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EFFECT OF *MACROPHOMINAPHASEOLINA* ON GERMINATION, GROWTH AND PHYSIOLOGY OF *CAPSICUM FRUTESCENS* L.

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A B S T R A C T

The current investigationwas carried outto evaluate the effect of collar rot fungus *Macrophomina phaseolina* (Tassi) Goid. on germination, growth and physiology of *Capsicum frutescens* L. Two commercial varieties DY1143 California hybrid and DY1143 were used for the experiment. The experiment was designed as completely randomized (CRD) with three replicates per treatment in Petri plates and pots. In Petri plate experiment considerable decline in germination rate, germination percentage, seed vigor, length and weight was observed in 10-days old seedling ofboth the tested varieties of *C. frutescens*. Physiological parameters i.e. protein content, proline content, sugar content and chlorophyll content also significantly suppressed under *M. phaseolina* stress. The present investigation concluded that DY1143 California hybrid variety of *C. frutescens*is more sensitive as compared to DY1143 variety.

Keywords: Biotic stress, Chili, Charcoal rot, Germination, Seed borne disease, Pakistan

INTRODUCTION

*Capsicum frutescent s*L. (chili) belongs to family Solanaceae is an annual herbaceous vegetable commonly grown in sub-tropical and tropical regions of the world (Than *et al*., 2008). Chilies are used as fresh and dried powdered form world over. *C. frutescens* is rich in carbohydrates, lipids, fibers, minerals, protein and vitamins (A, B) (El-Ghoraba*et al.,* 2013). In Pakistan ,*C. frutescens* is commonly grown species with an annual production of 186.5 thousand tones(Anonymous, 2010).

Chilies are very sensitive to cool weather and susceptible to a number of diseases, including collar rot, root rot and charcoal root rot (Hussain and Abid, 2011;Hussain*et al*., 2012;Hussain*et al.,* 2013a). Fungal pathogens especially *Phytophthora capsici, Rhizoctonia solani* and *Macrophomina phaseolina* attack on root, leaves, stem and fruits of chilli plants and produced upto 70% yield lose (Liu and Lu. 2003). *Macrophomin aphaseolina* (Tassi) Goid is an anamorphic fungus belongs to ascomycetes causes collar rot disease in chilli. *M. phaseolina* affected more than 500 wild and cultivated plant species worldwide (Purkayastha *et al*., 2006). This fungus produces micro-sclerotinia in the soil which is a resistant structure (Shaner *et al.,* 1999). *M. phaseolina* induced biotic stress in large number of crop plants and affects various physiological functions like chlorophyll, proline and sugar content (Kuldeep*et al*. 2012).

So, the aim of this study was to examine the effect of *M. phaseolina*on germination, growth (fresh and dry weight) and physiology (contents of chlorophyll, proline, sugar and protein)of two varieties of *C. frutescens*.

MATERIALS AND METHODS

The experiment was conducted in Fungal Biotechnology Research Laboratory, Department of Botany and green house at Lahore College for Women University Lahore, Pakistan.

The fungal culture of *Macrophomina phaseolina*(FCBP-751) was collected from FirstFugal Culture Bank of Pakistan, Institute of Agricultural Sciences, Quaid-e-Azam Campus, University of Punjab, Lahore, Pakistan. The culture was retained on 2% Malt extract agar (MEA) in 9 cm Petri plates.

The seeds of DY1143 variety of *C. frutescens* were collected from Punjab Seed Corporation, Sahiwal (Pakistan) and seeds of DY1143 California Hybrid were procured from Seed Corporation, Sheikhupura (Pakistan). The experiment was design in randomized

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complete block design with three replicates per treatment.

Petri plate experiment: Twenty seeds were surface sterilized andplaced randomly in Petri plates of 9cm diameter on filter paper moistened with fungal suspension. *M. phaseolina* suspension was prepared by using fresh culture and 1×10^5 conidia per mL of the test fungus was adjusted. In control treatment distilled water was used only. The Petri plates were incubated for 10days in incubator at 20±2 ºC. Seed germination data was noted after every 24 hour. After 10 days incubation period; germination %age, root and shoot length, tolerance index and root, shoot dry weightwere determined. Seed germination percentage was calculated by using the formula given by Close and Wilson(2002).A modified Timson index was used to estimate the germination rate (Khan and Ungar, 1984).The root and shootlengths (cm) of the seedlings were measured after 10 days of sowing.Seed vigor was calculated by using the method of Abdul-Baki and Anderson (1973).Formula given by Turner and Marshal(1972) was used to determine the tolerance index.After ten days incubation period, fresh weight was calculated with the help of electrical balance. Dry weight was recorded by drying the seedlings at 65ºC for 72 hours.

