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Cell Membrane Injury In Sorghum (*Sorghum Bicolor*) associated with infection by *Macrophomina phaseolina* , Charcoal Rot

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Abstract: Cell membrane injury was observed in naturally infected (weakly, moderately and heavily) and artificially inoculated seedlings of sorghum at different time of infection. The cell membrane injury increased with increased time of infection in both naturally infected and artificially inoculated seedlings by *Macrophomina phaseolina.*

Keywords: Sorghum, Cell membrane injury, *Macrophomina phaseolina*

Introduction

Sorghum bicolor (L). Moench is the crop for grain for human and animal consumption. Sorghum is the fifth most important cereal crop in the world after rice, wheat, corn and barley. It is the main cereal food for over 750 million people living in semi-arid tropical regions of Africa, Asia and Latin America (CCCF, 2011). *Macrophomina phaseolina* (Tassi) Goid., is a devastating plant pathogen that causes charcoal rot disease in more than 500 agriculturally important crops including common bean, soybean, chickpea, sunflower, maize, tomato and sorghum (Kaur *et al.* 2012; Jordaan *et al.* 2019). Sorghum (*Sorghum bicolor* L. Moench) is one of the most afected crops by *M. phaseolina* after soybean and can cause up to 100% yield loss under favourable conditions (Patil and Kamble 2011). Plant cell wall is a highly dynamic structure that besides providing mechanical support needs to respond to various environmental and developmental cues and fulfils important functions in signaling events, the defence against biotic and abiotic stresses and growth (Bosch *et al*., 2011). Cell wall defense structures involve morphological changes invaded by the pathogen. The outer layer of cell wall of epidermal cells in contact with incompatible pathogen swells and produces an amorphous and fibrillar material that surrounds and trap the pathogen and prevents them from multiplying.

Material and Method

Raising of crop: The crop was raised in earthern pots (height 30 cm, diameter 20 cm) filled with sterile coarse sand (pH 8.3). Two sorghum samples Sg23 and Sg49 (naturally infected) and artificially inoculated were taken for conducting studies.

Artificial inoculation: Artificial inoculation of *Macrophomina phaseolina* was made in sorghum seeds of sample Sg70. Naturally infected sorghum seeds were used for the isolation of test pathogen *Macrophomina phaseolina*. Their pure culture were raised on PDA medium. For inoculation, seeds from healthy lots were surface sterilized with 1% aqueous solution of NaOCl and soaked in suspension (1×10^5 spores/ml) from 15 days old sporulating cultures pf the pathogen for 24 h.

Estimation of cell membrane injury: Seedlings of healthy, naturally infected (weakly, moderately and heavily) and artificially inoculated sorghum plants were taken for following physiological studies at different time of infection. Cell membrane injury was estimated by the method described by Sullivan. Six leaves per replication of sorghum categorized healthy, naturally infected (weakly, moderately and heavily) with *Macrophomina phaseolina* and artificially inoculated were taken for detecting cell membrane injury. Electrical Conductance (EC) was measured by conductivity bridge of normal and autoclaved tissues. The percent injury of tissue was calculated by the following formula.

% Injury in normal tissue= Conductivity of the tissue/ Total conductivity ^{x} 100

% Injury in stressed tissue= Conductivity of the tissue/ Total conductivity ^{x} 100

% uninjured tissue= 100 - % injury

% Membrane injury = 100 – [(% injured stressed tissue)/ (% uninjured normal tissue) ^{x} 100)]

Result

The cell membrane injury increased with increased days of infection. The changes were very much evident in both samples Sg23 and Sg49 of naturally infected by *Macrophomina phaseolina* as well as in artificially inoculated. It was higher at 30 days of infection in both naturally infected and artificially inoculated seedlings of Sorghum. The cell membrane injury was highest in heavily infected seedling of naturally infected. An increase in cell membrane injury was associated with disease development. The results indicate that increased Electrolyte leakages are one of the initial response of Sorghum to infection by *Macrophomina phaseolina*. The increase in cell membrane injury in heavily infected seedlings may be due to different physiological interactions.

