Interaction between infection of phaseolus vulgaris l. plant with trichoderma rossum and fusarium moniliforme and some important viral diseases in A.R.E

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All the cultivated plants under natural conditions in the field may be infected with more than one pathogen.So the present studies were carried out to isolate and identify some virus infecting bean plants (Phaseolusvulgaris L.) in Egypt and to study the interaction between these viruses and two fungi, Fusariummoniliforme and Trichothecium rosewn which were found tocause damping-off and root rot disease of bean seedlings. ReSUlts obtained in the present investigation couldbe summarized as follows:1- Mosaic symptoms were found to spread on plants cultivatedat Kalubia snd Giza Governorates. SO two differentviruses were isolated and identified as common bean mosaic virus (CBMV) and bean yellow mosaic virus(BYMV). 2- CBMV has a narrow hos t-range only among F.amilyLeguminosae whereas, BYMV infected ’different hostsbelonging to leguminose,Cucurbitaceae and Chenopod_iaceae but they did not .infect those belonging toGraminaea, .Mal vace ae or Solanaceae.3- CBMV induced only systemic symptoms on six susceptiblehosts whereas BYMV infected 11 hosts systemicallyand induced local lesions on the two hosts andin this respect 5 hosts were susceptible to infectionwith both viruses and 7 were susceptible only ofthem.4- Both viruses were transmitted mechanically whilecarborandum powder increased the transmissibilityof CBMV, also !![. persicae transmi tter of,CBlV(a’nId;.~;BYMVto 407 and 801 of insect-inoculated plants respectively.In this respect, BYMV was not transmittedthrough the seeds of both cultivara (Swiss Blanc andContender eva.) while CRMV was found to be transmittedthrough the seeds of both cultivars and the highest percentage in case of Swiss Blanc cv.5- No external virus symptoms were observed on any ofthe seedlings inoculated by infested soil with amixture of !.moniliforme or !.roseum spores and/orinfectious sap of CBMV and BYMV, while the infectioussap of CBMV or BYMV caused a reduction in thenumber of infected plants by both fungi and thiseffect was lost by boiling the infectious saps oftwo tested viruses however·”the effect of CBMV wasmore effective than BYMV •6- Thermal inactivation point (TIP) of CBMV was between58°C and 59°C and dilution end poind (DEP) was foundto be between 10-3 and 10-4, while longivity ill vitro(LIV) was between 36 and 48 hrs.7- Thermalinactivation point (TIP) of BYMV was between59°C and 60°C and dilution end point (DEP) was foundto be between 10-3 and 10-4, while Io.ngavf, ty !.!:! vitro(LIV) was between 48 and 60 hrs.When seedlings of Swiss Blanc and Contender evs.beanplants were grown in infested soil with !.moniliforme or!.roseum and inoculated with either of CBMV or BYMV,theobtained results could be summarized as follows:1- The treatments of Swiss Blanc and Contender cva.with!.moniliforme and BYMV + !.moniliforme caused increasein plant lengths whereas, the most harmful I treatmentswhich caused considerable decrease were CBMV+!.roseum1n case of Swiss Blanc cv. and together with -T..r-o..s..e.-u-.m=treatment in case ot Contender ev.2- All the treatments reduced the leaves number exceptin treatments with !.moniliforme or BYMV + E.moniloforme in case of the two cUltivars and alsoCBMV treatment 1n case ot Swiss Blanc cv3- The surface area of the first and the second leavesof the two cUltivars decreased in all treatmentsspeCially in case of CBMV and

CBMV+l.roseum •4- It was noticed that tbe treatments that induced theplants to give the highest numbers of flowers,causedthe lowest number ot pods as in the case of SwissBlanc cv. inoculated by l.moniliforme or BYMV and byCBMV,CBMV+ !.moniliforme or BYMV treatments in Caseof Contendercv. However, the highest numbers ofSwiss Blanc pods ~minduced by CBMV and CBUV +1.roseum and CBMV+P-.moniliforme and BYMV+P.moniliforme ~ •... _-----treatments in case ot Contender cv.5- The shoots fresh and dry weights increased under alltreatments except the infection with CBMV in case ofSwiss Blanc cv. and with BYMV in case of Contendercv. which gave lowest fresh weight and highest dry weights.6- Total carbohYdrates