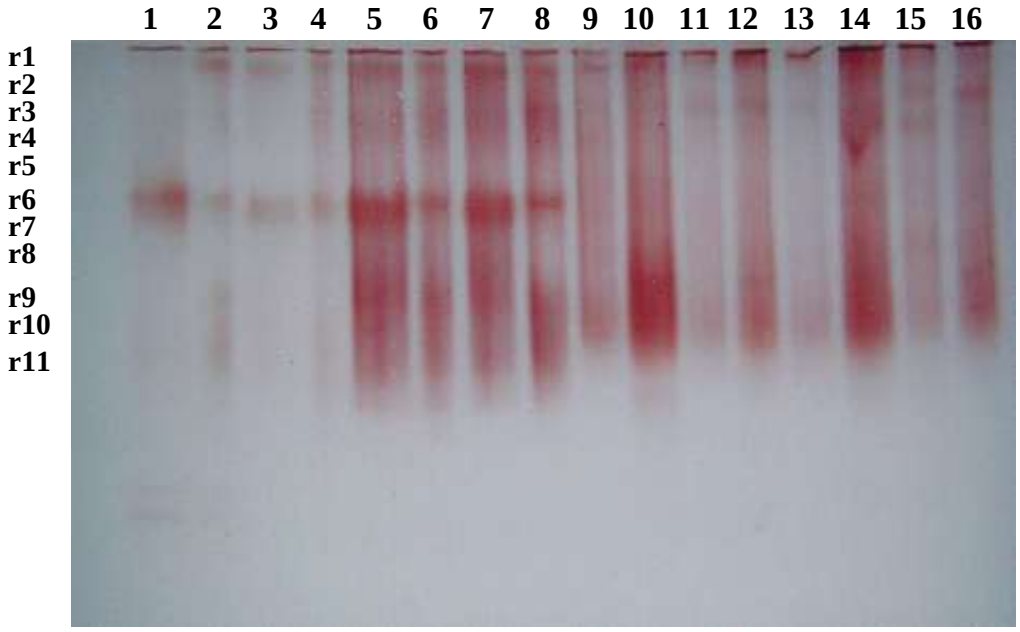


Fig. (76): Example of electrophoretic banding profile (zymogram) of the studied isozyme (peroxidase) for french bean (*Phaseolus vulgaris* L.) cvs. Nebraska and Bronco and soybean (*Glycine max* L.) cvs. Giza 22 and Crowford, 1 day after inoculation with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* separately (i.e. cultivar-pathogen/ susceptible (non induced) combination) at 20 °C for french bean and at 30 °C for soybean.

Lanes:

1 = *Phaseolus vulgaris* L. cv. Nebraska (healthy)

2 = *Phaseolus vulgaris* L. cv. Bronco (healthy)

3 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with *F. solani* f.sp. *Phaseoli*

4 = *Phaseolus vulgaris* L. cv. Bronco inoculated with *F. solani* f.sp. *Phaseoli*

5 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with *F. solani* f.sp. *glycine*

6 = *Phaseolus vulgaris* L. cv. Bronco inoculated with *F. solani* f.sp. *glycine*

7 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with *Colletoterichum lindemothianum*

8 = *Phaseolus vulgaris* L. cv. Bronco inoculated with *Colletoterichum lindemothianum*

9 = *Glycine max* L. cv. Giza22 (healthy)

10 = *Glycine max* L. cv. Crowford (healthy)

11 = *Glycine max* L. cv. Giza22 inoculated with *F. solani* f.sp. *Phaseoli*

12 = *Glycine max* L. cv. Crowford inoculated with *F. solani* f.sp. *Phaseoli.*

13 = *Glycine max* L. cv. Giza22 inoculated with *F. solani* f.sp. *glycine.*

14 = *Glycine max* L. cv. Crowford inoculated with *F. solani* f.sp. *glycine.*

15 = *Glycine max* L. cv. Giza22 inoculated with *Colletoterichum lindemothianum*

16 = *Glycine max* L. cv. Crowford inoculated with *Colletoterichum lindemothianum*

Table (16) wide table

Fig. (77): Electrophoretic banding profile (zymogram) of peroxidase isozyme for french bean (*Phaseolus vulgaris* L.) inoculated with Fusarium potato isolate for 4h, then inoculated with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* separately (i.e. cultivar-pathogen / resistant (induced) combination) for 24 hours at 20 °C.

Lanes:

1 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with Fusarium potato isolate (mock control).

2 = *Phaseolus vulgaris* L. cv. Bronco inoculated with Fusarium potato isolate (mock control).

3 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *Phaseoli*.

4 = *Phaseolus vulgaris* L. cv. Bronco inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *Phaseoli*.

5 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *glycine*.

6 = *Phaseolus vulgaris* L. cv. Bronco inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *glycine*..

