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INHIBITION OF SESAME SEEDLING GROWTH BY *XANTHOMONAS CAMPESTRIS* PV. *SESAMI* CULTURE SECRETIONS

I. Rehman, F. Deeba, M. I. ul Haque* and S. M. Saqlan Naqvi

Department of Biochemistry, PMAS Arid Agriculture University Rawalpindi
*Department of Plant Pathology, PMAS Arid Agriculture University Rawalpindi
Corresponding author: saqlan@uaar.edu.pk

ABSTRACT

Xanthomonas campestris pv. *sesami* (Xcs) is rod shaped, gram negative bacterium. This bacterial pathogen causes the bacterial blight of sesame and significantly reduces its yield. Severely infected leaves defoliate and spots are formed on the twigs. Present study was conducted to check the effect of culture filtrate of *Xcs* on germination and seedling of sesame. Different concentrations of culture filtrates of *Xcs* in MS (Murashige and Skoog) medium were used to monitor its effect on seed germination, height and root length of sesame seedlings. It was observed that *Xcs* greatly affects the length of root and length of whole seedling and slows down the process of germination. Smallest root and seedling height was obtained with 4% culture filtrate while there was normal growth in control sesame seedlings.

Key words: Sesame, *Xanthomonas campestris*, culture filtrate, seedling.

INTRODUCTION

Sesame commonly known as "till", is the most ancient oilseed crop domesticated and cultivated in Asia for more than 5000 years. The world production of sesame is over 3.84 million metric tons with India as the leading producer. Pakistan ranks 14th among major sesame producing countries in the world. In Pakistan, the sesame is grown on about 76,000 hectares with a total production of about 31,000 tons and yielding only 402 kg/h (FAO, 2011). Its utilization is more than its indigenous production. The low yields in the country are due to its susceptibility to biotic and abiotic stresses. Sesame suffers from many diseases like leaf spot (Ojiambo *et al.,* 2003), leaf and stem blights, fusarium wilt (El-Bramawy, 2006), charcoal rot and root rot. Among the major constraints, bacterial blight and bacterial spot caused by *Xanthomonas campestris* pv. *sesami (Xcs)* and *Pseudomonas syringae* pv. *sesami* respectively are very serious diseases in most sesame growing regions and dramatically decrease the sesame yields during monsoon season.

Bacterial blight of sesame caused by *Xcs* was first reported in Pakistan by Mirza and Akhtar (1987). *Xcs* can affect crop at any growth stage. Bacteria infects plants through stomata and wounds, spreads in intercellular space outside plant cell wall (Boureau *et al*., 2002), generate virulence factors and results in the formation of small and irregular spots on leaves. Severe infection results in leaf defoliation and seedling death. Severity of bacterial blight is related to soil moisture and relative humidity. A study on disease assessment showed that 75.6% of the development of disease severity in sesame is contributed by *Xcs* and *Pseudomonas syringae* pv. *sesami* (Bashir *et al*., 2007). Firdous *et al.* (2009) studied the pathogenesis of *Pseudomonas syringae* pv. *sesami* associated with sesame bacterial leaf spot.

In Pakistan, the bacterial blight of sesame caused by *Xcs* is generally observed during monsoon season. Currently this disease has become more threatening and is greatly affecting the sesame production in Pakistan. Several studies have been conducted regarding the effect of *Pseudomonas syringae* and *Xcs* on leaves defoliation alone and in combination (Bashir *et al.,* 2007; Firdous *et al.,* 2009) but no serious and systematic study has been conducted on finding the effect of pathogen on germination of seeds, root and seedling length. Keeping in view the potential threat of *Xcs* to sesame production, the present study was designed to evaluate the effect of different concentrations of culture filtrate of *Xcs* on sesame seed germination, effect on root and seedling length.

MATERIALS AND METHODS

Bacterial Isolates and growth conditions: Stock culture of *Xanthomonas campestris* pv. *sesami* (*Xcs*) was streaked on nutrient agar (NA) medium (5g/L peptone, 3g/L meat extract, 15g/L agar, pH 7) and incubated at 37°C for 48 hours to obtain the isolated culture. To obtain pure isolated colonies, 1:10 dilution of dissolved culture was spread on NA medium again and incubated for 48 hours at 37°C. Isolated colonies were picked and incubated at 37°C in 50 mL nutrient broth medium (5g/L peptone, 3g/L meat extract, pH 7) for 7 days on orbital shaker.