in SWiss Blanc cv. was decreased-under most treatments specially with BYMV+I.roseumin shoots and pods while BYMV+!.roseum and CBMV+F.moniliforme treatments caused the greatestdecrease in Contender cv. shoots and pods totalcarbohydrates respectively.7- Almost all the treatments decreased total solublesugars in Swiss Blanc cv. shoots and pods speciallyCBMV+ !.moniliforme and BYMV+T.roseum while in caseof Contender cv. the same treatments decreased totalsoluble sugars in shoots but CBMV+!.moniliformetreatments increased it in pods.8- Percentages of reducing sugars increased in SwissBlanc cv. shoots as a result of infection by allcausals except in case of CBMV + !.moniliformewhile in case of pods all treatments decreased it.As for contender shoots and pods, all treatmentsdecreased reducing sugars percentages except thoseof CBMV and !.roseum treatment> in shoots and CBMV+I.monillforme treatment, in pods.In conclusion, it could be noticed that some of the~nteractlon was created between viral and fungalreffects in plants carbohydrates products in such amanner to be an antagonism.9- Total nitrogenpercentages increased under all treatmentsin the shoots of the two eultivara in case ofBYMV infected Swiss Blanc ev. while the highest percentageswas reached by infection of Swiss Blanc cv. with CBMV + !.moniliforme and Contender with BYMV+!.roseum _.10- All treatments decreased the percentage of totalnitrogen in the pods of the two cultivars while thetwo viruses decreaGed this percentage specially CBMVtreatment in case of Contender but their combinationwith either of ~.moniliforme or 1.roseum increasedthe total nitrogen percentages in Swiss Blanc pods.11- Phosphorus percentages in Swiss Blanc and Contendercvs. shoots under all treatments showed greatincrease .except in case of Contender treatmentswi th CBMV + 1.roseum and CBMV +,l.;m:.;:o;,::D:w;h;i:I:ei:;,:l:;,:i:;.:f:.;pod phosphorus percentages decreased under alltreatments in both cultivars except CBMV+!.moniliformeand CBMV treatments in case of Swiss Blanc and- CBMV + -T..r;:;o;,,;s:;,,e;:;u,,;;m,-= treatment in case of Contender cv.which caused considerable increase.12- A reduction was noticed in potassium percentage ofthe shoats of the two cUltivars under all treatmentsbut the greatest decrease was noticed under treatmentwith T.roseum alone or in combination with CBMV in ----.••..•...•.•...case of Swiss Blanc cv. and in combination with BYMVin case of contender cv.As for the pods all treatments almost increased thepotassium percentages in Swiss Blanc cv. except !.roseumtreatment while in case of Contender cv. all treatmentscaused reduction in potassium peroentage except !.roseumand -F.moniliforme combined with CBMV or BYMV treatments.from the previous results ,it could be concluded thatthere are great differences in the response of each cultivarto the infection by the different viruses or fungi,or their combinations.Also the following data were obtained when Swiss Blanccv , was grown in infested soil with !.moniliforme and!.roseum or their combination and inoculated with CBMVeither mechanically or seed borne and could be summarizedas fo110ws/:-gave the shortest ones.5- The heaviest shoots fresh weight as obtained byinoculating the plants with !.moniliforme or!~moniliforme + CBMV (mechanical inoculation) whiletreated plants with CBMV' (mechanical inoculation)and ~.moniliforme + !.roseum decreased the shootsdry weight than healthy plants and similar resultswere obtained for fresh and dry weights in theroots.6- As regards E.moniliforme treatments which encouragedplant growth ,fresh and dry weig~ts,total nitrogen,phosphorus and potassium contents was attributed tosecretion of gibrillic acid specially when combinedwith CBMV which caused plant resistant action againstbad effect of post-emergence damping-off , but notgibrillic acid secretion and action.As for the number of the leaves,no significant differenceswas noticed while F.moniliforme and F.moniliforme - _ .--. •••••••• O= •••••••• oiiiooiiOiioiiio+ CBMV (mechanical inoculation) treatments gave thehighest number and this effect was noticed in the leavesaverage surface area, also similar results were obtained1n fresh weight however as for the dry weight, it wasnoticed that !.moniliforme + CBMV and !.roseum +