Discussion

The plant cell wall acts as a barrier to penetration by fungal pathogens. These include the secretion of a range of plant cell wall degrading (Depolymerases) and the production of the fungal toxins such as oxalic acids by fungal pathogens. Schillerg *et al.,* also supported that develop strategy that could have potential to reduce pathogen infection is immunomodulation, the expression of genes encoding antibodies or plant bodies that could bind to pathogen virulence products. Ibrahim ElBasyoni *et al* 2017 states that the increased permeability and leakage of ions out of the cell has been used as a measure of cell membrane stability (CMS) and as a screen test for stress tolerance. Ranjeet *et al* 2012 supports that when tissues are subjected to high temperature, electrical conductivity (EC) increases due to damage to cell membranes and consequent solute leakage. Papadakis 2005, Auld 2003 and Xiaozhong Liu 2000 reported that electrolyte leakage from heat stressed wheat leaves was caused by changes in membrane permeability and was related to alterations in lipid and fatty acid composition.

Table 1.1: % Cell membrane injury in seedling of healthy (control), naturally infected (Weakly, moderately and heavily) and artificially inoculated on 20th and 30th day of sowing (sample acc. No. Sg.23)

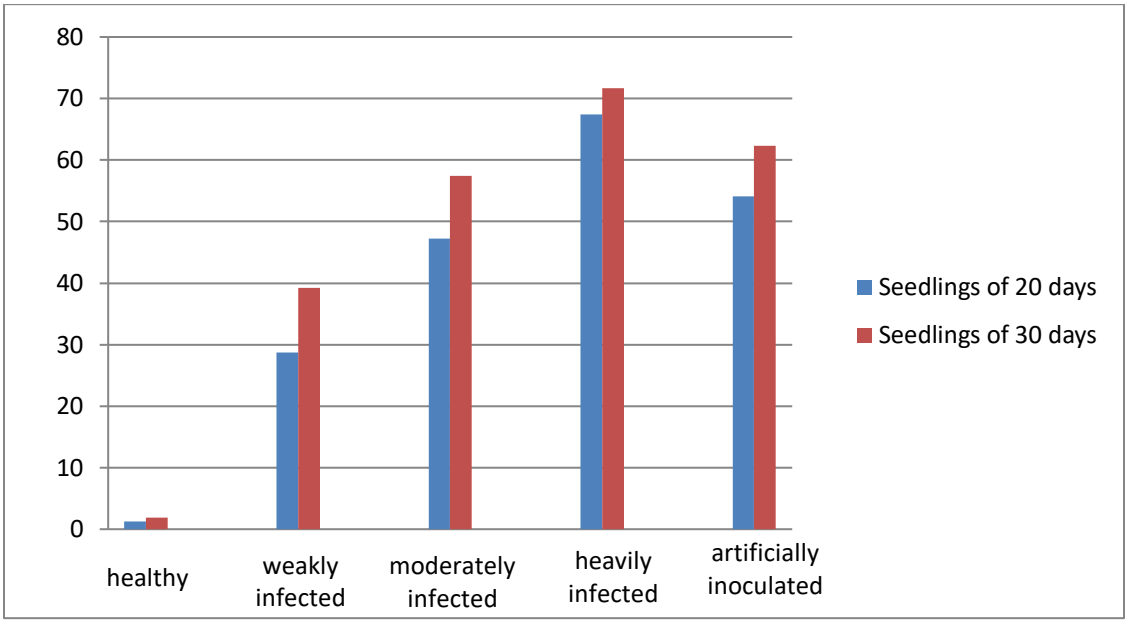
S.No.	Category of seedlings	% Cell Membrane Injury in	
		Seedling of days	Seedling of 30 days
1.	Healthy	1.23 $\pm$ 0.41	1.88 $\pm$ 0.07
2.	Weakly infected	28.69 $\pm$ 0.72	39.23 $\pm$ 0.56
3.	Moderately infected	47.23 $\pm$ 2.22	57.42 $\pm$ 1.33
4.	Heavily Infected	67.43 $\pm$ 1.66	71.67 $\pm$ 1.89
5.	Artificially inoculated	54.11 $\pm$ 1.69	62.33 $\pm$ 1.00