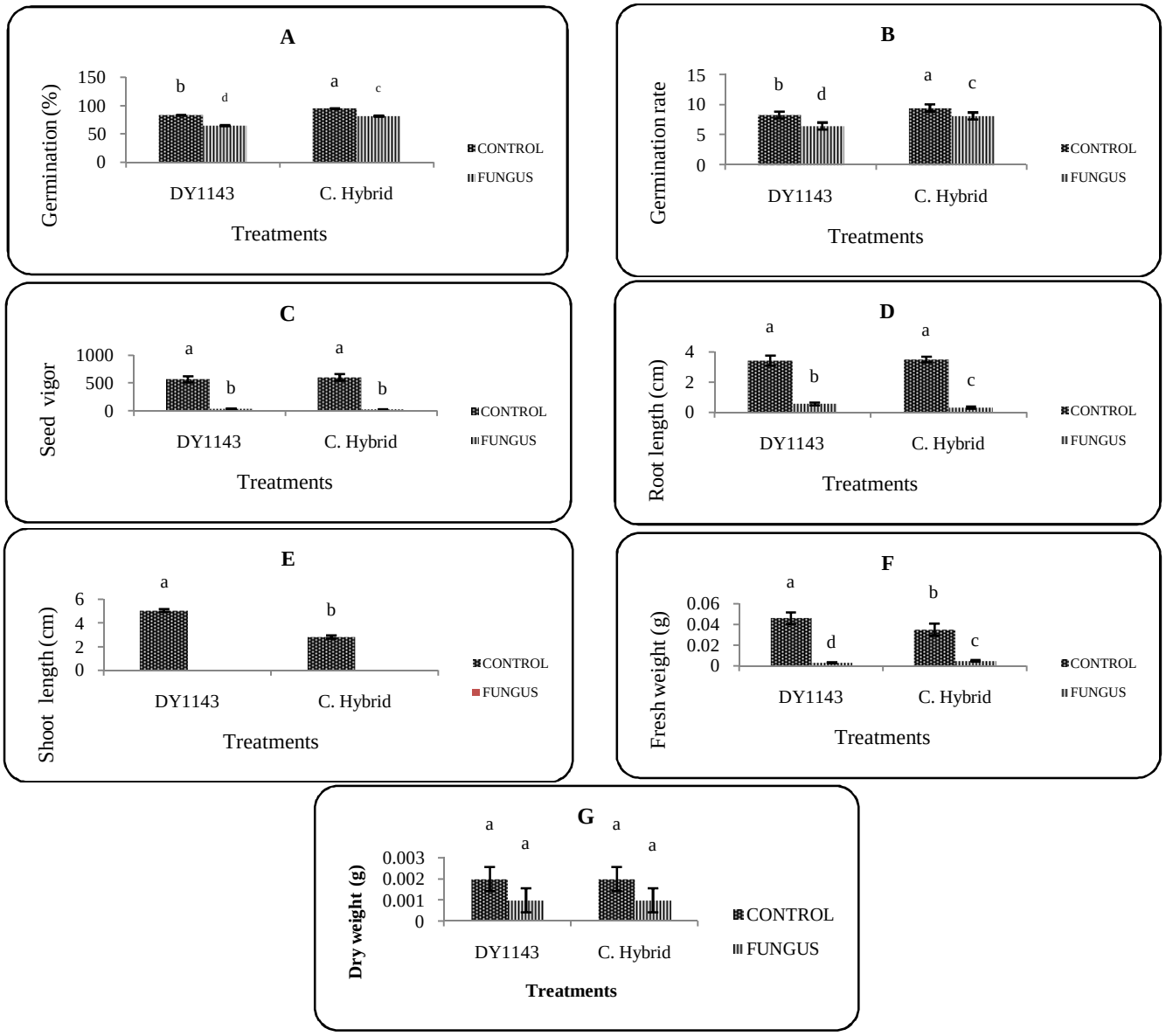

Figure 1A-G. Effect of *Macrophomina phaseolina* on germination (%) germination rate, seed vigor, root length, shoot length, fresh weight and dry weight of*Capsicum frutescens*.

