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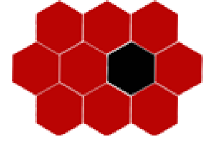

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# Variation in oxalic acid production by groundnut isolates of *Sclerotium rolfsii*

Saraswathi M and Jaya Madhuri R.

**Abstract**

Thirty isolates of *Sclerotium rolfsii*, the causal agent of stem rot of groundnut obtained from different Rayalaseema areas of Andhra Pradesh, India were categorized into four groups, on the basis of oxalic acid production in the culture filtrate and severity of patrhogenicity on groundnut seedlings. The group four was more dominant than other groups and highly virulent to groundnut seedlings. There was a positive correlation between oxalic acid production and the virulence of the isolate.

**Key words:** *Sclerotium rolfsii*, Stem rot, Groundnut, Oxalic acid, Virulence.

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## INTRODUCTION

*Sclerotium rolfsii* is a ubiquitous soil-borne fungal pathogen known to cause disease on worldwide range of agricultural and horticultural crops (Kaveriappa, 1979). It infects more than 500 plant species in 100 families throughout the world (Punja, 1985; Sarma *et al*., 2002; Adandonon, 2005; Ganesan *et al*., 2007). Most *S.rolfsii* diseases have been reported on dicotyledonous hosts, but with several monocotyledonous species also being infected (Aycock, 1966). In 1983, Patil and Rane reported that all the 35 hosts, including important cultivated crop plants, were susceptible to the pathogen, indicating the wide host range of parasitism of *S.rolfsii*. Among the numerous secondary hosts, majority are economically important crops like food crops, ornamentals, etc. Doidge and Bottomly (1931) were the first to report that *S.rolfsii* was the causal agent of stem rot or southern blight of groundnut in South Africa. The disease occurs throughout groundnut growing areas of the world in the tropic and in warmer part of the temperate zone.

Oxalic acid is a key factor of the early pathogenic stage in a wide variety of fungi like *Sclerotium rolfsii* (Bateman and Beer., 1965; Punja and Jenkins., 1987; Ansari and Agnihotri, 2000; Lehner *et al*., 2008; Malcolm *et al*., 2005; Rajane and Stolz., 2004 ), *Aspergillus* (Leagon *et al*., 1999), *Sclerotia minor* (Malcolm *et al*., 2005), *Poria placenta* (Espejo and Agosin., 1991), *Sclerotinia sclerotiarum* (Diad *et al*., 2006) and *Sclerotinia trifoliorum* (Franklin *et al.,* 1991). There are few reports which indicate the variability in the population of *S.rolfsii* of different host and in the same host (Kaveriappa, 1979; Patil and Rane, 1983; Upadhayay and Mukhopadhyay, 1985; Singh and Dwivedi, 1987; Ansarti and Agnihotri, 2000). However, no work has been done to study the variation in production of oxalic acid among groundnut isolates of Indian origin. Hence, a study was undertaken on variation in the oxalic acid production by the isolates of *S. rolfsii* and to correlate it with the virulence of the isolates.

## MATERIALS AND METHODS:

### Isolation of pathogen:

During the survey of groundnut fields around Srikalahasti and Tirupati areas of Chittoor District, and also other Rayalaseema Districts of A.P., *S. rolfsii* was found to be associated with the infected hypocotyls region of groundnut variety TMV-2 at early stages of growth and development. The plants showing stem rot or southern blight symptoms were brought to the laboratory for making isolation according to the tissue segment method on PDA. Pure culture was obtained by transferring the sclerotia to PDA plates. Thirty isolates were isolated and stock cultures were maintained on PDA slants in refrigerator at 10ºC and subcultured for every two months.

### Variation in pathogenicity:

For patrhogenicity test, oat meal-sand medium of each isolate was thoroughly mixed with sterilized soil at 10% level. This inoculum-soil mixture was then distributed in 12” diameter earthenware pots and left undisturbed for two days. After this period, 10 days old seedlings of TMV-2 variety of groundnut grown in seed pans were transplanted into the pots and covered with a polythene bag to maintain high humidity and incubated at room temperature. Samples were collected at random at ‘0’ hours, 2 days, 5 days, 9 days and 11 days after inoculation. All most all the seedlings collapsed by 11 days after transplantation. All the samples at each stage was compared with the control plants to observe the severity of the disease.