The values indicated in the table, mean of three replications with standard error of mean (SEM)

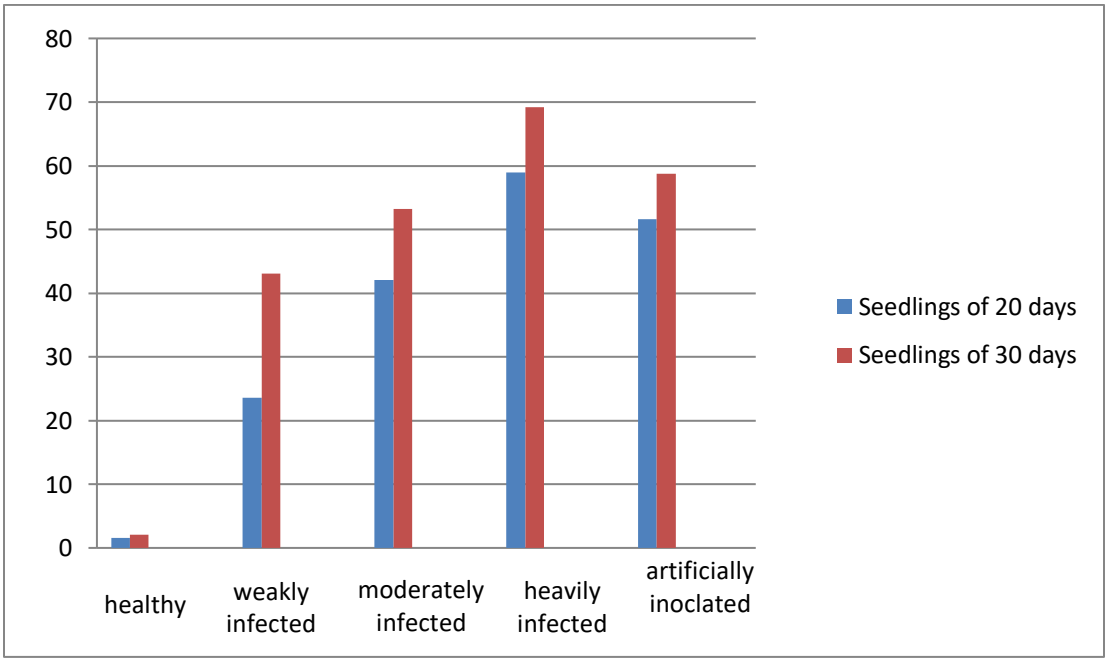
Table 1.2: % Cell membrane injury in seedling of healthy (control), naturally infected (weakly, moderately and heavily) and artificially inoculated on 20th and 30th days of sowing (sample acc. No. Sg. 49)

S.No.	Category of seedlings	% Cell Membrane Injury in	
		Seedling of days	Seedling of 30 days
1.	Healthy	1.53 $\pm$ 0.48	2.12 $\pm$ 0.04
2.	Weakly infected	23.56 $\pm$ 0.69	43.11 $\pm$ 0.69
3.	Moderately infected	42.11 $\pm$ 1.53	53.27 $\pm$ 1.69
4.	Heavily Infected	58.93 $\pm$ 2.13	69.23 $\pm$ 1.77
5.	Artificially inoculated	51.57 $\pm$ 1.89	58.72 $\pm$ 0.83

The values indicated in the table, mean of three replications with standard error of mean (SEM)



% Cell Membrane Injury in Naturally Infected Seedlings Sample Sg23



% Cell Membrane Injury in Naturally Infected Seedling of Sample Sg49

Conclusion

Our findings further agree with the fact that the permeability properties of cell membranes are fundamental to normal functioning of the cell.

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