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Effect of combined biotic and abiotic stress on some physiological aspects and antioxidant enzymatic activity in mungbean (*Vigna radiate L.*)

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Stomata conductance (g_s), Shoot water content (SWC), chlorophyll pigments (chl a,b) and enzymes involved in anti-oxidant photo-protection were determined in two mungbean genotypes (Kawmay-1 and VC2010) under greenhouse conditions. The two genotypes were subjected to water deficit stress (20, 40 and 80% of field capacity) and two root-knot nematode (*Meloidogyne javanica*) infection levels (non-infected and infected at 15000 juveniles per pot). Both water deficit and nematode infection resulted to a fast decline in the chlorophyll pigments, g_s and SWC in both genotypes; however, VC2010 was recorded as being comparatively resistant. Increase in antioxidant enzymes activity was detected for superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and polyphenol oxidase (PPO) in both stresses, but this activity was more pronounced in water deficit stress than nematode infection, especially at 40% field capacity. APX and PPO production peaks recorded at 20% of irrigation in VC2010 were highest. This revealed that VC2010 genotype was tolerant to environmental stresses compared to Kawmay-1. It was conceived from the present study that water deficit stress significantly hampered the physiological representatives of plant health, while on the other hand enzymatic alterations to cope with the biotic and abiotic stresses in plants could be used for better tolerability and plant health. The results indicated that oxidative damage (ROS) produced under environmental stress can be minimized by increasing the antioxidant enzymatic activities in mungbean.

Key words: Enzymatic activities, Nematode infection, Mungbean, reactive oxygen species (ROS), stomata conductance, water deficit.

INTRODUCTION

Drought stress is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally death of plant (Kaya et al., 2006; Jaleel et al., 2008). Water deficit is frequently the primary limiting factor for crop production. However, the response to stress depends on the intensity, rate and duration of exposure, as well as the plant growth stage (Hussain et al., 2004).

Drought problems for agricultural crops are on the increase with the rapid expansion of water stressed areas around the world. Limited water supplies and increasing demand sectors impose innovative and efficient water

employment in agriculture. Root-knot nematodes (*Meloidogyne* ssp.) are obligate endoparasites, which spend a greater part of their life cycle inside the host plant and parthenogenetically reproduce by mitotic divisions (Blaxter, 2003; Strajnar et al., 2009). More than 2,000 plants, comprising of major crops are attacked by *Meloidogyne* ssp., which is responsible for $157 billion (more than 50%) of overall damage caused by nematodes (that is, agricultural losses annually). Formation of root galls increases wilting reduces growth, nutrient and water uptake, which result to mineral deficiency and low yield. Among the various *Meloidogyne* ssp., *Meloidogyne javanica* is a potential threat to pulse crops. The biochemical and physiological activities of the host plants are hampered by *M. javanica* infection. In *Vigna radiata*, 23 to 49% yield losses has been reported to have resulted from *M. javanica* infection (Sharma et al., 2000).

Mungbean (*V. radiata* L.) originates from South Asia, and is being cultivated in the short rainy season in Southern Asia. Now, it has also been introduced in South East Asia, Austronesia, Africa, China, Egypt and South America (Lambridge and Godwin, 2006). Among the favourable characters of cultivating mungbean are: Short-term growth, nitrogen fixation capability, soil reinforcement and prevention of soil erosion (Hoorman et al., 2009). Mungbean seed is a rich source of protein (23.86%), carbohydrates (62.62%), minerals (K, P, Mg, and Ca), vitamins (C and A) and dietary fibre (Khattak et al., 2009). It enhances human body immunity, lowers the cholesterol level and protects against diabetes. Plant tolerability to various abiotic and biotic stresses at cellular, as well as at plant level is a complex issue. This complexity is due to interactions among various physiological, molecular, metabolic and morphological phenomena affecting growth and development under stress factors. Oxidative damage (ROS) produced under environmental stress) cause a serious problem in plants and can be minimized by increasing the antioxidant enzymatic activities (Hernandez et al., 2010). A systematic study is inevitable to understand the physiological response of mungbean under drought and nematode stresses. The present study was designed to investigate the physiological response of mungbean under drought-nematode coupled stress, under Saudi Arabian conditions.

MATERIALS AND METHODS

This greenhouse study was carried out in the Department of Botany and Microbiology, Faculty of Science, King Saud University, Riyadh, Saudi Arabia (24.710 N and 46.720 E) in 2012 to 2013.