For oxalic acid estimation and quantification, each isolate was grown individually in Czapek-Dox broth. 100 ml of broth was poured in 250 ml Erlenmeyer flasks and sterilized at $121^0$C for 15 min. Each flask was inoculated individually with 5 mm mycelia mat of 5 day old culture grown on PDA plates and incubated at room temperature for 10 days. The mycelia mat was removed by filtering the broth through Whatman No.1 filter paper and the aliquots were centrifuged at 5000 g for 10 min to remove the mycelia fragments, if any. To 10 ml of culture filtrate, 8 ml of calcium chloride-acetate buffer (pH 4.5) was added and mixed thoroughly. The mixture was allowed to stand overnight and then centrifuged at 5000 g for 10 min, supernatant was discarded and the residue was washed with 10 ml of 50% acetic acid saturated with calcium oxalate and centrifuged. The residue was dissolved in 10 ml of $H_2SO_4$. The solution was transferred to 100 ml conical flask and heated at $80^0$C on a water bath. While hot, it was titrated with 0.02 N potassium permanganate until a faint pink colour persisted. The amount of oxalic acid present in the culture filtrate was calculated as 1 ml of 0.02 N potassium permanganate reacted with 1.265 mg of oxalic acid (Mahadevan and Sridhar, 1986).

Three replicates were maintained for each isolate.

## RESULTS AND DISCUSSION:

Thirty isolates showed wide variation in disease development when inoculated to same age seedlings of groundnut. Isolates 2, 5, 15, 18, 20 and 27 isolates were more virulent compared to other isolates and the isolates are also produced higher amount of oxalic acid. All the isolates tested for oxalic acid production in culture filtrates showed variation in its production. Few isolates belonging to group 1 produced low amounts of (0.1265mg/ ml), while some of group 4 produced at the rate of 0.480 mg/ml in the culture filtrate. On the basis of quantity of oxalic acid produced in culture filtrate they were divided into four groups **(Table 1).** There are few reports which indicate that the isolates vary in oxalic acid production in the culture filtrate (Punja and Jenkins, 1984; Ansari and Agnihotri, 2000). Oxalic acid is one of the impediments responsible for degrading the host tissue. It clearly indicates that the low producers will degrade the plant tissue less or slowly for its establishment, whereas the more producer establish faster and cause more damage. Such type of wide variation in oxalic acid production was also noticed among Indian isolates of *Sclerotinia* (Ameer *et al.,* 2008). As indicated by some workers that there are differences in virulence of isolates from same host (Ansari and Agnihitri. 2000) as well as from various hosts (Punja, 1985), the present result confirms that there are definite variations among the isolates of *S.rolfsii* of the groundnut, in terms of oxalic acid production and a positive correlation was observed between rate of oxalic acid production and virulence of the isolate.

| Oxalic acid (mg/ml) | Isolate No. |
|---|---|
| 1.0 to 0.2 | 1(0.1771), 3(0.1664), 4(0.1265), 7 (0.1664), 9(0.1897), 11(0.1664), 17 (0.1664), 19(0.1664), 23(0.1265), 25 (0.1265) |
| 0.2 to 0.3 | 6(0.2125), 8(0.215), 12(0.253), 13 (0.240), 14(0.227), 16(0.227), 22 (2.277), 24(0.265) ,26(0.204), 28 (0.240), 29(0.265), 30(0.253) |
| 0.3 to 0.4 | 10(0.3921), 21(0.3195), |
| 0.4 and above | 2(0.4554), 5(0.408), 15(0.4807), 18 (0.408), 20(0.430), 27(0.4427) |

## REFERENCES:


**Adandonon A, Theresa ASA, Nicolaas A and Gina Sanders 2005.** Genetic variation among *Sclerotium* isolates from Benin and South Africa, determined using mycelial compatibility and ITS rDNA sequence data. *Australian plant path.* 34:19-25.

**Ameer Basha S, Sarma BK, Singh KP and Singh UP. 2006.** Variation in biochemical composition among Indian isolates of *Sclerotium sclerotiorum*. *Microbiology* 34(3):114-119.