CBMVtreatments caused considerable increase due to theinteraction between CBMV with.each of the two fungi.7- Inoculation with CBMV by the two ways (mechanicalor seed borne) and its combination with !.roseumincreased leaflets ~hedding,whereas clear decrease1n leaflets DROPping were noticed in any combinedinoculation with -F.moniliforme •8- The considerable changes in chlorophylls and carotenoidswere due to fungal inoculation and methodsof CBMV infection (mechanical inoculation or seedborne). In this respect, l.moniliforme treatmentcaused increase in chlorophylls and carotenoidsafter 15 days of viral inoculation and decreasedthem after 30 days, however,opposite erfecta wereobtained with !.roseum with their combinationincreased them.9- The total carbohydrates percentages of the leavesdecreased in all treatments after 15 days and 30d~ys except CBMV seed borne treatment and its combination with F-.moniliforme after 15 days andthere is an negative correlation between carbohydratesand carotenoids xcontent of bean plant leaves under all treatments.10- Number of flowers decreased 8r gave similar resultsas healthy plants under all treatments except thatof CBMV (mechanical inoculation) and !.moniliforme +!.roseum + CBMV (seed borne); while the number and weight of pods in all treatments reduced except incombined inoculation of the two fungi together withCBMV either by mechanical inoculation or seed bornein case of pods number •11- Total nitrogen in leaves decreased after 15 days ofviral inoculation with mechanical inocuation ofCBMV while the increase was noticed after 30 days,the opposite occurred with CBMV seed borne infectionhowever all the other treatments increased totalnitrogen after 15 days and decreased it after 30days except !.roseum and l.moniliforme +T.roseumtreatments.12- All treatments increased phosphorus percentages inleaves after 15 days and)0 days except in combinedtreatments of the three casuals ,also seed borneCBMV and r.moniliforme + CBMV (seed borne) treatementdecreased it after 15 days.1)- Potassium percentages in leaves increased greatly ininfection with the two fungi while CBMV (by the twoways) either alone or combined with the two fungi,decreased this percentage fafter 15 and)0 daysexceot single seed borne infection by CBMV after 15days.14- Results as regards total nitrogen percentages in theroots were on contrast with that in the leaves, asall the treatments decreased these percentages exceptin case of CBMV (mechanical inoculation) and!.moni1iforme •15- All treatments decreased roots phosphorus percentageafter 15 days of viral inoculation comparing withhealthy plants while the contrast was noticed after)0 days.16- Potassium percentages in roots increased in case ofCBMV (mechanical inoculation) and the other combinedtreatemnts containing !.roseum after 15 days alsounder all treatments after 30 days than healthyplants •It could be concluded that infection with CBMV playsa role in the different tested minerals (elements)togetherwith F.moniliforme and !.roseum. Also a clear role played by CBMV in nitrogen metabolism was noticed.Also when the present work tried to clear the relationbetween CBMV infection and new protein(s) productionand their inhibiting effect against purified PG enzymesof some fungi by using the new very accurate methods ofprotein determination, results obtained could be summarizedas follows:-1- No detectable .differences in protein profiles betweenhealthy and infected plants with CBMV or witheach of the two tested fungi (!.moniliforme and~.roseum) were noticed.2- In case of CBMV infect”si plants eithar by seedtreansmission or by artifical inoculation, two extraprotedn bands were noticed with molecular weights of80,000 and 55,000 daltona compared with healthyp~antB.J- Plants showing virus symptoms after inoculation withCBMV sap and each of fungal filtrate either 20 minutesbefore inoculation of the sap or inoculationwith both the filtrate and virus infectious sap in thesame time gave· appearance to the majour protein bandsof molecular weight approximatly(60,OOO daltonsJ,whilethis hand was not·recognized 1n case of plants whichshowed no symptoms.4- l.moniliforme and T.roseum have the ability toproduce polygalacturonase