

Biotechnological and pathological studies on some pear rootstocks

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This study was conducted at Tissue Culture Unit, Horticulture Department, Faculty of Agriculture Moshtohor, Zagazig University during the period from 2000 to 2003 to establish micropropagation and acclimatization protocol for some pear rootstocks (*P. communis*, *P. betulaefolia*, and *P. calleryana*). Also, studying and inducing natural resistance to fire blight disease in some pear rootstocks. New growing shoots from healthy *communis*, *betulaefolia*, and *calleryana* pear rootstock trees were taken, prepared and washed by running water as well as surface sterilized and dipped in sterilized water three times. The shoot tips were excised as explant. These explants were subjected to anti-oxidant treatments, cold pre-treatments, organic additives during establishment stage. Cytokinin types, 2-iP concentrations, medium strength, GA3 concentrations, medium state, auxin type, and NAA concentration were studied. Also, rooted plantlets were subjected to fire blight disease treatments under aseptic conditions. Diseased *P. communis* and *P. betulaefolia* pear plants show symptoms of fire blight as necrosis/cankers on young shoots or older pear branches were collected from different trees at the Farm of the Research and Experimental Station where the causal agent was isolated. The isolated bacteria from infected *P. communis* pear plants, were identified according to their morphological, cultural and biochemical characteristics. Also, they confirmed using Hypersensitivity in tobacco (HR) and small immature pear fruits. Then, in vitro plantlets of either *communis* or *betulaefolia* was infested through hypodermic injection or leaf spray. Three isolate types (toxins, virulent bacteria, or attenuated bacteria) were used for infection. Moreover, *communis* pear and *betulaefolia* pear plants were evaluated to their resistance to fire blight disease. In addition, acclimatization was tested using different agricultural media. Moreover, artificial re-inoculation with fire blight causal agent of acclimatized plantlets was involved. Besides, 115 Summary and Conclusion biochemical and molecular genetic fingerprints for the three pear rootstocks were performed using either polyacrylamide gel electrophoresis or RAPD fingerprint. The obtained results can be summarized as follows: V.1. Establishment stage: 1- *communis* and *betulaefolia* pear rootstock explants responded positively than *calleryana* pear in encouraging good explant development and greening while reduced necrosis and browning. 2- Pre-treating the explants with anti-oxidant solution and culturing it on medium supplemented with PVP was the best anti-oxidant treatment which reduced necrosis and browning as well as improved explant development parameters. 3- Cold treatment (5°C) of *calleryana* pear for 4 or 6 days was very effective in reducing phenolic compounds accumulation and encouraging an increase in explant development parameters as well as reducing necrosis and browning. 4- Addition of glutamine to the culture medium is preferred for maximizing explant development and greening while succeeded in reducing necrosis and browning. V.II. Proliferation: 1- Addition of 2-isopentenyladenine (2-iP) to the culture medium was superior than kinetin and 6-benzylaminopurine in increasing proliferation while kinetin was effective in increasing both growth and greening beside reducing necrosis. 2- Culture medium supplemented with either 2.0 or 4.0 mg/L of 2-iP enhanced the highest proliferation, growth and greening parameters. 3- The highest growth and proliferation parameters were induced when *betulaefolia* pear explant was used as compared with the other pear rootstocks. 4- *Calleryana* pear rootstock explant was negatively response to different cytokinin types or 2-iP concentrations as it showed an increase of necrosis with less growth, proliferation, and

greening parameters which led to get side of calleryana pear rootstock from the following experiments.