7 = *Phaseolus vulgaris* L. cv. Nebraska inoculated with Fusarium potato isolate for 4 h, then inoculated with *Colletoterichum lindemothianum*

8 = *Phaseolus vulgaris* L. cv. Bronco inoculated with Fusarium potato isolate for 4 h, then inoculated with *Colletoterichum lindemothianum*

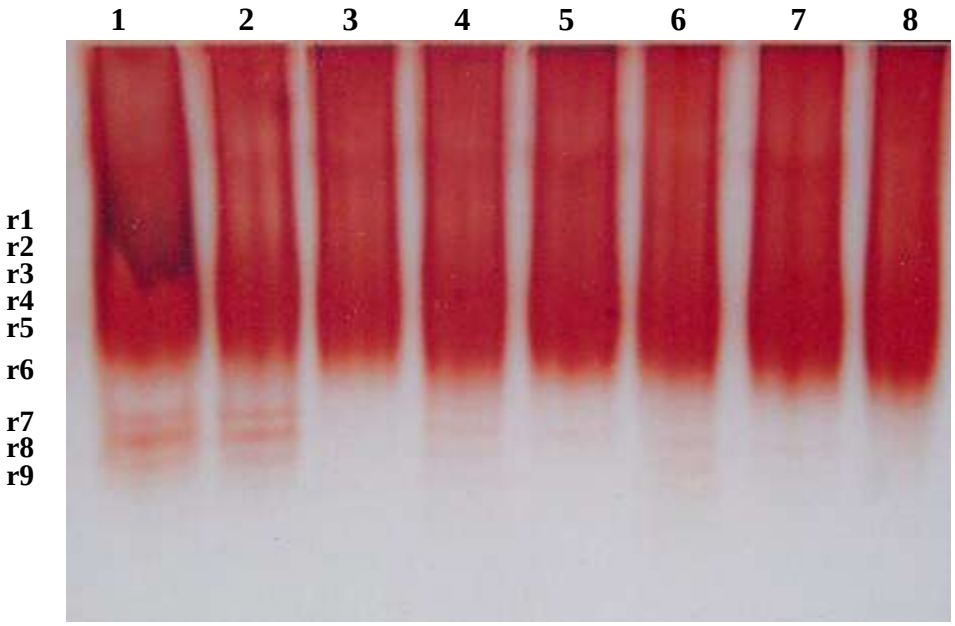


Table 17: (1) and (0) presence and absence of band in the position corresponding to peroxidase isozymes of french bean cvs Nebraska and Bronco inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*, and *F. solani* f.sp. *glycine* and *C. lindemothianum* separately (i.e. cultivar-pathogen / resistant (induced) combination) for 24 hours at 20 °C.

Rows (No. of bands)	French bean (*Phaseolus. vulgaris* L.)							
	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
r1	1	1	1	1	1	1	1	1
r2	0	0	0	1	1	1	1	1
r3	1	1	0	0	0	0	0	0
r4	0	1	0	1	1	1	0	0
r5	1	0	0	0	0	0	1	1
r6	1	1	0	0	0	0	0	0
r7	1	1	0	1	1	1	1	0
r8	1	1	0	1	1	1	1	0
r9	1	0	0	0	1	1	0	0

Fig. (78): Electrophoretic banding profile (zymogram) of peroxidase isozyme in soybean cvs. Giza 22 and Crowford inoculated with Fusarium potato isolate for 4h, then inoculated with *F. solani* f.sp. *phaseoli*, *F. solani* f.sp. *glycine* and *C. lindemothianum* separately (i.e. cultivar-pathogen / resistant (induced) combination) for 24 hours at 30 °C.

Lanes:

1 = *Glycine max* L. cv. Giza22 inoculated with Fusarium potato isolate (mock control).

2 = *Glycine max* L. cv. Crowford inoculated with Fusarium potato isolate (mock control).

3 = *Glycine max* L. cv. Giza22 inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *Phaseoli*

4 = *Glycine max* L. cv. Crowford inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *Phaseoli*

5 = *Glycine max* L. cv. Giza22 inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *glycine.*

6 = *Glycine max* L. cv. Crowford inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *glycine.*

7 = *Glycine max* L. cv. Giza22 inoculated with Fusarium potato isolate for 4 h, then inoculated with *Colletoterichum lindemothianum*

8 = *Glycine max* L. cv. Crowford inoculated with Fusarium potato isolate for 4 h, then inoculated with *Colletoterichum lindemothianum*