Plant material

Seeds of two mungbean genotypes; Kawmay-1 and VC-2010, imported from Egypt and Thailand, respectively were surface sterilized with 0.1% sodium hypochlorite (NaOCl) for 5 min, washed with 0.1 M MgSO4 solution thrice and dried in open air. These seeds were sown in 30 cm plastic pots filled by soil-peat moss, with a 2:1 mixture; sterilized by steam under pressure at 126°C for 30 s. The experiment consisted of three replications and 36 pots arranged in a Randomized Complete Block Design (RCBD) under factorial arrangement with three factors: genotype (A), irrigation (B) and nematode infestation (C) (Gomez and Gomez., 1984). Pots were filled with recommended fertilizers and maintained to 6 seedlings per pot, two weeks after sowing.

Drought stress induction

Three weeks after sowing, the plants were arranged for imposing drought stress. Three irrigation levels (80, 40 and 20% of field capacity) were used as imposed drought stress in mungbean based on pretested field capacity of soil-peat moss mixture. All pots were irrigated weekly according to the treatments earlier given. After 45 days of nematode infection, data were recorded for the aforementioned parameters.

Stomata conductance (mmole/m^2/s)

Stomata conductance was measured using Leaf Porometer, Model: SC-1 (Decagon Devices USA). The measurements were taken between 10:00 to 11:30 a.m. using three leaves for each treatment.

Shoot water content measurement, SWC (%)

Shoot water contents (SWC) was measured by sampling three similar fully expended leaves per plot. Leaf samples were sealed in plastic bags, placed above ice in a cooler and transported to the lab for the determination of fresh weight. Leaf samples were dried at 70 ±5°C until constant weight was reached. SWC were calculated according to the following formula: SWC=[(FW-DW)/(FW)] × 100.

Chlorophyll contents (a and b)

Chlorophyll a and b contents were determined according to the technique described by Metzner et al. (1965). UV/Visible Spectrophotometer - LKB (Biochrom 4050) was used at wavelengths of 663 and 644 nm, chlorophyll contents were computed using the following equations:

Chlorophyll (a) = 10.3×OD663 - 0.918×O.D644 = ug/g
Chlorophyll (b) = 19.7×OD644 - 3.87×O.D663 = ug/g

Root-knot nematode inoculum

The *M. javanica* population was derived from single egg mass and cultured on tomato (*Solanum lycopersicum*) seedlings in a greenhouse. After 60 days of culturing, eggs were extracted from

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tomato by shaking infected roots for two minutes in 0.5% sodium hypochlorite (NaOCl) solution using an electric shaker (Hussey and Barker, 1973) followed by thorough washing under distilled water; a modified technique described by Mcclure et al. (1973). The eggs suspension was then incubated at 28 ± 2°C on cotton-wool filter to obtain freshly emerged juveniles. To avoid contamination and water evaporation, petri dishes were covered with glass lids. After 72 h of incubation, second stage juveniles were collected from suspension and stored under recommended conditions for further use (Walters and Barker, 1993). Number of juveniles was counted using counting dish, and 20 ml water suspension was prepared for 15,000 juveniles per pot. Suspension was poured uniformly in root zone 5 cm below the soil surface. Half of the mungbean pots were inoculated with root-knot nematodes four weeks after sowing, while the other half were kept non-inoculated as control.

Enzyme extraction and estimation

According to McCord and Fridovich (1969), 0.50 g of fresh leaves were collected and frozen in nitrogen liquid; then the sample was ground to make it soft. The sample was extracted by 6 ml of liquid composite of 50 mmol of potassium phosphate pH 7 + 0.1 mmol of EDTA + 4% of PVP + 0.2 mmol of ascorbic acid. Then, all were extracted in centrifuge at 1200 for 20 min. The pure liquid was kept in a refrigerator at 4°C till estimation.

Catalase (CAT)

CAT was determined by spectrophotometer according to Aebi (1984), where a mixture of 2 ml of enzyme extraction and 3 ml of interaction mixture (1.0 mmol Potassium phosphate pH 7 + 15 mmol of H_2O_2) were used.

Superoxide dismutase (SOD)

Superoxide dismutase was analyzed according to methodology described by Weisiger and Fridovich (1973), using viresitokrom and xanthine oxidase as a source of activation of SOD, followed by potassium cyanide for estimation of enzyme activity in hydrogen peroxide.