Detached leaf method for pathogenicity: Six-to eight week old fully expanded sesame leaves were used for

pathogenicity test. Leaves were washed with 70% ethanol and then rinsed with sterilized distilled water three times (Agrios, 1997). The leaves were placed in petri plates containing double filter paper to retain the moisture level. Inoculum was injected into the leaves with the help of syringe dipped in the bacterial suspension. Infiltrated leaves were incubated at 28 °C for 12 hours under light. Control leaves were inoculated with sterilized distilled water and were incubated using the same method employed for seedlings inoculated with bacterial suspension.

Preparation of bacterial culture filtrate: *Xcs* liquid culture was centrifuged at 52 xg for 10 minutes. The supernatant was filtered through Whatmann No. 1 filter paper and then through 0.2 µm syringe filter. The final filtrate was stored at 4ºC until used.

Growth of sesame seeds on media containing bacterial culture filtrate: Sesame GP 12 seeds were washed with 70% ethanol for 1 minute. Then seeds were surface sterilized under aseptic conditions in laminar flow cabinet with 25% Clorox for 10 minutes and rinsed 6 times, each for two minutes with autoclaved distilled water. Finally seeds were dried on filter paper. Five flasks containing MS medium (MS salt 4.4g/L, vitamin B5 10mL/L, sucrose 30g/L, agar 6g/L) were labeled as; A: control having 0% filtrate (96 mL MS medium + 4 mL nutrient broth), B: 1% filtrate (99 mL MS medium + 1mL filtrate), C: 2% filtrate (98 mL MS medium + 2mL filtrate), D: 3% filtrate (97 mL medium + 3 mL filtrate), E: 4% filtrate (96 mL MS medium + 4mL filtrate). Each medium containing filtrate was poured into the relevant labeled petri plate. In each petri plate, thirteen sterilized seeds were transferred. Petri plates were kept at 25°C in growth chamber for 72 hours. Completely Randomized Design (CRD) was followed. Data regarding seed germination, and root and seedling length was recorded after 72 hours.

Statistical analysis: To analyze effect of culture filtrate on root and seedling length, analysis of variance (ANOVA) was used followed by Least Significant Difference (LSD) test (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Sesame is a flowering plant that is grown throughout the world and cultivated for its seeds which contain about 50–60% odorless and colorless oil (Uzun *et al.*, 2003). Pakistan is facing acute shortage of edible oil. Demand of edible oil is increasing but its production is decreasing day by day. This low yield is attributed to prevalence of parasitic diseases. Sesame crop is severely affected by bacterial diseases. Mostly *Xanthomonas campestris* pv. *sesami* (*Xcs*) and *Pseudomonas syringae* pv. *sesami* cause bacterial leaf spot or blight disease which effect the plants at all growth stages. Small and irregular spots are formed on leaves and severely infected leaves defoliate. Bashir *et al.* (2007) studied the role of *Xcs* and *Pseudomonas syringae* pv. *sesami*, alone and in combination in symptoms development of bacterial blight in sesame. They found that highest leaf infection occurred in plants inoculated with both the pathogens together as compared to individual inoculations. Present study was carried out to evaluate the impact of culture filtrate of *Xcs* on sesame germination and seedlings. Phytopathogenicity of isolated *Xcs* was confirmed by detached leaf method in which same symptoms were produced as in the sesame plants when re-inoculated. By culturing on NA medium, same colonial features were observed confirming the pathogen to be the same as that causing bacterial blight.

The effect of culture filtrate of *Xcs* was observed on the growth of sesame GP 12 seeds. After 72 hours the petri plates were observed. Seed germination and growth was normal in control (Fig. 1 A) but progressively decreased in MS containing increasing concentration of culture filtrate (Fig. 1 B-E). Germination/growth was slowest in petri plate containing 4% filtrate (Fig. 1 E) but all seeds were germinated in control and treated plates.