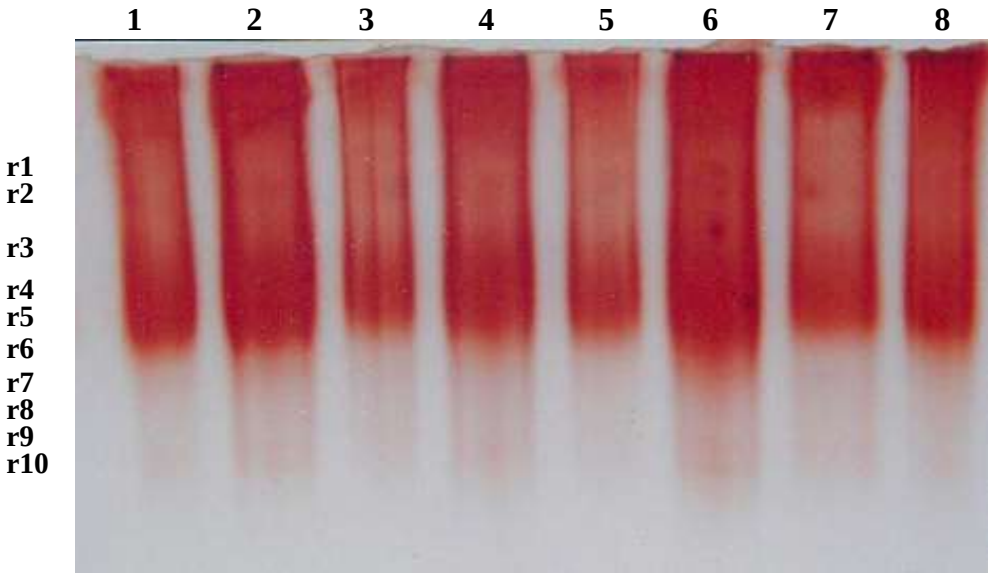


Table 18: (1) and (0) presence and absence of band in the position corresponding to peroxidase isozymes of soybean bean (*Glycine max.* L.) cvs Giza 22 and Crowford inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*, and *F. solani* f.sp. *glycine* and *C. lindemothianum* separately (i.e. cultivar-pathogen / resistant (induced) combination) for 24 hours at 30 °C.

Rows (No. of bands)	Soybean (*Glycine max.* L.)							
	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8
r1	1	1	1	1	1	1	1	1
r2	1	1	1	1	1	1	1	1
r3	0	1	0	1	0	1	0	1
r4	0	0	1	0	1	0	0	0
r5	0	0	1	0	1	0	0	0
r6	0	1	0	1	0	1	0	1
r7	1	1	1	1	1	1	1	1
r8	1	1	0	0	0	0	0	0
r9	0	1	0	0	0	1	0	0
r10	0	1	0	0	0	1	0	0

Conclusion

The presence of the bands r_1, r_3, r_5, r_7, r_8 and r_9 with the non pathogen/ cv. Nebraska combination alone indicates the role of this non pathogenic fungus (Fusarium potato isolate) in inducing the peroxidase isozymes which is regarded one of the defence response induced as a result of the non pathogen elicitation (Table 16 and 19). But with cv. Bronco, r_3, r_4, r_7 and r_8 were the indicators for the induction of peroxidase isozymes production.

Non host resistances in the combinations studied exhibited induction of peroxidase isozymes 24 h after the challenge with the non pathogen. Whereas the induced resistance that was expressed in french bean cultivars combinations, was characterized by the induction of peroxidase isozymes as revealed by the presence of r_2, r_7 and r_8. However, cv. Bronco combination expressed induction of the peroxidase isozymes represented by r_4 and r_7. This indicates that the specificity of cultivar particular tested for the induction of peroxidase isozymes. Further more that r_8 was associated with the other pathogenic fungi tested.

For the soybean combinations, cv. Giza 22, *F. solani* f.sp. *phaseoli* or *F. Solani* f.sp. *glycine*, r_4 and r_5 were regarded as indicators for the induction of peroxidase isozymes as a result of the resistance induced against the tested fungi (Table 16 & 19). R_6 is specifically associated with cv. Crowford regardless the pathogen involved. This based on its absence with cv. Giza 22. It is interesting that r_7 characterized the induced resistance developed in all of the tested interactions.

VI. RAPD and genetic stability:

On the basis of the number, intensity and amplified of the RAPD bands, three primers (OPA-02, OPA-09 and OPA-18) were selected out of twelve primers, which were previously tested (Data not recorded). Bands with some mobility were treated as identical fragments. Weak bands with negligible intensity and smear bands were both excluded from the final analysis.

VI.1. French bean (*Phaseolus vulgaris* L.) cultivars

Figures 79, 80 and 81 demonstrate of RAPD profiles obtained with three different primers (OPA-02, OPA-09 and OPA-18). The number of scored bands varied from 14 to 17 with an average of 20 bands per primer and an average of 5.3 polymorphic bands per primer (Tables 20 and 21). The analysis of healthy and induced (inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*) two cultivars Nebraska and Bronco, the total number of bands scored was 31 with a size range from 4.25 to 2087.83 bp. Sixteen bands were polymorphic which represent 52% average RAPD polymorphism. Six common (monomorphic) bands (approximately 48.14, 4.25, 538.61, 420.65, 833.44 and 454.04 bp) (Table 20 and Figs.79, 80 and 81) were found in each of healthy and induced two cultivars. Five unique bands (approximately 82.47; 29.20; 1641.84; 1508.14, and 569.17 bp) were found in cv. Nebraska healthy plant (resistant), while were absent with the rest of induced ones. As well as seven bands (approximately 657.95, 304.90, 224.15, 125.90, 2087.46, 1712.91 and 602.26 bp) were found in induced cv.Nebraska plants, while were absent with in healthy ones.

On the other hand, one unique band (approximately 429.10 bp) was found in healthy Bronco cultivar (susceptible), while absent with the rest of healthy and induced ones. As well as three bands (approximately, 261.43, 96.18 and 406.05 bp) were found only in induced cv. Bronco plants.

