selection in vitro resistant genotypes of chocolate spot disease in vicia faba

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Chocolate spot disease caused by Botrytis fabae Sard. is a serious disease in our country, causing great annual losses to broad bean (Vicia faba L.) especially in the North of Delta. Selection of resistant embryogenic cell cultures to Botrytis fabae in vitro through tissue culture technique provide our country with cell-line resistant to chocolate spot disease. The results obtained in this work could be summarized as follows: I- Callus characters of broad bean as affected by different cultivars, gamma-irradiation dose, media and cultivation period:(1)-The average of callus production of Giza 402 cv. was greater than Giza 2 cv., Giza 3 cv. and Giza 461 cv. Average of callus formation with MS 1 and MS4 were significantly higher than that obtained with MS7. No significant differences were recorded among 0, 50 and 100 Gy on the callus production. There were significant differences among 2 and 6 weeks of cultivation period.(2)-No browning of calli were observed in the four tested cultivars with the three media used and gamma-irradiation doses after 2 weeks of cultivation but after 6 weeks gamma rays decreased the percentage of browning calli of Giza (2) and Giza (3) with MS1 and inhibit calli browning of Giza (402) and Giza (461) with MS1 and MS4.(3) The average of friable calli of Giza 402 was significantly exceeded that of the other cultivars. The average of friability withMS1 media was significantly higher than that of MS4 and MS7. Gamma rays had a significant increase on friability than control (0 Gy).(4)Significant increase was recorded with MS 1 media in the average of embryogensis. The maximum average was obtained with Giza 461 cv. Gamma rays at 100 Gy showed significant increase in average embryogensis and this average after 6 weeks of cultivation was significantly higher than after 2 weeks.(5)The average of adventitious shoot formation of Giza 402 calli exceeded that of the three other cultivars and MS7 was the best media for formation of calli shooting. Significant increase was obtained by gamma-rays at 100 Gy.(6)Calli root formation of Giza 3 cv. was significantly higher than that of the other three tested cultivars. Gamma rays had significant increase in calli root formation as compared with the control (0 Gy). Cultivation period affected significantly calli rooting. The highest average was recorded with Giza 3 cv. with MS4 and 50 Gy. II- Regenerated plantlets (Indirect regeneration):The best media for regenerated plantlets was MS 1. Giza 461 cv. and Giza3 cv. exhibited the highest average. Significant increase was detected with the three doses of gamma-rays.Average of regenerated plantlets after 18 weeks of cultivation was significantly greater than that after 10 weeks.III- Callus selection under pathologic condition:(1) Data revealed that no calli browning was found with the four cultivars at all the tested doses of gamma-rays at (0)% concentration of culture filtrate of B. fabae (that had grown on potato dextrose liquid medium) after 1 and 5 weeks of cultivation while percentages of browning calli was increased by increasing culture filtrate concentration and cultivation period. The doses(50 and200 Gy) decreased the percentage of browning calli. This mean that these doses increase calli tolerance to B. fabae culture filtrate while 200 Gy dose had the greatest effect, there were no calli browning after 1 week and 5weeks of cultivation at the highest concentration.The same result recorded in case of calli culture on MS1 medium supplemented with potato dextrose liquid medium was similar to that recorded in case of calli cultured on MS 1 medium supplemented with culture filtrate of B. fabae grown on faba bean leaves extract liquid medium.(2)-The maximum friable calli average was obtained with Giza3, (in case of supplemented MS medium with culture filtrate of B. fabae grown on potato dextrose liquid medium). Significant decrease was

detected after 5 weeks of cultivation. Averages of friable calli under concentrations of filtrates of B. fabae decreased with increasing the concentration of culture filtrate. The maximum average was obtained at 200 Gy which means that 200 Gy increase calli tolerance to B. fabae.-The difference among the averages of friable calli of Giza 461, Giza 3 and Giza 2 were not significant, while Giza 402 recorded the lowest average (in case of supplemented MS medium with culture filtrate of B. fabae that had grown on faba bean leaves extract liquid medium). Significant decrease was detected on the average after 5 weeks of cultivation period and with increasing the concentration of the fungal filtrate. Meanwhile, gamma rays at 50 and 200 Gy caused significant increase in the average of friable calli while the average of friable calli at 200 Gy was greater and significant than at 50 Gy (200 Gy increase calli tolerance to B. fabae)•-Data revealed that the highest averages were recorded were with Giza 3 and Giza 2, (in case of supplemented MS 1 medium with culture filtrate of B. fabae that had grown on Czapek's dox liquid medium and sucrose used as carbon source). Average of friable calli after 5 weeks was significantly less than after 1 week. The average of friable calli at 200 Gy significantly exceeded that of 0, 50 and 100 Gy dose (this mean that 200 Gy increased resistance of calli to the pathogen).-Maximum averages were recorded with Giza 461 and Giza 3, (in case of supplemented MS medium with culture filtrate of B. fabae that had grown on Czapek's dox liquid medium and glucose was used as carbon source). The averages of the four cultivars at 50% concentration were significantly less than the averages at the other concentrations applied. Significant decrease was found after 5weeks of cultivation. Both 50 and 200 Gy caused tolerance to B. fabae.(3)- Lysis did not appear on the four cultivars at the different doses of gamma-rays when the culture filtrate of B. fabae . That had grown on Czapek's dox liquid medium and sucrose used as carbon source was not incorporate to the tissue culture medium. There was an increase in calli by increasing the concentration of culture filtrate. Gamma rays on doses had effects on this characters as follows:At 0 Gy (control): Giza 461 was the most tolerant variety for B. fabae culture filtrate.At 50 Gy dose there was an increase in calli tolerance to B. fabae culture filtrate for all cultivars except of Giza 461.The 100 Gy increases calli susceptibility to the filtrate for all varieties except of Giza 461 calli.The 200 Gy dose caused tolerance to B. fabae with the four cultivars calli.-Lysis of calli cultured on MS medium supplemented with 50% concentration of B. fabae culture filtrate that had grown on Czapek's dox liquid medium and glucose as a carbon source at 0 Gy was appeared in the third degree only with Giza 461 and Giza 402 after 5 weeks of cultivation. Gamma rays at 50 Gy, lysis in the third degree appeared at the same concentration only with Giza 461 after 5 weeks of cultivation.At the same concentration of fungal filtrate at a dose of 100 Gy lysis an the third degree appeared with Giza 2, Giza 3 and Giza 402, the lowest percentage was with Giza 3 after 5 weeks, while at a dose of 200 Gy, all the lysis detected were in degree (1) with all the cultivars. V-For estrase isozymes: More bands and higher enzyme activity (more intense and longer bands) were detected with susceptible calli of all the tested cultivars under experiment, while resistant forms showed less bands, slight and very slight activity of estrase isozyme.