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PHYSIOLOGICAL RESPONSE OF *VIGNA RADIATA* L. WILCZEK TO SOUTHERN BLIGHT DISEASE

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Abstract

Effects of *Sclerotium rolfsii* on two commercial varieties of mungbean *Vigna radiata* (L.) Wilczek in Pakistan *viz.* NM 2006 and NM 2011 were evaluated *in vivo*. In pot experiments, various biochemical and physiological parameters like chlorophyll, sugar content, proline, relative water content, osmotic potential and disease index were examined at reproductive stage. Both the tested varieties NM 2006 and NM 2011 were found to be susceptible against southern blight. All the investigated parameters such as chlorophyll and carotenoid content, plant height, number of pods, number of seeds per pod, 100 seed weight and membrane stability index were reduced under biotic stress.

Mungbean [*Vigna radiata* (L.) Wilczek] is an important pulse crop which can fix atmospheric nitrogen through symbiotic relationship with microorganisms and enhance the soil fertility (Yadav *et al.* 1994). It contains 51% carbohydrate, 26% protein, 4% minerals, 10% moisture and 3% vitamins (Ali *et al.* 2010). Several biotic and abiotic constraints are responsible for its low yield in Pakistan. Mungbean is susceptible to about 26 diseases in the world. Several fungal phytopathogens such as *Sclerotium rolfsii, Rhizoctonia solani, Alternaria alternata, Colletotrichum capsici* and *Macrophomina phaseolina* are involved in causing severe damage at reproductive stage in the mungbean crop (Iqbal and Mukhtar 2014).

Sclerotium rolfsii Sacc., [teleomorph: *Athelia rolfsii* (Curzi)] belonging to Basidiomycetes is a significant soil-borne phytopathogen which causes Southern blight disease in large number of economically important crops. This fungus has at least 500 host species in 100 families and among those crucifers, cucurbits and legumes are the common hosts (Bhuiyan *et al.* 2012). *S. rolfsii* commonly infect the basal stem region of host species causing wilt and root rot diseases in numerous horticultural and agricultural crops (Ciancio and Mukerji 2007). It forms round, small, brown Sclerotia and white mycelium on basal cells of diseased plants under hot and humid environment (Songvilay *et al*. 2012) Infection rigorously damages the plant by causing a decline in photosynthesis rate and finally plant growth. Therefore, the present study was aimed at evaluating the effects of Southern blight disease caused by of *S. rolfsii* on the physiology of *V. radiata*.

The experiment was conducted at Department of Botany, Lahore College for Women University Lahore, Pakistan during the growing season of mungbean (May-Aug) under natural conditions. Seeds of two varieties of mungbean NM 2006 and NM 2011 were collected from Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad. The pure culture of *S. rolfsii* was collected from First Fungus Culture Bank, University of the Punjab, Lahore, Pakistan. Then sub culturing of *S. rolfsii* in liquid and solid medium was done. Experiment was conducted in pots (30 cm) and comprised of 3 sets of treatments with three replicates each containing 10 plants per pot. First set of pots contained sterilized sandy loam soil while second set of pots contained unsterilized