Genetic similarity among resistant and susceptible cultivars as well as healthy and induced ones, cultivars was estimated using dissimilarity coefficient matrix based on RAPD bands scored. Pairwise values of dissimilarity coefficients ranged from 0.13 for cultivars with the same scored bands to 0.33 for the most distant cultivars. The dendrogram was constructed based on the dissimilarity matrix, using the **Nei & Li (1979)** dissimilarity coefficient and clustering via UPGMA (unweighted pair group method using arithmetic means; **Sneath & Sokal, 1973**), using the computer program MVSP version 3.11e (Kovach Computing Services, Anglesey, UK). The healthy and induced of two cultivars divided into three different clusters (Fig.82). The induced cultivar were the most distant the rest of the cultivars falling under two major groups. The first group contained only one induced cv. Nebraska. The second major cluster was further separated into two subgrouips. The first subgroup contained healthy cv. Nebraska and healthy cv. Bronco and exhibit the highest (0.27) genetic dissimilarity as well, while the induced cv. Bronco was highly diverged from them and has represented the second subgroup (Fig. 82).

Table (20): Number and molecular weights of amplified bands with french bean (*Phaseolus vulgaris* L.) hypocotyls that inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f. sp. *Phaseoli* for 24 hours at 20 °C.

RAPD-Primers	Molecular weight (bp)	*Phaseolus vulgaris* L.			
		cv. Nebraska (Resistant)		cv. Bronco (Susceptible)	
		Healthy	Induced	Healthy	Induced
OPA-02 5`TGCCGAGCTG3`	657.95	-	⊕	-	-
	304.90	-	⊕	-	-
	261.43	-	-	-	⊕
	242.07	+	-	+	-
	224.15	-	⊕	-	-
	125.90	-	⊕	-	-
	96.18	-	-	-	⊕
	82.47	⊕	-	-	-
	76.36	-	+	+	-
	48.14	+	+	+	+
	29.20	⊕	-	-	-
	15.18	+	-	+	+
	6.27	-	-	+	+
	4.25	+	+	+	+
OPA-09 5`GGGTAACGCC3`	1150.83	-	-	+	+
	701.94	+	+	-	-
	689.65	-	-	+	+
	538.61	+	+	+	+
	420.65	+	+	+	+
	406.05	-	-	-	⊕
OPA-18 5`AGGTGACCGT3`	2087.46	-	⊕	-	-
	1712.91	-	⊕	-	-
	1641.84	⊕	-	-	-
	1508.14	⊕	-	-	-
	1105.52	+	+	-	+
	833.44	+	+	+	+
	713.50	-	+	+	+
	602.26	-	⊕	-	-
	569.17	⊕	-	-	-
	454.04	+	+	+	+
	429.10	-	-	⊕	-
Total amplified number	31	15	17	14	15
% of amplification		48	54	45	48

Table (21): Genotype polymorphic (% of polymorphic) among two cultivars of french bean (*Phaseolus vulgaris* L.)

Cultivars / RAPD Primers		cvs. of *Phaseolus. vulgaris* L.							
		cv. Nebraska				cv. Bronco			
		Healthy		Induced		Healthy		Induced	
	TAF	MAF	PAF	MAF	PAF	MAF	PAF	MAF	PAF
OPA-02	14	4	2	3	4	6	-	4	2
OPA-09	6	3	-	3	-	4	-	4	1
OPA-18	11	3	3	4	3	3	1	4	-
Total amplified bands	31	10	5	10	7	13	1	12	3
% polymorphic		32	16	32	23	42	3	39	10

TAF = Total amplification fragments.

MAF = Monomorphic amplification fragments or (Common amplification fragments).

$$\% \text{ MAF} = \frac{\text{No. of MAF}}{\text{No. of TAF}} \times 100.$$

PAF = Polymorphic amplification fragments or (specific amplification fragments).

$$\% \text{ PAF} = \frac{\text{No. of PAF}}{\text{No. of TAF}} \times 100.$$

Fig. (79): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profiles of stained agarose gel containing PCR Products of nucleic acid (DNA) of french bean (Primer OPA-02).

Fig. (80): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profiles of stained agarose gel containing PCR Products of nucleic acid (DNA) of french bean (Primer OPA-09).

Fig. (81): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profiles of stained agarose gel containing PCR Products of nucleic acid (DNA) of french bean (Primer OPA-18).

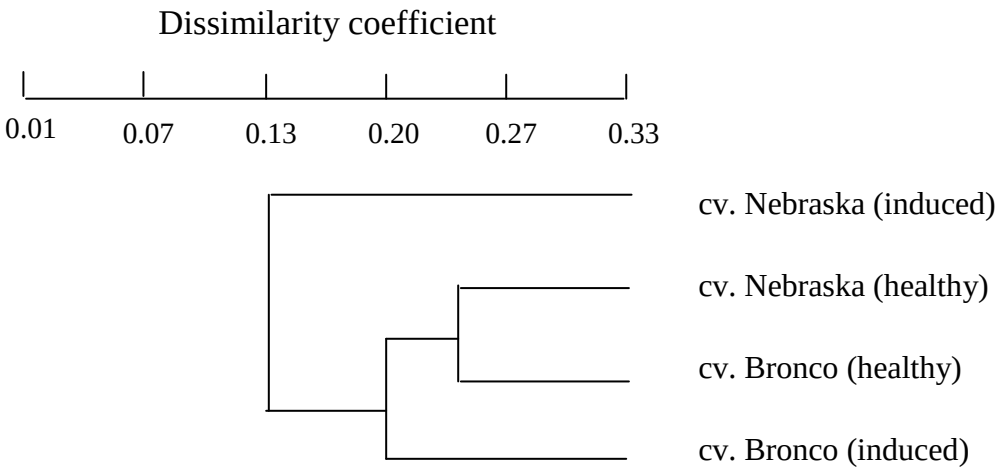


Fig. (82): Dendrogram showing genetic relationships among healthy and induced two cultivars of french bean based on RAPD data analysis.