The root and seedling length of ten best seedlings was measured and the effect of culture filtrate on the length of root and whole seedling was analyzed. The control seedling showed normal root length while the root length was significantly reduced when treated with different concentrations of culture filtrate ($p<0.05$), 4% filtrate resulted in much less root length as compared to control (Fig. 2). The root length of control seedling was 3.66 while length of seedlings treated with 1%, 2%, 3% and 4% culture filtrate was 2.19, 1.61, 1.69 and 0.80 cm respectively (Fig. 3). Similarly the length of whole seedlings treated with 1%, 2%, 3% and 4% culture filtrate was significantly less ($P<0.05$) as compared to control (Fig. 2). The length of treated seedlings with 1%, 2%, 3% and 4% was measured as 6.00, 6.04, 5.74 and 4.10 cm respectively while the whole seedling length of control was 7.74 cm (Fig. 4). These results showed that the effect of culture filtrate on seedling length is significant. Many bacterial pathogens produce non host selective toxic metabolites considered as secondary metabolites (Bender *et al*., 1999). Many Pseudomonas species produce secondary metabolites which lead to chlorotic symptoms (Arrebola *et al.*, 2003). Virulence factors produced by *Pseudomonas syringae* pv. *sesami* and *Xcs* have been reported by Firdous (2009). The results of present study shows that *Xcs* culture filtrate slowed down the process of germination and significantly reduced the root growth and seedling length. The growth retardation of sesame seedling in this highly controlled study can safely be attributed to the presence of some potent phytoinhibitory factor released by *Xcs*. Further studies may be focused on identification and characterization of the inhibitory

substance(s) released by *Xcs* responsible for retardation of sesame growth, and their status and contribution in *in vivo* infections and their management.

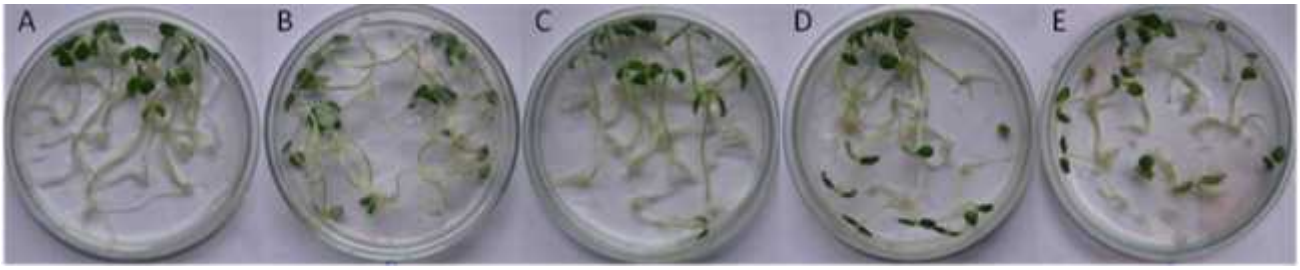

Fig. 1: Sesame seed germination on MS media without or with different concentrations of culture filtrate of *Xanthomonas campestris* pv. *Sesami* (A) control (B) 1% filtrate (C) 2% filtrate (D) 3% filtrate (E) 4% filtrate . Seed germination was slowest with 4% culture filtrate.

Fig. 2: Effect of different concentrations of culture filtrate on length of root and whole seedling (A) control (B) 1% filtrate (C) 2% filtrate (D) 3% filtrate (E) 4% filtrate. The length of roots and seedling was lowest with 4% filtrate.

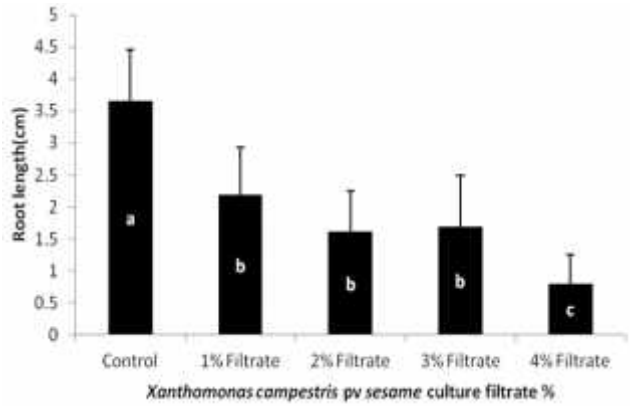

Fig. 3: Effect of different concentrations of culture filtrate of *Xanthomonas campestris* pv. *sesami* on root length of sesame seedling. Control was without filtrate. Bars with different lower case letters are significantly different (P<0.05) according to LSD test (LSD $_{0.05}$= 0.6292). Bars with same lower case letters are not significantly different. Error bars represent Standard deviation.

