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لم ترد بالأصل

ADVANCED STUDIES ON BARLEY STRIPE MOSAIC VIRUS (BSMV)

BY

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THESIS

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In the present study, Barley stripe mosaic virus (BSMV) was from naturally infected barley plants, collected from the Agriculture Research Experimental Station Center, Giza. The virus infected nine plant species belonging to three different families. It was inactivated by ten minutes exposure to 65°C, withstood dilution up to 10⁻³ but not 10⁻⁴ and infectivity was retained for 21 days. The virus was detected using serological methods (DAS-ELISA) and transmitted mechanically and through barley grains at percentage ranging from 4 to 23%. The electron microscopy revealed ultra-structural changes. The morphology of plastids was altered during infection, the grana become disorganized. Plastids developed vesicles. The cytoplasm contained inclusion bodies with disordered masses of virus. The palisade cells were deformed and seemed shorter than normal when semithin sections were examined using light microscope. Using three different purification procedures, BSMV yield was 3.7, 7.0 and 2.3 mg/100g of barley leaves, respectively. Electron micrograph of purified BSMV showed rod-shaped particles. The polyclonal antibodies raised against BSMV had a virus-specific titer of 1:2000. The concentration of IgG conjugate with alkaline phosphatase was 1/1000. The prepared antiserum was used for detection of BSMV by tissue-blotting immunobinding assay (TBIA) and Dot blotting immunobinding assay (DBIA) on nitrocellulose membrane (NCM) and was also used to detect BSMV within different parts in mature seed and non -seed parts. The embryos and lodicules were infected with BSMV in all cultivars. Nucleic acid sequence comparison between the two strains (G119 and ND18) of BSMV revealed that: the sequence of the intergenic region of the β RNAs was highly conserved, but the sequenced portion of the β b gene showed diversity between the two strains. Five bases were found to be different, 18 out of 116 amino acids of the β b protein were different, three amino acids insertion, and one amino acid deletion. The 5' untranslated regions, of RNA γ a of the two strains were nearly identical. Only six nucleotides were changed, 12 out of 162 amino acid of γ a protein were differed and one amino acid deletion. The coat protein of G119 strain was 23 (KD) as tested by western blotting. Virus infection significantly reduced the number of grain/spike of cvs G117 and G119. The mean number of grains/spike was reduced from 55.26 to 42.93 in G117 and the main number of grain/spike was reduced from 57.06 to 43.33 in G119. Also BSMV infection significantly reduced the grain weight/spike of cvs G117 and G-119 compared with healthy plants that it was reduced from 3.33 to 2.11 in G117 and from 2.73 to 2.00 in G119. On the other hand no significant differences were observed between diseased and healthy plants. The effect of thermotherapy on barley grains (cv. G119) on elimination of BSMV was tested. Exposure of the grains to 60°C for 60 min resulted in the reduction in percentage of both germination and virus transmission through barley grains. The effect of chemotherapy on BSMV elimination was testes. All chemical compounds tested gave promising results in virus elimination. Kinetin was the most effective component (90% reduction), whereas ribavirin and 8-azoguanine had the lower effect. Thiouracil reduced infection by 80%. Benzoic acid was effective at lower than in higher concentration.

Key words:-barley stripe mosaic virus, hordeivirus, seed transmission, serology, western blotting, molecular biology, and virus elimination

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INTRODUCTION