VI.2. Soybean (*Glycine max* L.) cultivars:

Figures 83, 84 and 85 demonstrate of RAPD profiles obtained with three different primers (OPA-02, OPA-09, and OPA-18). The number of scored bands varied from 17 to 22 bands with an average of 26 bands per primer and an average of 8.66 polymorphic bands per primers (Table 22 and 23). The analysis of healthy and induced (inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*) two cultivars Crowford and Giza 22, the total number of bands scored was 44 with a size range from 4.27 to 2808.29 bp. Twenty six bands were polymorphic which represent 59% average RAPD polymorphism. Six common (monomorphic) bands (approximately 4.27, 654.07, 493.10, 398.94, 1137.19 and 798.86 bp) (Figs.83, 84, 85 and Tables 20,21) were found in each of healthy and induced plants of two cultivars. Five unique bands (approximately 894.97, 68.04, 17.04, 2654.02 and 2087.46 bp) were found in cv. Crowford healthy plants (resistant), while were absent with the rest of induced ones. As well as three bands (approximately 26.02, 6.52 and 704.12 bp) were found in induced cv. Crowford while were absent with in healthy ones. On the other hand, nine bands (approximately 121.15, 1609.56, 1499.80, 1302.23, 2808.29, 2473.04, 1838.26, 1255.38 and 610.82 bp.) were found in cv. Giza 22 healthy plants (susceptible) while absent with induced ones. As well as nine unique bands (approximately, 215.69, 49.27, 1853.76, 510.82, 2768.90, 2508.22, 2000.85, 1347.25 and 585.48 bp.) were found in induced cv.Giza 22 plants while absent with healthy ones.

Genetic similarity among resistance, sensitivity for Fusarium infection as well as healthy cultivars of *Glycine max* L. was estimated using dissimilarity coefficient matrix based on RAPD bands scored. Pairwise values of dissimilarity coefficients ranged 0.13 for cultivars with the same scored bands to 0.33 for the most distant cultivars. The dendrogram was constructed based on the dissimilarity matrix using UPGMA method (unweighted paired group method with arithmetic averages, using the **Nei & Li (1979)** coefficient). The induced and healthy of two cultivars divided into three different clusters (Fig.86). The induced cultivars were the most distant the rest of the cultivars falling under two major groups. The first group contained only one induced cv. Crowford. The second major cluster was further separated into two subgroups. The first subgroup contained healthy and induced cv. Giza 22 and exhibited the highest (0.20) genetic dissimilarity as well, while the healthy cv.Crowford was highly diverged from them and has represented the second subgroup (Fig.86).

Table (22): Number and molecular weights of amplified bands with Soybean (*Glycine max* L.) hypocotyls inoculated with Fusarium potato isolate for 4 h, then inoculated with *F. solani* f. sp. *Phaseoli* for 24 hours at 30 °C.

RAPD-Primers	Molecular weight (bp)	*Glycine max.* L.			
		cv. Crowford (Resistant)		cv. Giza 22 (Susceptible)	
		Healthy	Induced	Healthy	Induced
OPA-02 5`TGCCGAGCTG3`	894.97	⊕	-	-	-
	215.69	-	-	-	⊕
	199.73	+	+	-	-
	121.15	-	-	⊕	-
	76.36	-	+	+	-
	68.04	⊕	-	-	-
	49.27	-	-	-	⊕
	39.71	+	+	+	-
	26.02	-	⊕	-	-
	17.04	⊕	-	-	-
	13.53	-	+	+	+
	6.52	-	⊕	-	-
	4.27	+	+	+	+
OPA-09 5`GGGTAACGCC3`	1853.76	-	-	-	⊕
	1609.56	-	-	⊕	-
	1499.80	-	-	⊕	-
	1302.23	-	-	⊕	-
	1110.90	+	+	-	-
	1053.58	-	-	+	+
	931.04	+	-	+	-
	704.12	-	⊕	-	-
	654.07	+	+	+	+
	510.82	-	-	-	⊕
	493.10	+	+	+	+
	398.94	+	+	+	+
OPA-18 5`AGGTGACCGT3`	2808.29	-	-	⊕	-
	2768.90	-	-	-	⊕
	2654.02	⊕	-	-	-
	2508.22	-	-	-	⊕
	2473.04	-	-	⊕	-
	2087.46	⊕	-	-	-
	2000.85	-	-	-	⊕
	1838.26	-	-	+	-
	1737.38	+	-	-	+
	1445.84	+	-	+	-
	1347.25	-	-	-	⊕
	1255.38	-	-	+	-
	1137.19	+	+	+	+
	946.42	+	+	-	-
	845.30	+	+	+	-
	798.86	+	+	+	+
	610.82	-	-	⊕	-
	585.48	-	-	-	⊕
	480.43	+	+	-	+
Total amplified bands	44	20	17	22	19
Percentage of amplification		45%	38%	50%	38%

Table (23): Genotype polymorphic (% of polymorphic) among two cultivars of soybean (*Glycine max*. L.).

