

On C_2 -family basis, the percentage of tetraploid progenies was 3.5 in 6.8 pH colchicine solution 2.2 and in 3 pH solution (table 1). Thus, the results show a slight decrease in the percentage of ploidy induction at low pH condition. Percentage of C_1 plants showing complete sterility was also low (4.8) at 3 pH (table 1).

Taira and Larter¹ observed maximum frequency of chromosome doubling among the wheat \times rye hybrids at pH 5.5. Effect of temperature on the effectiveness of colchicine² in arresting mitosis is reported by many workers.

Effect of colchicine as a mutagen was observed at both the pH levels by screening chlorophyll mutants like albina and xantha and other morphological mutants like plants with small and compact spikes, short awns and thin grains in C_2 generation.

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CHANGES IN TOTAL PHENOLIC CONTENTS OF SORGHUM CALLUS RESISTANT AND SUSCEPTIBLE TO DOWNY MILDEW

M. S. C. PRABHU, P. VENKATASUBBAIAH*
and K. M. SAFEEULLA

*Department of Applied Botany, University of Mysore,
Manasagangotri, Mysore 570 006, India.*

* *Present Address: Division of Microbiology, Central Food
Technological Research Institute, Mysore 570 013, India.*

PERONOSCLEROSPORA sorghi (Ito) Shaw, incitant of downy mildew of sorghum and maize causes great loss to sorghum and maize¹. Certain cultivars exhibit natural resistance to downy mildew which has been correlated to the presence of high amount of phenolics². Changes in phenolic contents of the leaves and roots of downy mildew susceptible and resistant cultivars of sorghum and maize have been reported². However, no *in vitro* studies have been conducted in this regard, since this particular obligate parasite has not been grown axenically. In the present investigation, tissue cultures were used as the host-parasite

systems to study the total phenolics in relation to downy mildew infection. Further it was proposed to look for a possible correlation with the age of the callus and disease resistance.

Meristematic tips of three-week-old sorghum plants (healthy, systemically infected-DMS-652 and resistant-QL-3) were excised aseptically and surface-sterilized using 0.1% $HgCl_2$ solution for 3 min followed by six washings in sterile-distilled water. After drying on sterilized blotters, they were transferred to 100 ml Erlenmeyer flasks containing 30 ml modified Murashige and Skoog medium³ with 5 ppm of 2,4-D; 3 ppm of NAA; 10 mg/l of glycine and 100 ml/l of coconut milk. The flasks were incubated at $20 \pm 1^\circ C$ under 12 hr light and 12 hr dark conditions. The callus produced was subcultured frequently on the same medium, but with 2 ppm of 2,4-D. To study the changes in total phenolics in relation to age, young and old callus (20 days and 120 days old respectively) were selected from susceptible (both healthy and diseased) and resistant varieties.

One gram of each sample was taken, cut into pieces and put into boiling 80% methanol. After 5 min, it was cooled, ground in a mortar with pestle, filtered through cheese cloth and the residue was re-extracted with methanol. The combined filtrate was made to 5 ml and total phenols were estimated by employing Folin-Ciocalteus reagent method⁴. The results were statistically analysed to find out the significance.