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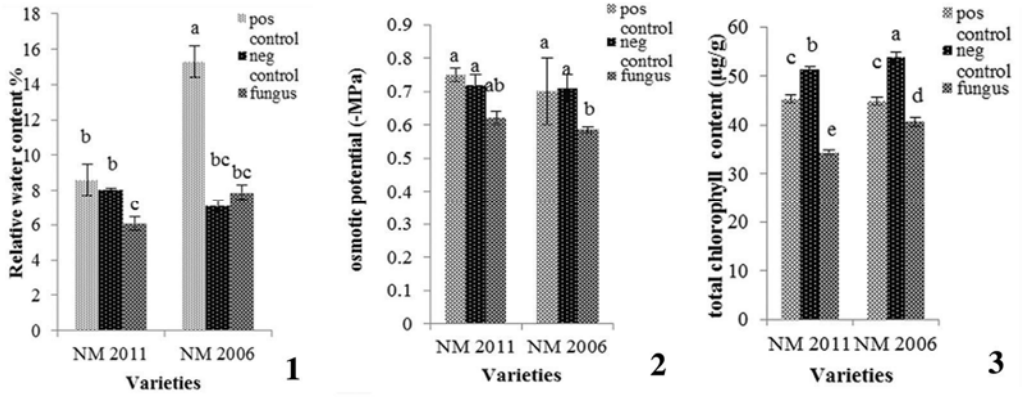
soil and third set of pots contained sterilized soil inoculated with fungal propagules. Pot of sterilized soil act as positive control and unsterilized soil used as negative control. Pots were organized in RCBD. Seeds of two varieties NM 2006, NM 2011 of mungbean plant were surface sterilized with sodium hypochlorite, thoroughly rinsed with distilled water and soaked in conc. 1×10^5 spores per ml suspension of *S. rolfsii* for 3 hrs. For control set, seeds were soaked in distilled water and then sown in pots (Younas *et al.* 2016). Sampling was done at 65 DAS. Following parameters were determined at reproductive stage of the plant.

Chlorophyll content was calculated using formulas of (Arnon 1949). Equation of Lichtenthaler and Wellburn (1983) was followed to estimate carotenoid content. Membrane stability index (MSI) of leaf was determined according to the method of Sairam (1994). Sugar content of leaves was determined by the method of Dubois *et al.* (1956). Proline content was determined by the method of Bates *et al.* (1973). For the estimation of protein content method of Lowry *et al*. (1951) was used. Three plants from each pot were uprooted at reproductive stage, washed with tap water for observation. Root length, root weight and number of nodules were recorded. Disease index was estimated on the basis of following formula:

$$\text{Disease index} = \frac{\text{Weight of diseased roots}}{\text{Weight of healthy roots}} \times 100$$

Moreover plants were harvested (80 - 90 DAS) at maturity from all pots and yield related parameters like height of plant, number of branches, number of pods, number of seeds per pod and 100-seeds-weight were determined. The data were analyzed by ANOVA and the mean values were assessed with DMRT using COSTAT software (Steel *et al.* 1997).

Results of this study revealed that *S. rolsfii* significantly affects the growth of mungbean. At reproductive stage reduction in chlorophyll *a, b* and total chlorophyll content was observed in both the varieties (Fig. 1). Total chlorophyll content in negative control of both varieties was increased as compared to positive control. Significant reduction in carotenoid content was also observed in both the varieties of mungbean.

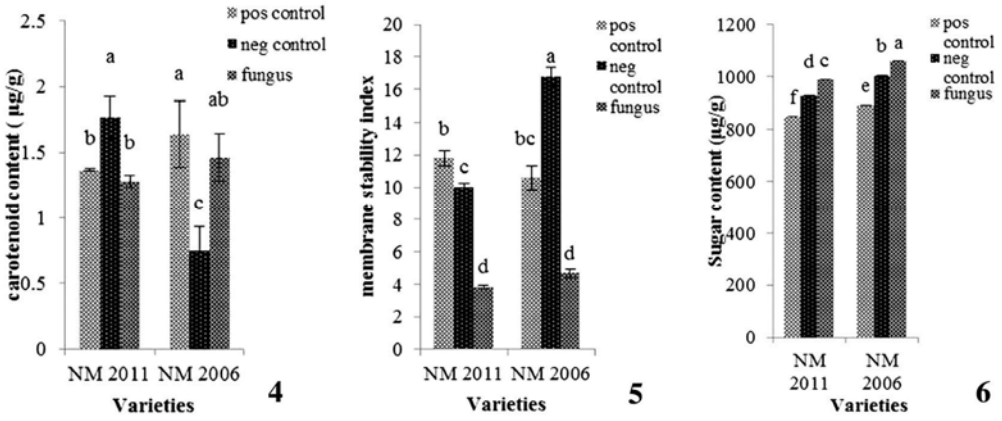


Figs 1-3. Effect of *Sclerotium rolfsii* on relative water content, osmotic potential and chlorophyll content of *Vigna radiata*.