Cultivars / RAPD Primers		*Glycine max L.*							
		cv. Crowford				cv. Giza 22			
		Healthy		Induced		Healthy		Induced	
	TAF	MAF	PAF	MAF	PAF	MAF	PAF	MAF	PAF
OPA-02 TGCCGAGCTG	13	3	3	5	2	4	1	2	2
OPA-09 GGGTAACGCC	12	5	-	4	1	5	3	4	2
OPA-18 AGGTGACCGT	19	7	2	5	-	4	5	4	5
Total amplified bands	44	15	5	14	3	13	9	10	9
% polymorphic		34	11	32	7	30	20	23	20

TAF = Total amplification fragments.

MAF = Monomorphic amplification fragments or (Common amplification fragments).

$$\% \text{ MAF} = \frac{\text{No. of MAF}}{\text{No. of TAF}} \times 100.$$

PAF = Polymorphic amplification fragments or (specific amplification fragments).

$$\% \text{ PAF} = \frac{\text{No. of PAF}}{\text{No. of TAF}} \times 100.$$

Fig. (83): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profiles of stained agarose gel containing PCR Products of nucleic acid (DNA) of soybean, (Primer OPA-02).

Fig. (84): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profiles of stained agarose gel containing PCR Products of nucleic acid (DNA) of soybean, (Primer OPA-09).

Fig. (85): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profiles of stained agarose gel containing PCR Products of nucleic acid (DNA) of soybean, (Primer OPA-18).

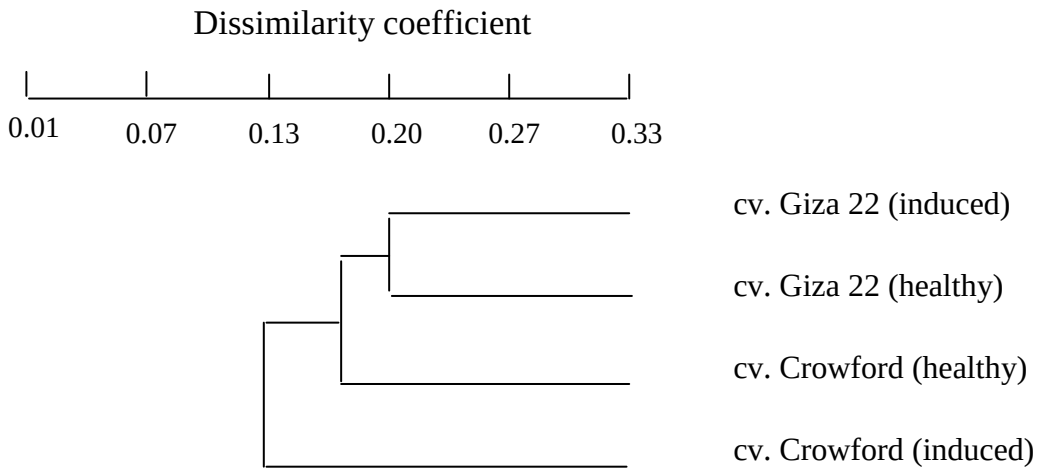


Fig. (86): Dendrogram showing genetic relationships among healthy and induced two cultivars of soybean (*Glycine max* L.) based on RAPD data analysis.

VI.3. *Fusarium solani* f.sp. *phaseoli*:

Figures 87, 88 and 89 demonstrate of RAPD profiles obtained with three different primers (OPA-02, OPA-09, and OPA-18). The number of scored bands varied from 5 to 16 with an average 13 bands per primer and an average of 7 polymorphic bands per primer. In our analysis of the two *Fusarium solani* f.sp. *phaseoli* isolates (original isolate and reisolated isolate from cv. Nebraska (resistant plant)). The total number of bands scored was 30 with a size range from 3.95 to 2730.06 bp. Twenty one bands were polymorphic which represent 70% average RAPD polymorphism. Nine common (monomorphic) bands (approximately 12.66, 5.81, 3.95, 1758.12, 1017.03, 620.33, 894.43, 754.61 and 441.39 bp) (Figs. 87, 88, 89 and Tables 24, 25) were found in each of original and reisolated isolates. Fifteen unique bands (approximately 192.19, 38.22, 1526.52, 914.79, 714.44, 510.82, 371.74, 2730.06, 2438.35, 1945.11, 1737.28, 1405.57, 1105.52, 987.39 and 569.17 bp) were found in original isolate of *F. solani* f.sp. *phaseoli* while absent in reisolated one. As well as six unique bands (approximately 328.52, 1573.72, 1255.38, 1044.74, 622.52 and 314.48bp) were found in the reisolated isolate while absent in the original one.