**Ansari MM and Agnihotri SK. 2000.** Morphological, physiological and pathological variations among *Sclerotium rolfsii* isolates of soybean. *Indian Phytopath.,* 53(1):65-67.

**Aycock R. 1966.** Stem rot and other diseases caused by *Sclerotium rolfsii* or the status of Rolf's fungus after 70 years. *N. C. Agri. Exp. Stn. Tech. Bull*.174.

**Bateman DF and Beer SV. 1965.** Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenicity by *Sclerotium rolfsii*. *Phytopath.,* 55:204-211.

**Dias BBA, Cunha WG, Morais LS, Vianna GR, Rech EL, Capdeville G de and Aragao FJL. 2006.** Expression of an oxalate decarboxylase gene from Flammulina sp. transgenic lettuce (Lactuca sativa) plants and resistance to *Sclerotinia sclerotiorum. Plant Path*., 55:187-193.

**Espejo E and Agosin E. 1991.** Production and degradation of oxalic acid by brown rot fungi. *Appl. and Envir. Micro.,* 57(7):1980-1986.

**Franklin EC and Dennis ER. 1991.** Use of a host-pathogen interaction system to test whether oxalic acid is the sole pathogenic determinant in the exudates of *Sclerotinia trifoliorum*. *Phytopath.* 81 (12):1546-1550.

**Ganesan S, Ganesh KR and Sekar R. 2007.** Integrated management of stem rot disease (*Sclerotium rolfsii*) of groundnut (*Arachis hypogaea* L.*)* using *Rhizobium* and *Trichoderma harzianum* (ITCC-4572). *Turk. J. Agric. For* 31:103-108.

**Kaveriappa KM. 1979.** Mutual aversion in brinjal

isolates of *Sclerotium rolfsii*. *Indian Phytopath.,*32:475-477.

**Leangon S, Maddox IS and Brooks JD. 1999.** Influence of the glycolytic rate on production of citric acid and oxalic acid by *Aspergillus niger* in solid state fermentation. *World J. of Micro. and Biotech.,* 15:493-495.

**Lehner A, Meimoun P, Errakhi R, Madiona K, Barakate M and Bouteau F. 2008.** Toxic and signaling effects of oxalic acid. *Plant Sign. and Beh.,* 3(9):746-748.

**Livingstone DM, Hampton JL, Phipps PM and Grabau EA. 2005.** Enhancing resistance to Sclerotinia minor in peanut by expressing a barley oxalate oxidase gene. *Plant Physiol.,*137:1354-1362.

**Mahadevan A and Sridhar R. 1986.** Methods in physiological Plant Pathology (3rdedn). Sivakami, Madras. 182.

**Patil MB and Rane MS. 1983.** Studies on host range effect of plantage on susceptibility and varietal reaction of groundnut to *Sclerotium rolfsii*. *Indian J. of Myc. and Plant Path.,*13(2):183-186.

**Punja ZK. 1985.** The biology, ecology, and control of *Sclerotium rolfsii. Ann. Review of Phytopath.,* 23:97-127.

**Punja ZK and Jenkins SF. 1984.** Influence of medium composition on mycelia growth and oxalic acid production of *Sclerotium rolfsii*. *Mycologia* 76:947-950.

**Rajane LG and Stotz HU. 2004.** Oxalate production by *Sclerotinia sclerotiorum* deregulates guard cells during infection. *Plant Physiol.,* 136:3703-3711.

**Sarma BK, Singh UP and Singh KP. 2002.** Variability in Indian isolates of *Sclerotium rolfsii*. *Mycologia* 94(6):1051-1058.

**Saude CN, Melouk HA, Chenault KD, and Meador CB. 2004.** Oxalic acid production by isolates of *Sclerotium rolfsii* and their patrhogenicity on peanut. *Proc. of the American Peanut Res. and Edu. Soc*., 36:51.

**Upadhyay JP and Mukhopadhyay AN. 1985.** Effect of nitrogenous fertilizers on some physiological activities of *Sclerotium rolfsii*. *Indian J. of Mycol. and Plant Pathol.,* 15(2):172-179.