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بسم الله الرحمن الرحيم

HANAA ALY

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USE OF ENDOPHYTIC BACTERIA IN CONTROLLING SUGARCANE SMUT DISEASE

By

SHADIA TAGHIAN AHMED MOHAMED

B.Sc. (Plant Pathology), Fac. Agric., Ain Shams University, 2002

M.Sc. Agric. Sc. (Plant Pathology), Zagazig University, 2009

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ABSTRACT

SHADIA TAGHIAN AHMED MOHAMED "Use of Endophytic Bacteria In Controlling Sugarcane Smut Disease" PhD, Thesis, Plant Pathology Department, Faculty of Agriculture, Ain Shams University, 2020

Sugar cane (*Saccharum* spp.) is considered as one of the most vital crops grown for sugar making all over the sphere. In Egypt the economic sugar cane smut disease is sugar cane smut disease caused by *Sporisorium scitamineum*. Atotal of 240 isolates of endophytic bacteria were isolated from 160 samples of sugarcane stalks (variety, GT-54-9). The isolated endophytes were screened for its antagonistic activity against *Sporisorium scitamineum, In vitro,* using dual culture method. Only 62 isolates showed different degrees of antagonistic activity. 29 isolates of endophytic bacteria were selected according to the efficacy of pathogen inhibition to study their potential in producing the enzymes as (β-1, 3 glucanase ,chitinase, polypenoloxidase, nitrogenase and peroxidase) as well as siderophores , salicylic acid and indol acetic acid). The *In vitro* studies showed that, isolate BAN32 followed by Q54, Sp-80-1842, Q15, Q14 and Q16 isolates were the highest in indol acetic acid production. while, isolate of LU23 followed by LU26, Sp-70-1143, BAN32, LU25, Q16, Q19, Q60 and LU51 were the highest in producing siderophores. The production of salicylic acid was detected in only nine isolates, LUX 26 and SOH isolates were the the highest production followed by BAN39, LUX25 BAN32, BAN3, LUX46 and BAN34 isolates. Chitinase activity was recorded with only eight isolates, (BAN39, BAN37 and MEX-2001-80 gave the highest activity followed by isolates of LUX46, LUX50. Only ten isolates were able to hydrolyze β-1, 3 glucanase (Q19, BAN33, BAN34, SOH28, LUX45, MEX-2001-80, Q17, Q62, Q56 and LU440.Q16 isolates). Almost all isolates where able to produce nitroginase, the highest nitroginase activity LUX24 followed by SOH28,

LUX23. and isolates of BAN33 and Sp-27-5181 gave the lowest nitrogenase activity.

Treated sugarcane plants with pathogenic fungus and endophytic bacteria led to produce of indol acetic acid (IAA) with bacterial isolates (SOH29, BAN32, LUX41, LUX45, LUX46, BAN37, Sp-27-5181, Q16, Q18 and LUX2) compared with untreated healthy and infected control, but highest salicylic acid (SA) levels were recorded with isolates of BAN33, LUX42, LUX43, LUX44, LUX49, Mex-2001-80, Q16, LUX24, LUX27 and SOH29 compared to control treatment and highest chitinase levels were recorded with isolates of Sp-79-2233, BAN33, Q16, Q19, LUX27, BAN39, Q15, LUX23, LUX26, LUX43, Q17, LUX50, Sp-27-5181 and SOH28 compared to control. While, the highest glucanase activity were recorded with isolates of LUX24, LUX45, BAN33, LUX27, Sp-27-5181, SOH29, Q19, Q17, LUX41, BAN37, Mex-2001-80, LUX43, LUX23, BAN32, LUX44, Q18, Q14, LUX50, BAN39 and Sp-79-2233 compared to control treatment, but the highest Peroxidase activity were recorded with isolates of LUX44, Sp-27-5181, SOH28, Mex-2001-80, LUX23, LUX41, SOH29, Q14, Q19, LUX42 and Sp-79-2233 compared to control treatment and highest Polypenoloxidase levels were recorded with isolates of Q56, LUX26, Sp-79-2233, Q17 and Q15 compared to control treatment. The incase of treated sugarcane plants with only endophytic bacteria, showed that, the highest IAA levels were recorded with isolates of Sp-79-2233, Q16 and LUX41 compared to the untreated healthy and infected, but highest SA levels were recorded with isolates of Sp-79-2233, SOH29, LUX27 Q17 and BAN33 compared to control treatment and the highest chitinase levels were recorded with isolates of Sp-79-2233, BAN33 and BAN39 compared to control treatment. Meantime, highest glucanase levels were recorded with isolates of BAN33, LUX24, LUX27, SOH29, Q19 and Sp-27-5181 compared to the untreated healthy and infected control, but highest Peroxidase levels were recorded with isolates of LUX46, LUX49, LUX44, SOH28, Q56, Q61, Q19, LUX50, Q14 and LUX42 compared with control treatment and the

highest Polypenoloxidase levels were recorded with isolate of Q56 compared with control treatment.

Also, all the tested endophytic bacteria isolates were completely reduced the disease except isolates Sp-79-2233, Q15, LUX26, SOH28, LUX43, LUX45 and LUX50 under artificial inoculation conditions. The number of tileries appeared the highest with Q18 isolate, but Sp-79-2233 and LUX44, Q14, Q18, LUX23 and LUX50 isolates led to increase diameter of stalk sugarcane compared with the control and Q19, BAN37, LUX41, LUX42, Q14, Q18 and BAN33 isolates were the most effective on plant length compared with other isolates. On the other hand, Mex-2001-80, Q16, Sp-79-2233, Sp-27-5181, Q15 and Q18 isolates were the most effective on leaf area, but LUX26, Q19, BAN32, Q61, Q18, LUX23, SOH29 and LUX49 isolates were the most effective on leaf area compared with other isolates and BAN33, Q17, BAN39, BAN32, LUX27, LUX49 and BAN37 isolates led to increase total chlorophyll of sugarcane plants compared with other treatments.

The isolates Mex-2001-showed a close identity to the genus Enterobacter, gave 100% similarity to *Enterobacter ludwigii* and the isolate the isolate LUX 27 and BAN 33 showed 100% similarity to *Enterobacter sp.* Also the isolates LUX 27, LUX 41, BAN 33 and BAN 39 recorded a similarity (100, 100, 99.88 and 100% respectively) to *Kosakonia radicictans.* On the other hand the isolate LUX 41 showed also a 100% similarity to *Kosakonia oryzae* and the isolate BAN 39 recorded a 100% similarity to *Klabsiella oxytoca..*

The isolates Q17 and Q24 shoed a high similarity to Bacillus sp.,the isolate Q17 showeda100% similarity to *Bacillus subtilis subsp. Inaquosorumwhile the isolate* Q24 showed a 100% similarity to *Bacillus anyloliquefucanes* and *Bacillus velezensis.* On the other hand, the isolate SOH 29 showed a high similarity (99%) to different two genera. *Pantoea sp. And Pseudomonas. Agglomerans.*

Key words: Endophytic bacteria, biological control, sugarcane, smut disease, *Sporisorium scitamineum* chitinase, β 1,3 glucanase, sidrophores ,Indol acetic acid (IAA),Saliciylic acid (SA) and phenylalanine ammonia lyase *Enterobacter sp Kosakonia sp., Bacillus subtilis, Bacillus anyloliquefucanes.*

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