However, an increase in carotenoid content was observed in NM 2006 at reproductive stage as compared to NM 2011 (Fig. 4). Faheed *et al.* (2007) also found that infected tomato seedlings showed decrease in chl. *a*, chl. *b*, carotenoids contents as compared to the control. Hossain *et al.*

(1999) had reported that biotic stress caused loss of carotenoids and chlorophyll contents: a noticeable phenomenon of senescence. Mycotoxin produced by fungi can disrupt the chlorophyll synthesis that caused discoloration of diseased plants. The disruption of chlorophyll might be due to the inhibition in grana formation (Shirurkar and Wahegaonkar 2012).

The biotic stress of *S. rolsfii* also significantly affected the membrane stability index of mungbean leaves at reproductive state (Fig. 5). Similar decrease in MSI induced by fungal stress has also been observed by Al-Hakimi and Alghalibi (2007). Significantly higher sugar content was observed in leaves of both tested varieties of mungbean under fungal stress at reproductive stage in comparison to control (Fig. 6). This higher sugar content in infested tissues might be due to the pathogen which acted as an extra sink. Fotopoulos *et al.* (2003) also reported that enhanced glucose uptake was observed in the *Arabidopsis* leaf tissue due to powdery mildew infection.



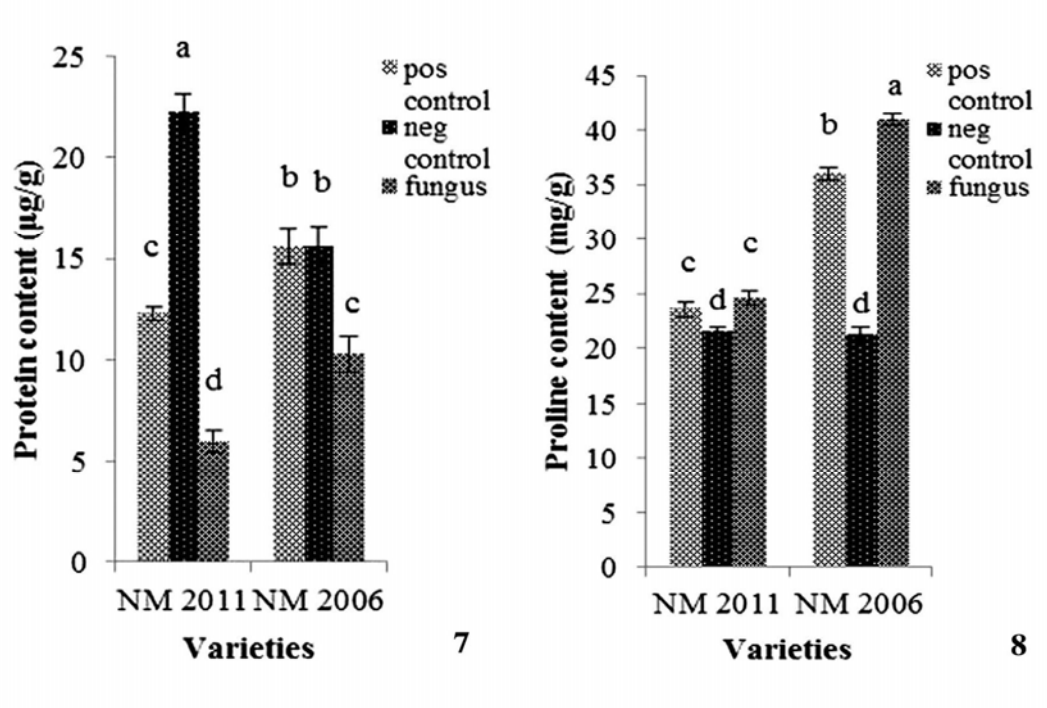
Figs 4-6. Effect of *Sclerotium rolfsii* on carotenoid content, membrane stability index and sugar content of *Vigna radiata.*

Significant reduction in proline content was observed in NM 2011 as compared to NM 2006 (Fig. 7). According to Fabro *et al.* (2004), proline addition in plant tissues may raise the plant tolerance to several biotic or abiotic stresses. Jaleel *et al.* (2007) suggested that accumulation of large amounts of proline (osmolytes) is an adaptive comeback in plants exposed to stressful conditions.