V.III. Rotting stage : V.I11.1. Shoot elongation : 1-Full and one-half medium strengths were the best medium strengths which induced the highest shoot elongation and improved greening.2-The culture medium containing GA3 at levels 2.0 or 4.0 mg/L encouraged the longest shoot elongation and maximized greening.V.11.2. Root formation:3-Liquid culture medium enhanced an increase in root formation but solid medium improved both growth and greening 4-Addition of either NAA or IAA to the rooting medium resulted in maximizing root formation5-Supplementation the culture medium with higher concentration of NAA (4.0 mg/L) encouraged the highest root formation while the lower concentration (1.0 mg/L) improved growth and greening.IV.V. Fire blight disease:1-Morphological, physiological and biochemical characteristics of ten bacterial isolated (isolated from pear trees which showed fire blight symptoms) revealed that isolates No. 1, 2, 3, 4, 5 and 6 were identified as *Erwinia amylovora*.2-All bacterial isolates of *Erwinia amylovora* except isolates 7, 8 and 9 gave, positive reaction on immature pear fruits and slices of pear fruits where necrotic tissue with bacterial oozing were developed.3-Hypersensitive reaction (HR) for tobacco leaves against all *Erwinia amylovora* isolates (except isolates No. 7, 8 and 9) showed rapid necrotic spots development within 24-48 hrs intercellular injection with bacterial cells.4-All six bacterial *Erwinia amylovora* isolates were pathogenic on plantlets of pear and affected the % of disease incidence on pear compared with control treatment. *Erwinia amylovora* isolate No.4, 6 and iso. No.5 were the most pathogenic isolates and caused 82.5%, 65% and 62.5% leaves wilted of pear plantlets. While *Erwinia amylovora* isolate No.2,1 and iso. No. 3 were the least pathogenic isolates (28.5%, 22.5% and 20% diseased leaves on pear plantlets). 5-Leaves sprayed with cell suspensions of pathogenic bacterium isolates were more effective in inducing fire blight symptoms (56.7%) than hypodermic injection (37%) on plantlets pear.6-The three virulent *Erwinia amylovora* isolates as cultured filtrated showed more pathogenicity on pear plantlets. *Erwinia amylovora* isolate No.6 was the most pathogenic isolates and caused 57.5% wilted leaves of pear plantlets. Isolates No. 5 and No. 4 of *Erwinia amylovora* were the least pathogenic isolates where 37.5% and 32.7% wilted leaves. 1- Hypodermic injection with culture filtrates of *Erwinia amylovora* isolates was more effective for the disease incidence (caused 53.3%) than by leaf spray (31.7%) on pear plantlets.8-*Erwinia amylovora* isolate No.5 and No. 6 were the most pathogenic isolates after treating with hot water at 50°C which caused 42.5% leaves wilted of pear plantlets. While, *E. amylovora* isolates No.4 was the least pathogenic isolates and caused 32.5% leaves diseased with fire blight.9-In general, hypodermic injection method with *Erwinia amylovora* isolates treated by hot water at 50°C was more effective for the disease incidence of fire blight disease and caused 60.0% than 18.3% disease by leaf spray with cell suspension.10-Concerning of explant stages results revealed that rooted plantlets stage was more tolerant to infection with *E. amylovora* than proliferated. On the other hand, completely died of pear plantlets cv. *Communis* was showed in shoot tip stage. II- Regarding the response of pear rootstock *Communis* and *Betulaefolia* cvs. to infection with the causal organism of fire blight disease results indicated that *Betulaefolia* plantlets was more resistant to infection with *E. amylovora* under the two inoculated methods than *communis* plantlets. 12- Healthy leaves of the susceptible pear cv. *Communis* to the infection with *E. amylovora* contained higher phenolic (Free, conjugate and total phenols) and sugar (reduced, non-reduced and total sugars) than the resistant pear cv. *Betulaefolia*. On the other hand, resistant plantlets containing higher amounts of both phenolic and sugar contents than diseased plantlets and control.VI.Acclimatization : 1- Using either BVI3 or peat-moss as agricultural media for acclimatization of either *communis* or *betulaefolia* pear rootstock plantlets were superior than agropeat as they increased survival percentages, plantlet length, shoot thickness, number of leaves, greening and rooting parameters.2 Disappearance of fire blight symptoms in re-inoculated *communis* pear plants showed resistance when in vitro inoculated with fire blight causal organism. VII.Fingerprint : 1- Most of isozyme results revealed a high level of polymorphism among the studied genotypes. Malate dehydrogenase electrophoretic pattern does not revealed polymorphism among the studied samples. Both poly phenyl peroxidase and profiles identified high polymorphism level in *Pyrus betulaefolia* samples and did not revealed any polymorphism among those of *Pyrus communis* and *Pyrus calleryana*. The three enzymes and

Summary and Conclusion peroxidase revealed a pronounced interspecific differences in both band's number and intensity within the studied rootstocks. 2-Results of the four isozymes patterns have proved to be effective in identifying the studied rootstocks *Pyrus betulaefolia*, *Pyrus communis* and *Pyrus calleryana* samples by a unique class pattern, which indicates that biochemical genetic fingerprinting, is a reliable technique to discriminate among these taxa. 3-Characteristic profiles for each *Pyrus betulaefolia*, *Pyrus communis* and *Pyrus calleryana* rootstocks, in terms of number and position of RAPD bands. Both the number and size of the amplified products varied considerably with the different primers. 4-Some banding patterns exhibited strong background or very faint patterns. These were difficult to score and were therefore discarded. A sum of 54, 41 and 31 polymorphic bands was generated by these primers in *Pyrus betulaefolia*, *Pyrus communis* and *Pyrus calleryana* rootstocks under study, respectively. Total of 14, 8 and 11 unique bands were identified out of the polymorphic ones in *Pyrus betulaefolia*, *Pyrus communis* and *Pyrus calleryana* rootstocks, respectively. These unique bands were used to discriminate among the studied *Pyrus betulaefolia*, *Pyrus communis* and *Pyrus calleryana* samples. Most samples of the rootstocks *Pyrus betulaefolia*, *Pyrus communis* and *Pyrus calleryana* samples were discriminated by one or more unique bands. 5-Isozyme analysis and RAPD analysis in *Pyrus communis* and between the two different treatments of *E. amylovora* represent were on differences in banding patterns and this mean resistance not inherited but induced in *pyrus* rootstocks.