Pathological studies on fusarium wilt of cucurbitaceae (watermelon)

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The present investigation was planned to study the Fusarium wilt disease of watermelon by inducing somaclonal variation in vitro of Egyptian is opinion. To produce some plants tolerant or resistant to Fusarium wilt disease using biotechnological techniques . The findings can be summarized as follows.Part I: Pathological studies:1.The obtained results indicated that 41 fungal isolates belonging to 5 genera were isolated from diseased plants collected from different localities. These isolates included Fusarium oxysporum (19 isolates), Rhizoctonia solani (6 isolates), Fusarium solani (4 isolates), Macrophomina phaseolina (5 isolates) and Verticillium dahlia (7 isolates).2.All the tested isolates of F. oxysporum were pathogenic to watermelon and significantly varied in their pathogenic abilities. In terms of % healthy survivals, isolate No. 7 (Minufyia, Sadat) and isolate No. 2 (Kafr El-Sheikh, Baltem) were the most virulent while, isolate No.10 (Minufyia, Khatatba) was the lowest one in this respect.3.Severity of both isolates (No.2 & No.7) was significantly increased as their inoculum levels were increased. Percentage of survivals recorded for both isolates was decreased from 75.0 & 72.5% to 5.0 & 7.5% by increasing their inoculum level from (1x103) to lx108 conidialml.4.Among the tested host plants, watermelon cultivars (Sugar Baby & Giza 1) were the only susceptible to infection with the two Fusarium oxysporum isolates (No. 2 & No.7). Sugar Baby was more susceptible to both isolates than Giza 1 cultivar. However, squash (Eskandarani), cucumber (Beta Alpha) and muskmelon (Honeydew) were not significantly affected compared with their respective controls.Summary 1185. Among the 7 tested watermelon cultivars, Calhoum most resistant since it recorded the lowest significant pre-emergence (7.5 & 10.0%), post-emergence (5.0 & highest % survival (87.5 & 85.0%) under stress of isolates No.2 & No.7, respectively. However, Sugar most susceptible one since it recorded the highest signi in % pr-emergence (65.0 & 62.5%), post-emergence (and produced the lowest % survival (10.0 & 7.5%) w infection with isolates No.2 & No.7, respectively. The 21, Giza 1, Odeum, Charleston Gray and Aswan show responses.Gray was the ecreases in % 5.0%) and the infection with Baby was the icant decrease 5.0 & 30.0%) en exposed to cultivars Giza d intermediatePart II: Tissue culture experiments for induce somaclonal variation:6. To find the suitable protocol for surface sterilization seeds to produce high germination seeds with free-cotyledons, Clorox and HCl at 20 & 30% were used disinfectants. The obtained results reveal that gf watermelon contaminated s seed surface rmination ofwatermelon seeds of both cultivars Giza 1 and Giza 21 was significantly decreased as concentration of t ese surface disinfectants increased. Moreover, all treatment resulted in 100.0% survived but contaminated cotyledons. Usin HC1 at 20% as seed disinfectant and 30% as cotyledon disinfec pant decreased survived cotyledons to 76.7 & 83.3% but also decreased their contamination to 1.7 & 5.0% concerning cultivars Gi a 1 and Giza 21, respectively. The decontaminated cotyledons ere used as explants in the following studies.7. All concentration of the tested cytokinins significa formation of the adventitious shoots and degree of h in both watermelon cultivars compared with the co (without cytokinin). Using BA at 2.0 mg/liter caus increase in the adventitious shoot formationhyperhydericity in both Gizal and Giza21 cultivarstly increased perhydericity trol treatment d the highest d degree of ompared withthe same concentration of IBA or 2iP. The incidence of hyperhydericity was higher in subculture 2 than subculture 1 and in Giza 21 than Giza 1 cv.8.The in vitro shoot proliferation and root formation of the 2 watermelon cultivars were affected significantly by BA (cytokinin) and IBA