Significant decrease in protein content was observed in fungal treatment of variety NM 2011 as compared to variety NM 2006 (Fig. 7). The variety NM 2011 was found more susceptible than NM 2006. Previous study had also suggested that less protein content was found in mulberry leaves due to infection of fungus (Ghosh *et al.* 2003).

Fungal stress significantly reduced the root length and weight as compared to control in lieu to estimate disease index (Table 1). Minimum reduction in length and weight of root was observed in fungus treated plant of variety NM 2011. Disease index of variety NM 2011 was less than NM 2006 i.e. 68.36 and 80.12%, respectively (Fig. 9a, b). This fungus parasitized on the basal region (roots) of plants; as a result roots become weaker as compared to the control plants.

In present experiments *S. rolfsii* attacks the roots of mungbean showed wilting, which results in fragile plant growth that is indicated by the decrease in root/shoot length, root/shoot dry weight.



Figs 7-8. Effect of *Sclerotium rolfsii* on proline and protein content of *Vigna radiata.*

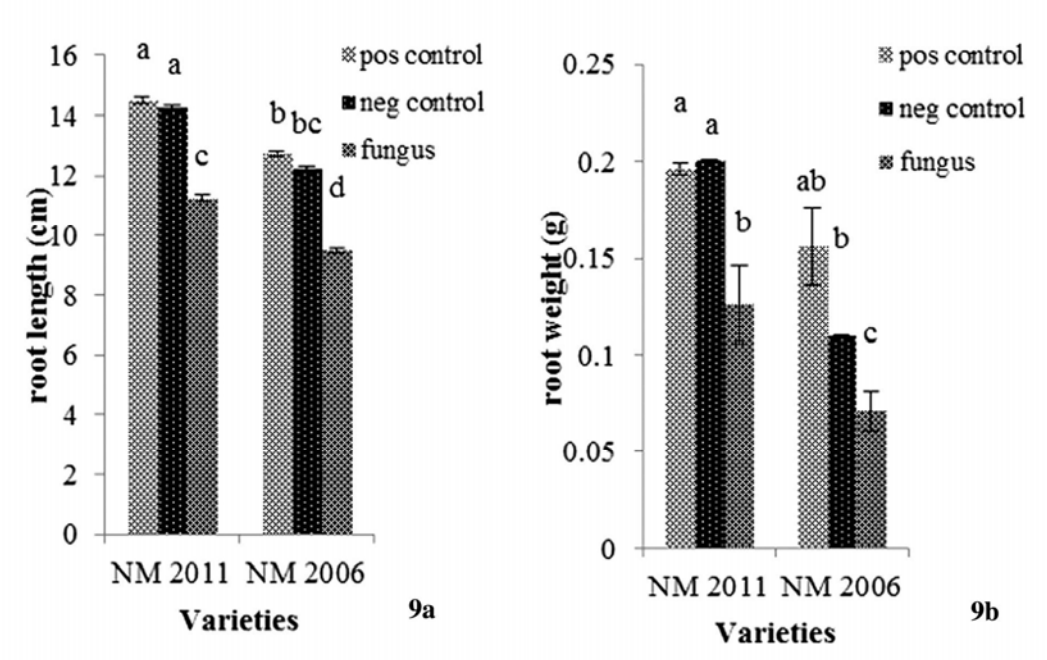


Fig. 9a,b. Effect of *Sclerotium rolfsii* on root length and root weight of *Vigna radiata.*