Pot experiment: Seeds of two varieties of *C. frutescens* (DY1143 and DY1143 California Hybrid) were firstly surface sterilized with sodium hypochlorite solution and then were thoroughly rinsed with distilled water. These surface sterilized seeds were soaked in *M. phaseolina* suspension (1×10^5conidia per mL) for 15 minutes. In control treatment seeds were soaked in distilled water. Two sets of plastic pots were prepared by adding 420 g sterilized soil. Five seeds of each variety were sown in each set of plastic pots of 9cm diameter and 11cm length with three replicates. Each variety was replicated thrice and the pots were arranged in randomized complete block design (RCBD) for 20 days.

The protein, proline, sugar, chlorophyll and carotenoid contents of chili seedlings were measuredby following methods after 20 days sowing period.Method of Bates and Coworkers (1973) was used to measure the proline content in leaves of *C. frutescens*. Protocol given byLowry *et al*. (1951) was applied to estimate the protein content. Sugar content was determined by using Dubois *et al.* (1956) method.Chlorophyll a, b and total chlorophyll content was estimated by the method of Arnon(1949). Lichtenthaler and Wellburn (1983) method was used to calculate the carotenoid content. All the data was statistically analyzed by using co-stat software. One-way (ANOVA) analysis of variance followed by Duncan's multiple range test (DMRT) at P<0.05% were applied (Steel and Torrie, 1980)