Table (24): Number and molecular weight of amplified bands with *Fusarium solani* f.sp. *phaseoli* as original isolate and the reisolated isolate that isolated from cv. Nebraska.

RAPD-Primers	Molecular weight (bp)	*F. solani* f.sp. *phaseoli*	
		Original isolate	Reisolted isolate
OPA-02 5`TGCCGAGCTG3`	192.19	⊕	-
	38.22	⊕	-
	12.66	+	+
	5.81	+	+
	3.95	+	+
OPA-09 5`GGGTAACGCC3`	1758.12	+	+
	1526.52	⊕	-
	1017.03	+	+
	914.79	⊕	-
	714.44	⊕	-
	620.33	+	+
	510.82	⊕	-
	371.74	⊕	-
	328.52	-	⊕
OPA-18 5`AGGTGACCGT3`	2730.06	⊕	-
	2438.35	⊕	-
	1945.11	⊕	-
	1737.28	⊕	-
	1573.72	-	⊕
	1405.57	⊕	-
	1255.38	-	⊕
	1105.52	⊕	-
	1044.79	-	⊕
	987.39	⊕	-
	894.43	+	+
	754.61	+	+
	622.52	-	⊕
	569.17	⊕	-
	441.39	+	+
	314.48	-	⊕
Total amplified bands	30	24	15
Percentage of amplification	-	80	50

Table (25): Genotype polymorphic (% of polymorphic) among original isolate and reisolated isolate of *F. solani* f.sp. *phaseoli*.

The causal pathogen / RAPD Primers	*Fusarium solani* f.sp. *phaseoli*				
		Original isolate		Reisolated isolate	
	TAF	MAF	PAF	MAF	PAF
OPA-02	5	3	2	3	-
OPA-09	9	3	5	3	1
OPA-18	16	3	8	3	5
Total amplified bands	30	9	15	9	6
% Genetic type	-	30	50	30	20

TAF = Total amplification fragments.

MAF = Monomorphic amplification fragments or (Common amplification fragments).

$$\% \text{ MAF} = \frac{\text{No. of MAF}}{\text{No. of TAF}} \times 100.$$

PAF = Polymorphic amplification fragments or (specific amplification fragments).

$$\% \text{ PAF} = \frac{\text{No. of PAF}}{\text{No. of TAF}} \times 100.$$

Fig. (87): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profiles of stained agarose gel containing PCR Products of nucleic acid of *F. solani* f.sp. *phaseoli*, (1) original isolate, (2) reisolated isolate from infected french bean cv. Nebraska (Primer OPA-02).

Fig. (88): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profile of stained agarose gel containing PCR Products of nucleic acid of *F. solani* f.sp. *phaseoli*, (1) original isolate, (2) reisolated isolate from infected french bean cv. Nebraska (Primer OPA-09).

Fig. (89): Gel documentation software analysis (Alpha Ease FC 4.0 software) showing-scanning profile of stained agarose gel containing PCR Products of nucleic acid of *F. solani* f.sp. *phaseoli*, (1) original isolate, (2) reisolated isolate from infected french bean cv. Nebraska (Primer OPA-18).

Fig. (90): RAPD profiles of french bean, soybean cultivars and *Fusarium solani* f.sp. *phasoli* (original and reisolated isolates) using the primer OPA-02, OPA-09 and OPA-18.

M = 1kb DNA marker

French bean *(Phaseolus vulgaris* L.)

1 = cv. Nebraska (healthy)

2 = cv. Nebraska induced (inoculated with Fusarium potato isolates for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*).

3 = cv. Bronco (healthy)

4 = cv. Bronco induced (inoculated with Fusarium potato isolates for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*).

Soybean *(Glycine max* L.)

5 = cv. Crowford (healthy)

6 = cv. Crowford induced (inoculated with Fusarium potato isolates for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*).

7 = cv. Giza22 (healthy)

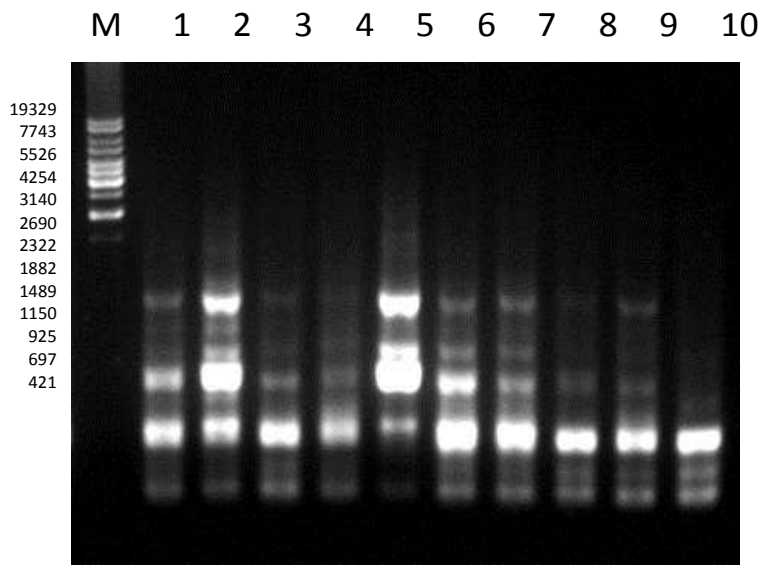
8 = cv. Giza 22 induced (inoculated with Fusarium potato isolates for 4 h, then inoculated with *F. solani* f.sp. *phaseoli*).