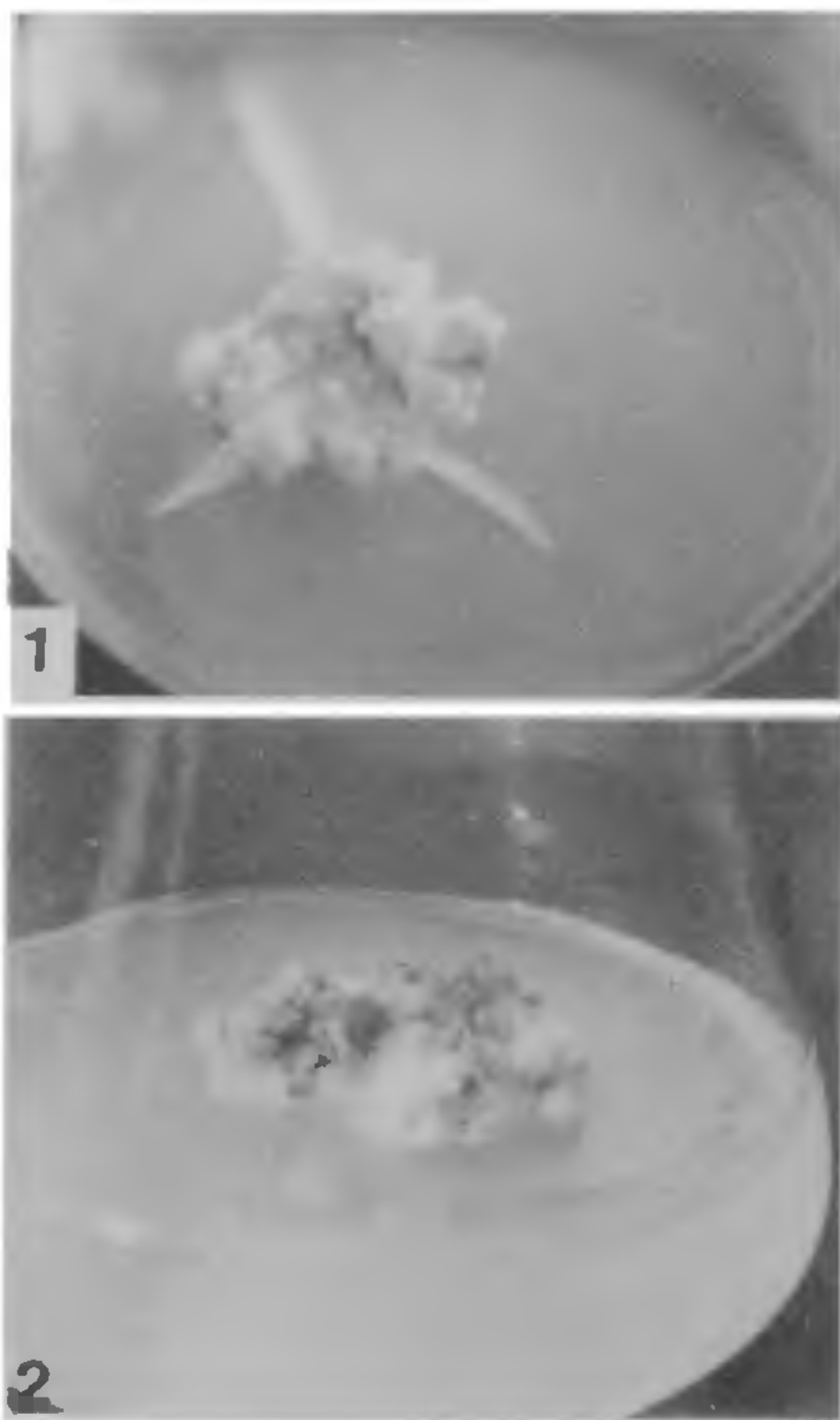
The callus initiation took place three days after placing on the medium in all the cases. The callus produced was of nodulated type and yellowish white in colour (figure 1). However, in the infected callus the mycelia appeared after 10 days as white cottony out growth (figure 2). Mycelia on the young callus developed profusely producing rich crop of asexual spores. Both in healthy and infected callus, as the age increased, it appeared reddish brown secreting certain pigments into the medium. But in the resistant callus the browning appeared at an early stage, leaching the coloured material into the medium. In the infected callus the mycelial growth reduced at later stages as evidenced by the shrinking of mycelia on the callus tissue and reduction in the production of asexual spores.

Table 1 indicates that the total phenolics increased due to infection. Infected callus contained more phenolics than their healthy counterparts. As the age of the callus tissue increased, total phenolics increased both in the susceptible and resistant cultivars. In the resistant variety the young callus contained very high amount of phenolics in contrast to the susceptible

Table 1 Total phenolics in the callus tissue of susceptible and resistant sorghum cultivars to *Peronosclerospora sorghi* in vitro.

Cultivar		Total phenolic content ($\mu\text{g/g}$ of fresh weight)*
Susceptible DMS-652	Young and healthy	70.51 \pm 4.51
	Old and healthy	130.95 \pm 2.19
	Young and diseased	94.97 \pm 1.53
	Old and diseased	190.01 \pm 2.81
Resistant QL-3	Young and healthy	249.63 \pm 2.01
	Old and healthy	303.33 \pm 6.24

* Mean \pm standard deviation.



Figures 1 & 2, 1. Healthy callus of sorghum. 2. Infected callus with profusely growing mycelia of *P. sorghi*.

variety. In the aged callus a substantial increase in the phenolic content was noticed, particularly in the susceptible variety where a two-fold increase was observed as against a slight increase in the resistant callus.

Phenolic compounds have been implicated as resistance factors in a number of host-parasite combinations⁵. The increase in the total phenolic compound in the diseased callus can be correlated with the increase in resistance to *P. sorghi* and this was evidenced by the reduction of mycelial growth and asexual spores on the callus tissue. This corroborates the study of Goodman *et al.*,⁶ where the inhibition of the growth of the pathogen was correlated with the rapid accumulation of phenolics. Shetty and Rasheed Ahmed², observed that sorghum and maize plants are very susceptible to downy mildew upto three weeks and become resistant after this period. This was due to the fact that following infection phenolics accumulate in the diseased tissue to check the fungal growth and develop resistance in the host tissue.