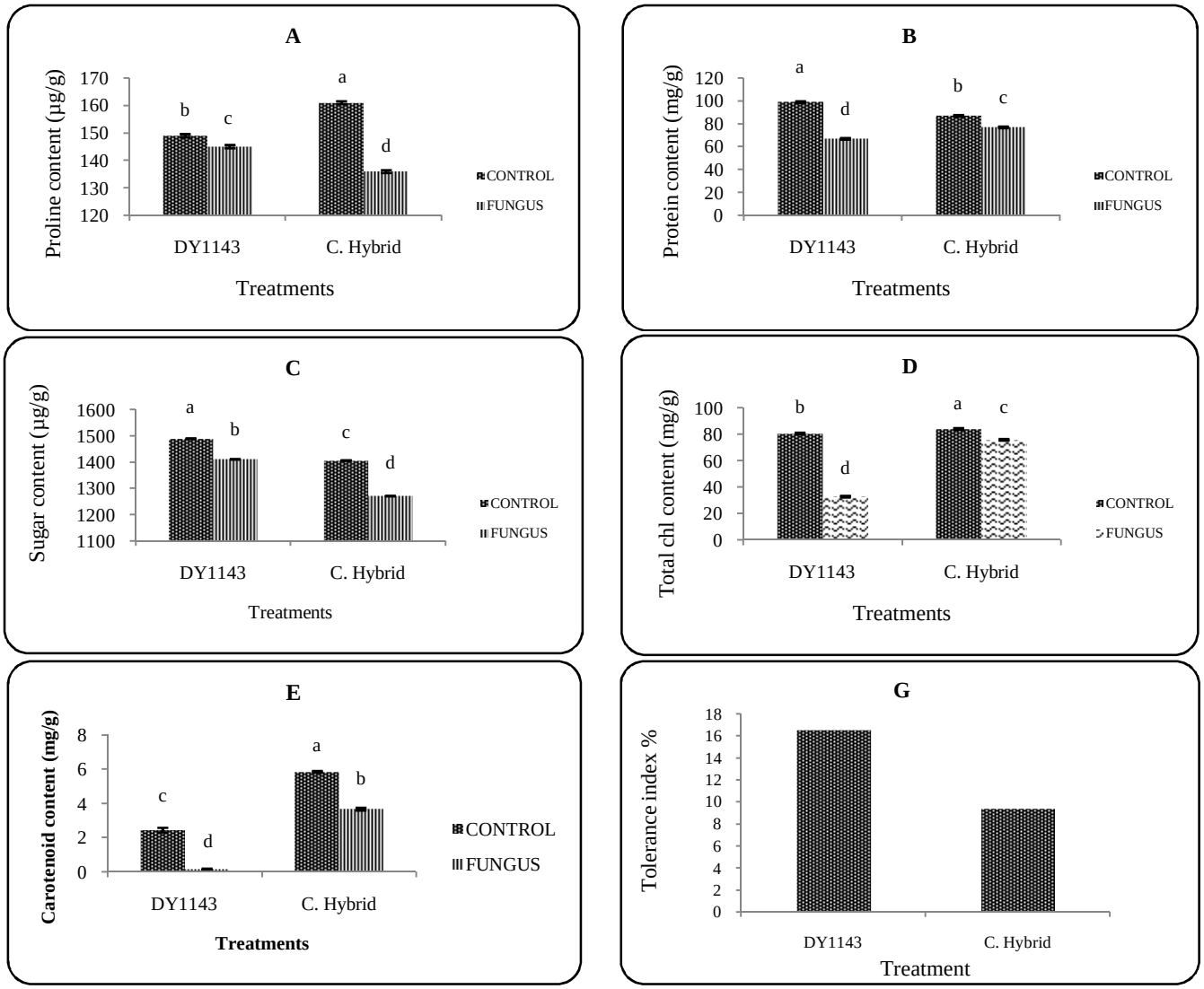

Figure 2A-G. Effect of *Macrophomina phaseolina* on content ofproline, protein, sugar, total chlorophyll, carotenoid (mgg^{-1}) and tolerance index in two varieties of *Capsicum frutescens*.

C. Hybrid = DY1143 California Hybrid

RESULTS AND DISCUSSION

Petri plate experiment: In Petri plate experiment, metabolites of *M. phaseolina* were noticeably decreased the *in vitro*germination rate, germination %age and seed vigor of both the test varieties of *C. frutescens* (Figure 1A-C). Earlier Shanthakumari *etal.* (2002) reported that *M. phaseolina* caused reduction in thegermination percentage of *Hibiscus schizopetalous*. Likewise, Al-Kassamand Monawar (2000) stated that certain seed-borne fungi are responsible of seed rot, a decline in seed germination leads to seedling death due to damping off.

A reduction in the root and shoot lengths as well as weights was observed under*in vitro* biotic stress of both the varieties when compared to control (Figure 1 E-G). Previously (Mavi,1986; Ahmad, 1996; Agrios,2005) reported that soil-borne diseases negatively affect the translocation of food in the plant body because they affect the root system of the plant. Inadequate food translocation affects physiological processes of plant.

Pot experiment: Proline, protein, chlorophyll and sugar contents were studied in Pot experiment. High proline content was observed in control set of both the test varieties. But the difference in prolinecontent was more significant in the variety DY1143 California Hybrid (Figure 2A). Significant decrease (67 mg/g-77 mg/g) in protein content of both the tested varieties of *C. frutescens* was observed under biotic stress(Figure 1B). Increased concentration of proteins and other biochemical in resistant variety may play a role in the inhibition of infection (Sharma and Sharma, 1994).Singh and Waraitch (1981) also reported that *Colletotrichum falcatum* infection caused reduction in the protein content of sugarcane leaves. The low sugar content was observed under biotic stress condition with respect to control set in both varieties (Figure 2C). Total chlorophyll content was decreased under *M. phaseolina* stress in comparison to control (Figure 2D). Likewise Jadan and Shah (2012) suggested that disease severity and symptom development on the leaves was correlated by the reduction in chlorophyll content. High carotenoid content was also observed in both the tested varieties as compared to control treatment (Figure 2E). Singh and Singh (1991) reported that infection by cucumber mosaic virus in *Capsicum,* reduced the chlorophyll a, b and total chlorophyll. Aguirreolea and Coworkers in(1995) stated that photosynthetic activity was decreased in the blighted bell pepper leaves and rice.None of the test variety of *C. frutescens* withstand against the biotic stress of *M. phaseolina* when tolerance index parameter was studied. Previously, Díaz *et al*. in(1997) stated that the phenolic metabolism of pepper is altered during the *Verticillium* wilt which might be responsible of low tolerance index of the tested *C. frutescens* varieties.

On the basis of the observed results present study concluded that collar rot disease is a serious threat to economically important chilli crop. This fungus badly affected the physiology of DY1143 and DY1143 California Hybrid.

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