Fusarium solani* f.sp. *phaseoli

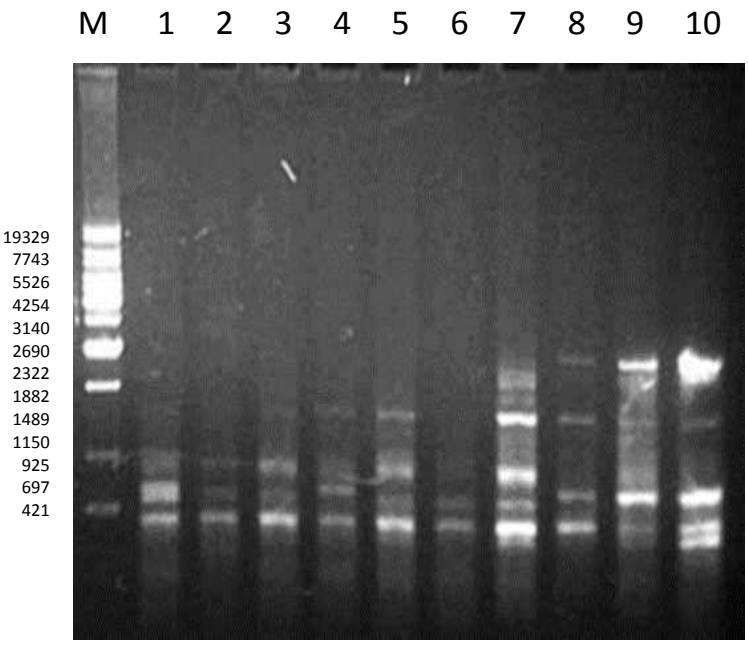
9 = Original isolate

10 = Reisolated isolate (isolated from french bean cv. Nebraska).

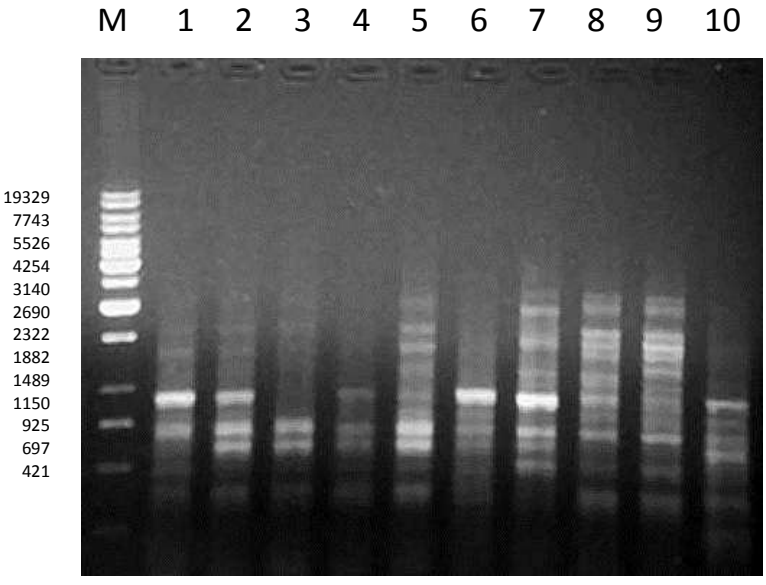
OPA-02
5`TGCCGAGCTG3`



OPA-18
5`AGGTGACCGT3`



OPA-09
5`GGGTAACGCC3`



Conclusion:

Four cultivar plants, Nebraska, Bronco (french bean) and Crowford, Giza 22 (soybean) showed variations response to inducing with the non pathogen (Fusarium potato isolate), then inoculation with the pathogenic fungus (*F. solani* f.sp. *phaseoli*). The genotype polymorphism was clearly observed in the studied combination system i.e. seven bands from 31 bands characterized the induced cv. Nebraska and 3 bands characterized the induced cv. Bronco than the other healthy and induced cultivars. In the other hand, three bands from 44 bands characterized the induced cv. Crowford and nine bands were observed with the induced cv. Giza 22 while absent with the other healthy and induced cultivars. In addition, six common (monomorphic) bands were shared and observed with all healthy and induced french bean cultivars, also six common bands were observed with all healthy and induced soybean cultivars tested in this study. Also, the genotypic polymorphism was clearly observed in our analysis of the two *F. solani* f.sp. *phaseoli* isolates (original and reisolated isolates) and appearance of polymorphic twenty one bands which represent 70% average RAPD polymorphism, fifteen unique bands characterized the original isolate and six unique bands characterized the reisolated isolate. These observations indicate the importance of this technique (RAPD analysis of the nucleic acid DNA) in developing molecular markers for the genome characterization that can be used for distinguishing between the plant cultivars and for molecular characterization of the fungal pathogen isolates.