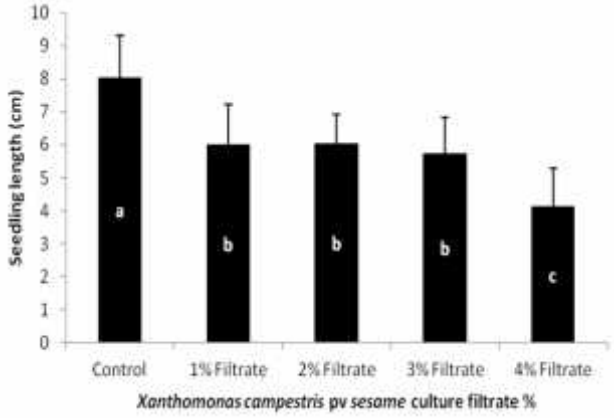

Fig. 4: Effect of different concentrations of culture filtrate of *Xanthomonas campestris* pv. *sesami* on length of whole sesame seedling. Bars with different lower case letters are significantly different (P<0.05) according to LSD test (LSD $_{0.05}$= 1.016). Bars with same lower case letters are not significantly different. Error bars represent Standard deviation.

REFERENCES

Agrios, G. N. (1997). Seedling pathology. 4th edition. Academic press, New York. USA.

Arrebola, E., F.M. Cazorla, V.E. Duran, E. Rivera F. olea, J.C. Codina, A. Perez-Garcia and A. Devicente (2003). Mangotoxin: a novel antimetabolie toxin produced by *Pseudomonas syringae* inhibiting ornithine/arginine biosynthesis. Physiol. Mol. Plant Pathol. 63: 117-127.

Bashir, S., M. Irfan-ul-Haque, T. Mukhtar, G. Irshad and M.A. Hussain (2007). Pathogenic variation in *Pseudomonas syringae* and *Xanthomonas campestris* pv. *Sesami* associated with blight of sesame. Pakistan J. Bot. 39(3): 939-943.

Bender, C.L., F. Alarcon-Chaidez and D.C. Gross (1999). *Pseudomonas syringae* phytotoxins: mode of action, regulation and biosynthesis by peptide and polyketide synthesis. Microbial. Mol. Biol. Rev. 63: 266-292.

Boureau, T., J. Routtu, E. Roinea, S. Taira and M. Romantschuk (2002). Localization of hrpA induced *Pst DC3000* in infected tomato leaves. Mol. Plant Pathol. 3: 451-60.

El-Bramawy, M.A.S. (2006). Inheritance of resistance to Fusarium wilt in some sesame crosses under field conditions. Plant Protection Sci. 42(2): 99-105.

FAO (2011). http://faostat.fao.org/site/567/Desktop Default. aspx?PageID=567#ancor.

Firdous, S. S., R. Asghar, M. I. Haq, A. Waheed, S. N. Afzal and M. Y. Mirza (2009). Pathogenesis of *Pseudomonas syringae* pv. *sesami* associated with sesame (*Sesamum indicum*) bacterial leaf spot. Pakistan J. Bot. 41(2): 927-934.

Firdous, S.S. (2009). Virulence anlysis of *Xanthomonas campestris* pv. s*esami* and *Pseudomonas syringae* pv. s*esami* the causal organisms of Sesame (*Sesamum indicum* L.) bacterial blight. Ph. D. thesis (Unpublished). Department of Botany, PMASAAUR Rawalpindi.89-108 pp.

Mirza, M. S. and M. A. Akhtar (1987). Susceptibility of Sesame to bacterial blight, A new disease for Pakistan. Pakistan J. Agri. Res. 8 (8): 87-89.

Ojiambo, P.S., R.K. Mibey, R.D. Narla and P.O. Ayiecho (2003). Field transmission efficiency of *Alternaria sesami* in sesame from infected seed. Crop Protection 22(9): 1107-1115.

Steel, R. G. D., J. H. Torrie and D. A. Dickey (1997). Principles and Procedures of Statistics. A biometrical approach. 3rd Ed., McGraw Hill Book Co., New York, USA.

Uzun, B., D. Lee and P. Donini (2003). Identification of a molecular marker linked to the closed capsule mutant trait in sesame using AFLP. Seedling Breed. 122: 95-97.