(auxin) combinations. In this regard, using BA at 1.0 mg/ liter alone or combined with IBA at 0.1 mg/1 produced the highest numbers of branches per shoot. While, BA at 0.1 mg/1 + IBA at o.1 mg/1 produced the highest increase in numbers of nodes and leaves and plantlet length particularly during the second subculture. Using BA at concentration more than 0.1 mg/liter significantly decreased plantlet length, of both watermelon Giza 1 and Giza 21 cultivars. On contrary, using IBA alone at 1.0 mg/1 followed by BA at 0.1 mg/1 + IBA at o.1 mg/1 were the best treatment for increasing number of roots/plantlet, respectively. However, BA (1.0 mg/1) + IBA (0.0, 0.1, 0.5 or 1.0 mg/1) produced the lowest root number/plantlet.9.The watermelon tetraploid plantlets could be produced in vitro by using the colchicine and dinitroalanine treatments. Production of tetraploid plantlets and callus formation increased proportionally with increasing concentration of these chemicals. On the opposite side, increasing concentrations showed negative effects on the in vitro growth (% survival, shoot number and length and number of leaves/shoot) of the resultant plantlets. Number of chloroplast in the guard stomatal cells was ranged 18.8-19.5 and 19.0-19.9 in the tetraploid plantlets of Giza 1 and Giza 21 cvs., compared with 10.8-13.4 and 9.2-11.8 in the diploid plantlet type of both cultivars, respectively.10.The tested temperature and germination-substrate significantly affected percentage of triploid seed germination. In general, the highest % seed germination was obtained at 32°C either on cottonsaturated with MS salts (62.0%) or distilled water (5. .0%) without significant differences in between.11. Number of adventitious shoot and hyperhydrici watermelon as affected by cytokinin type (BA, different concentrations (0.0, 1.0, 2.0 or 4.0 investigated. In general, BA at 2.0 mg/liter produc shoot number (4.67) followed by BA and Kin at 4.0 Using BA particularly at the higher concentration caused the highest degree of hyperhydricity (4.0).y of triploid iP or kin) atg/liter) was d the highest g/liter (2.33). (4.0 mg/liter)12. The hypocotyl-explants of Giza 1 and Giza 21 ormed higher percentage of callus (35.01-37.08%) than their coty edon-explants (26.24-26.68%). The callus formation was significantly affected byusing BA and IBA singly or in combination concentrations) compared with the control treatment 2.5 mg/I + IBA at 0.5 mg/1, however, produced the hi formation either from the hypocotyl-explants (80.0 cotyledon one (73.3 & 66.7%) of both watermelon 21 cultivars.(at different . Using BA at hest % callus 80.0%) or the iza 1 & Giza13.Percentage of callus differentiation, number of de and roots in both watermelon cultivars were signifi by concentrations of NAA. Using NAA at 0.05 and Gizal & Giza21 respectively recorded the differentiation (80.0%) whereas NAA at 0.05 & 0. best for increasing number of shoots/callus and r respectively in both cultivars.14.The cultural filtrate of F. oxysporum nevium c watermelon shoots in vitro. The wilt incide proportionally by increasing concentration of cultur 5 to 50 m1/1). However, more than 99% of shoots w of 40 ml/l. Dead shoots were significantly higher (55.07%) than Gizal cv. (53.46%).eloped shoots antly affected .1 mg/liter for ighest callus mg/I was the of formation,used wilt to ce increased filtrates from re dead at rate in Giza21 cv.Part III. Screening for resistance against watermelon fusarium will in vitro and ex vitro15.The triploid shoots were the most resistant against cultural filtrate of F. oxysporum nevium (91.0% survival) followed by the diploid shoots produced indirectly from callus (19.4 & 19.6%) and directly from cotyledon (16.4 & 15.2%) for Giza 1 and Giza 21 cvs., respectively. On contrary, the tetraploid shoots of both cultivars were completely susceptible (100.0% death).16.Among all treatments tested, IBA at 1.5 mg/liter was the most effective for whole plantlet development (100.0%), root number (7.0 & 5.3 roots/plantlet) and root length (6.7 & 4.9 cm) of both Giza 1 and Giza 21 cultivars. While, NAA at 0.1 mg/liter caused significant increase in plantlet survival of Giza 21 only (77.8%) compared with the control. Moreover, the root formation was completely failed on shoots (particularly in Giza 21 cv.) cultured on medium contained the highest concentrations of NAA (1.0 & 1.5 mg/liter).17.In the acclimatization study, the mixture contained sand and peatmoss at rate of (1:3 v/v) recorded highest significant increase in plantlet survival of Giza 1 (70.0%), Giza 21 (66.7%), and seedless watermelon (63.3%). While, the mixture of sand and peatmoss (1:1) recorded 20.0, 16.7 & 16.7% survived plantlets compared with 6.7, 3.3 & 6.7% in peatmoss alone for the watermelon cultivars Giza 1, Giza 21 and triploid (seedless), respectively.18.The diploid (direct or indirect), triploid and tetraploid watermelon plants of Gizal and Giza21 cvs were evaluated (after acclimatization) against the artificial infection with F. oxysporum nevium under greenhouse conditions. The triploid plants were the least susceptible (8.3%) followed by the diploid plants produced indirectly from callus which recorded 16.7

& 25.0% compared with 50.0 & 58.3% in the seed plants (control) of Giza 1 & Giza 21 cvs., respectively. On contrary, the diploid plants developed directlyfrom cotyledon as well as the tetraploid plants of were not significantly varied in this respect when c their control (seed) plants.19.As for phenols content, the triploid plants recorde amounts of free (4.57mg) and total phenols (7.86 with the 1.35 & 2.96 mg in the seed (control) plants The diploid callus-plants of Giza 1 and Giza 21 c recorded higher free (4.11 & 3.39 mg), conjugated mg) and total phenols (7.54 & 6.32 mg) than the tetra both cultivars. The diploid cotyledon-plants recorded amounts of different phenols.20.Regarding sugar content, the triploid plants (Giza 1 c plants of Giza 1 and Giza 21 cvs., recorded the highe reducing, non-reducing and total sugars in compari other investigated plants particularly the seed (control)21.Regarding peroxidase isozyme, Giza 1 and Giza 21 (peroxides protein band more than other test plants exc of Giza 21 resulted from callus.oth cultivars mpared withthe highest g) compared f Giza 1 cv. s., however, 3.43 & 2.93 loid plants of intermediate.) and callus t amounts of on with the plants.ontrol) had 1 pt the plants22.As for reaction against infection with wilt diseas conditions, the triploid plants of Giza 1 cv. sho resistant (0.0% wilt) compared with the tetraploid same cultivar (70.0% wilt). While, the local waterm Giza 1 and Giza 21 recorded 20.0 and 30.0% wilt, resp23.Concerning yield production, the same data reveal t cultivar Giza 1, however, produced the highest yiel number of fruits/row followed by the local cultivar I triploid and tetraploid plants, respectively. The triploid plants produced the highest fruit weight (3.8 value (13.4%) followed the local cultivars Giza 1 respectively. The bulb was more sweetish in Giza 21 fruits (taste 9) than Giza 1 (taste 8).under field ed complete lants of the ion cultivars ctively.at, the local feddan) and iza 21, then ost resistant g) and TSS nd Giza 21, and triploid.