and cellulolytic enZymeswhile !.moniliforme was more active in producingthese enzymes and 1.roseum produced them moreearlier.5-It is clear also that, pectin~ induced PG productionfollowed by NeFF whereas glucose was not effectivein this respect and PG enzyme production increasedby time •6- When both fungi were inoculated together. none ofthese enzymes (PG and eX) was secreted, indicatingan antagonistic effect.7- CBMV infected plants by the two ways were moreresistant to infection with each of the two tungiand the hypersensitive reaction of necrotic locallesions was noticed clearly on hypocotyls (Fig.9A&B)8- Soluble proteins of the viral infected plants causeda considerable inhibition effect on the activity ofthe commercial pectinase of !.niger, on contrastwith the case of virus free plants in which nodectectable inhibition was noticed.9-

The quality of soluble protein(s) in the treatedplant leaves with either virus sap or .fungal f~lt-rates or in combination was more lower than insolubleprotein(s) (c.w.b.p.) but its inhibitioneffect was greater, while a different situationoccured in pods as remarkable higher concentrationof cell wall-bound PG-inhibitor'W8s found under thesame treatments.10- The viral symptoms correlated possitively with theincrease in the percentage of PG inhibition eitherin the leaves or in the pods.11- Artificial inoculation with F.moniliforme and CBMVsap increased both PG inhibitor proteins and CBUVparticles infectivity as noticed in the pronounced viral symptoms •12- The inoculation with F.moniliforme filtrate increasedthe virus infectd plants on contrast twith I·roseumspecially in case of 20 minutes after viral inoculations.13- The purification of endopolygalacturonase extractedfrom F.moniliforme after the elution from theCMCellulosecolumn fractions were assayed fer activity of the pure enzyme (Fig.ll) &8 measured by the vicometerwas (1.170) RVU/mg. and (16.30) RGU/mg whenmeasured as reducing groups unit by Nelson-Somog!method, while the elution profiles of the comrnercialenzyme of A.niger show the single peak of the pure enzyme(Fig.12) and its activity as measured by theviscometer was 4370 RVU/mg and 2300 RGU/mg whenmeasured as reducing groups unit by Nelson-Somogimethod, when they were subjected in SDS-polyacrylam±de gel elevtrophoresis (Fig.13) ,two bands ofproteins were noticed with molecular weight of39,500 and 42,000 daltons in case of I·moniliformeand one band of proteins was noticed with molecular weight of)),500 da1tons in case of A·niger •14- These results show that there are differencesbetween PG enzymes secreted by the different fungi.15- !.moniliforme caused the greatest damage to virusfree seedlings of bean plants on contrast to the caseof CBMV infected ones , meaning induced resistance.16- The two cultivars (Contender and Swiss Blanc) shOWdifferences in their activity in producing bothsoluble and insoluble (cell wall-bound)proteins)inhibitor protein and Swiss Blanc sus.cV. was moreactive than Contender Res.cV. in this respect.17- Although the quantity of soluble proteins was lowerin virus infected seedlings (10 days old) comparedwith healthy plants, while the activity of its inhibitioneffect against the purified !.moniliformePG was more in both cultivars (Swiss Bland andContender cvs.).18- The inhibition effect of inhibiting protein fromvirus infected plants 15 and 40 days old1in insolubleproteins (c.w.b.p.)was active than those fromvirus free plants.the inhibiting protein remained adsorbed to(Sepharose 4B-polygalacturonase) column and was el~ted by 0.2 M glycine pH 2.2, which showed asingle peak with much more active effect in inhbitingl.moniliforme PG about 105,000/ for every mg of inhibiting protein.23- This was assured by an experiment on some bean pods,which show clearly that a very low concentration ofthis protein (0.00025 mg) caused complete inhibitionto the infection by E.moniliforme compared with theuntreated wounded parts of the pods (Fig.17). 24- When the purified inhibiting protein was sUbjectedin SDS-Po1yacrilamide gel electrophoresis two bandscorresponding to molecular weights of 55,000 and60,000 daltona respectively were noticed (Fig. 14)which were similar to those bands discovered at thebegining of this study.