The browning reaction in diseased tissues involves the oxidation of polyphenolic substances by polyphenol oxidase to highly reactive quinones, which then polymerize to form brown colored melanins⁷. In the present study also browning was observed in the old healthy callus tissue and in the highly infected callus.

It has been reported that there is no appreciable difference in the phenolic contents of resistant and susceptible cultivars of healthy sorghum and maize². In contrast the present study depicts the increase in phenolics in the resistant cultivar at an early stage when compared to the susceptible cultivar and this was further evidenced by the formation of brown pigments at the early stage of callus development in the resistant cultivar. This might be due to the resistance mechanism which is genetically predetermined in the resistant variety resulting in the production of high amount of phenolics as active resistant factors in defence mechanism of plants against the pathogen.

To study the host-parasite interaction of downy mildew, one has to depend on the infected plant materials, since the pathogen has not been grown axenically. Recently, dual cultures have been used as simplified systems to study the morphology, cytology and also to study the viability of the seed borne downy mildew mycelium⁸⁻¹⁰. For the first time Arya and Tiwari¹¹ used the dual cultures of pearl millet and *Sclerospora graminicola* to determine the amino acid contents of healthy and infected callus tissues. The present investigation clearly indicates that the dual cultures can be successfully utilised to study the

biochemical changes incurring in the host-parasite interactions of obligate parasites.

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SUGARCANE MOSAIC VIRUS STRAIN H—A NEW RECORD FROM INDIA

E. KONDAIAH and M. V. NAYUDU

*Department of Botany, S. V. University,
Tirupati 517 502, India.*

SUGARCANE is affected by more than 100 diseases in this country¹. Of these, mosaic is most widely distributed. The mosaic virus (SCMV) has various strains which differ in their ability to incite infection and induce deterioration in sugarcane. Strains A, B, C, D, E, F and G were identified² and revised³. Strain G was a variant of B. Later, strain H and I from Louisiana^{4,5}, J from Ohio⁶, K from Meridian, Mississippi⁷, L from

Meigs, Georgia⁸ and strain M from Louisiana⁹ were reported. Strains A, F¹⁰, D¹¹, B¹², C and E¹³ and J¹⁴ were identified from India. We now report the occurrence of a strain which was not recorded earlier from India.

The isolate was collected from sugarcane cv. Q49 from Tungabhadra Project Area (TBP), Basapur Village, Karnataka and maintained on the same cultivar since 1980 by vegetative propagation in the insect proof wire mesh. On sugarcane, the infected plants exhibited mild mosaic symptoms with indistinct white stripes (figure 1).

The healthy sugarcane differentials *viz* CP 31/294, CP 31/588 and CO 281 were obtained from the world germplasm maintained at west coast Regional Station, Cannanore, Kerala. Seeds of sorghum differentials—Rio, Atlas, Collier, Sart and Johnsongrass were obtained from Dr A. G. Gillaspie, Jr., Beltsville, Maryland, Dr J. L. Dale, Dr J. O. York, Arkansas, Dr Voigt, Dr S. M. Alcorn, Tucson, Arizona, Dr D. M. Broadhead, Mississippi and Prof. M. N. Prasad, TNAU, Coimbatore.

Eighteen healthy commercial sugarcane varieties were collected from ARS, Perumallapalli, Tirupati and maintained in 23 cm pots by single bud propagation. The discovery of sorghum Atlas as a local lesion host for SCMV-E¹⁵, led to an intensive search with nearly 250 lines of sorghi obtained from different sources (world collection) for this new isolate.

To prepare inoculum, leaves were washed with cold distilled water, blotted dry and cut into small strips. Weighed amounts were ground using mortar and pestle in 0.005 M phosphate buffer (pH 7.5) along with 0.005 M DIECA (sodium diethyl dithiocarbamate), 0.005 M sodium chloride and 0.01 M 2-mercaptoethanol. The sap was filtered through two layers of muslin cloth; carborundum (400 mesh) was directly added to the sap. Matz's method¹⁶ was used to inoculate sugarcane plants of 1–2 leaf stage for 2–3 times at one week-interval. The sorghi were inoculated by drawing the leaves between the thumb and forefinger. The symptoms on sugarcane were read at weekly intervals for 4–8 weeks and those on sorghi for 4 weeks.

Farrag *et al.*¹⁷ reported that soybean (*Glycine max*) and green gram (*Vigna radiatus*) as the local lesion hosts for SCMV. So, a preliminary search with 20 lines of green gram (supplied by Associate Director, APAU, Tirupati) was made with this new isolate. The cotyledonary leaves were inoculated and the symptoms were read at 1 week interval.

Physical properties were assayed on sorghum Rio,