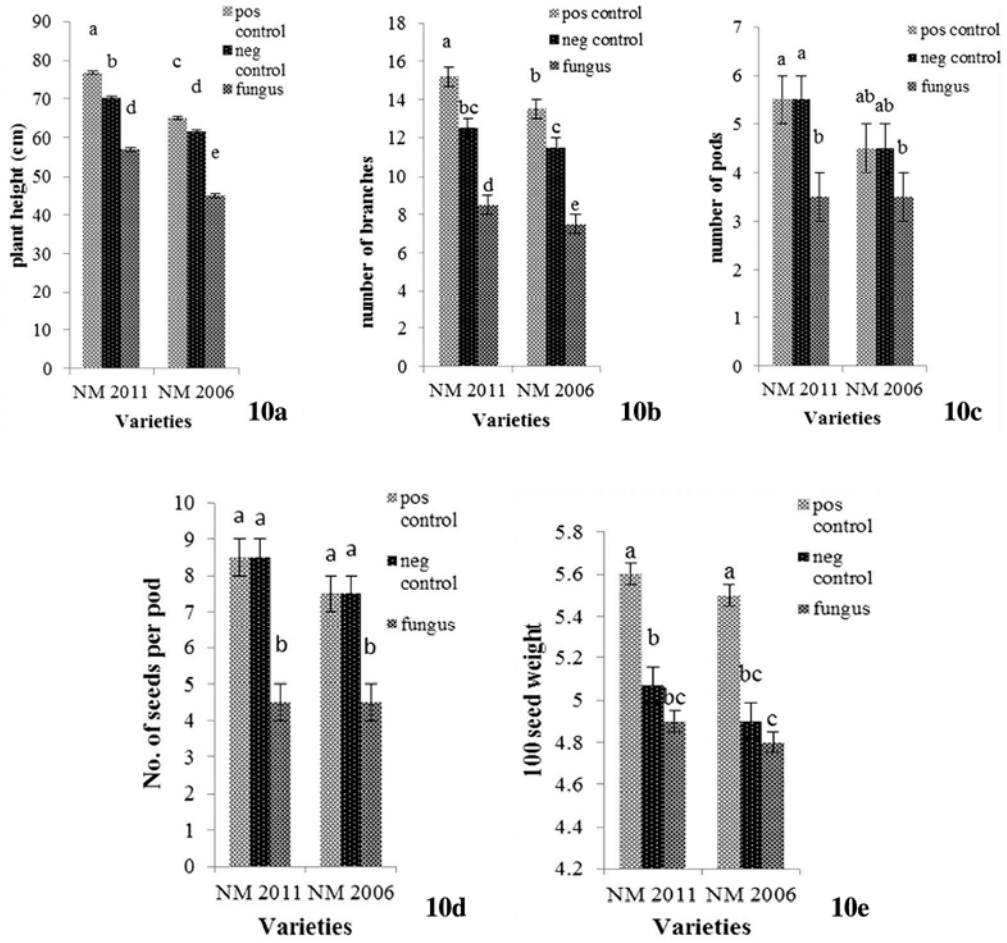


Fig. 10a-e. Effect of *Sclerotium rolfsii* on plant height, number of branches, number of pods, number of seeds per pod and 100-seed weight of *Vigna radiata.*

Table 1. Effect of *S. rolfsii* on tolerance index and disease index of two varieties of *V. radiata*.

Varieties	Tolerance index (%)	Disease index (%)
NM 2006	44.1	80.12
NM 2011	49.35	68.36

Height of plant, number of branches, number of pods, number of seeds per pod and 100-seed weight significantly decreased under fungal stress (Figs 10a-e). Faheed *et al.* (2005) had reported that biotic stress reduced all growth and yield parameters in infected tomato plants as compared to uninfected plants. Khan *et al.* (2017) suggested that fungus produce metabolites that caused degradation of seeds and decreased the seed viability by the synthesis of cell wall degrading enzymes.

From the present study it may be concluded that *S. rolfsii* significantly affect the physiology of mungbean and variety NM 2006 is more susceptible to *S. rolfsii* than NM 2011.

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