Ascorbate oxidase (ASO)

According to Saher et al. (2004), ASO enzyme activity was measured in a mixture of 50 mmol of potassium phosphate pH 7 + 1.5 mmol of ascorbic acid and 1.0 mmol of nitrogen peroxide. Then, the reading was done at 265 nm using a spectrophotometer.

Polyphenol oxidase (PPO)

The activity of enzyme was measured according to the method of Hernandez (2010), comprising 5 ml of catechol liquid with concentration 0.01 M + 2 ml of regulator liquid of phosphate and 0.50 ml of enzyme extraction, all were put in a test tube and measurements were taken after 3 min at 470 nm, using a spectrophotometer.

Statistical analysis

Analysis of variance (ANOVA) was performed by Statistical Analysis System (SAS, 2013) software. Means were compared using Fishers LSD analysis at 5% probability.

RESULTS AND DISCUSSION

According to Table 1 and Figure 1, the genotypic differences are significant for stomatal conductance (g_s) and SWC at 5% LSD while water deficit stress and nematode infection have been recorded as being highly significant (1%) for all mentioned parameters. Interaction between G and I stood significant for g_s, SWC, CAT and SOD, however, G*T recorded significant for stomatal conductance and SOD only, while all other parameters were non-significant. Irrigation by nematode interaction were computed as highly significant except for g_s and chlorophyll b. Stomatal conductance and shoot water contents exhibited almost similar trends, water deficit stress significantly hampered both parameters in descending order, from 80% < 40% < 20%. Infection by nematode also significantly reduced both parameters. VC2010 stood prominently resistant against water and nematode stress. G*I was recorded as significant for both; however, G*T and I*T were recorded as non-significant for stomatal conductance and SWC, respectively; whereas three way interaction was found as totally non-significant. Stomatal conductance (g_s) is an important and frequently varying plant parameter under both biotic and abiotic stresses and responds immediately. It regulates a number of physiological as well as biochemical process simultaneously e.g., rate of carbon assimilation, radiation absorption, transpiration. These findings were supported by the reports of Kaya (2006) and Jaleel (2008). photosynthetic pigments were restricted by water deficit stress, 20% irrigation reduced both chlorophyll a and b by more than 50% in comparison to control irrigation.

A similar trend was seen for nematode infection. However, no significant genetic differences were recorded. Genotype by Irrigation and Genotype by Nematode infection interactions were completely non-significant for both pigments; however, I*T was computed as highly significant for chlorophyll a but non-significant for chlorophyll b. Three factorial combined interactions were non-significant. Thalooth et al. (2006), Naresh et al. (2013) and Ahmed et al. (2009) reported almost similar results. These results indicated that stresses, drought and nematode infection have much effect on both genotypes. Water relations and chlorophyll content are very important in plant photosynthesis and hence crop productivity, so all physiological traits were affected by both stresses. These finding agree with many other authors (Sharma et al., 2000, Kaya et al., 2006; Jaleel et al., 2008) who found that severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally death of plant.

Reactive oxygen species (ROS) produced under environmental stresses can cause a serious effect to plant metabolism and hence higher reduction in crop productivity. It is known that oxidative stress results from the disruption of cellular homeostasis of reactive oxygen

Table 1. Analysis of variance summary for mungbean genotypes under water deficit and *Meloidogyne javanica* stresses.

Sources of Variation	df	Mean squares (Summary)							
		Stomatal conductance	Shoot water contents	Chl_a	Chl_b	Catalase	Superoxide dismutase	Ascorbate oxidase	Polyphenol oxidase
		µmol/m^2/s	%	µg/g	µg/g	µmol/mg/min	µmol/mg/min	µmol/mg/min)	µmol/mg/min
Genotypes (G)	1	971.4**	0.11**	32100^{NS}	1002.7^{NS}	1372.7*	1995.1^{NS}	0.174^{NS}	1.1736^{NS}
Irrigation (I)	2	1668.0**	0.38**	46135**	29226.4**	3041.4**	56761.3**	166.661**	39.5836**
G*I	2	80.8*	0.001**	150^{NS}	4386.11^{NS}	45.4**	128.1*	0.257^{NS}	0.4053^{NS}
Disease (T)	1	1013.4**	0.22**	34627**	95069.4**	974.5**	12026.8**	18.063**	6.3336**
G*T	1	1013.4**	0.001^{NS}	30334^{NS}	69.4^{NS}	0.7^{NS}	676.0**	0.003^{NS}	0.0136^{NS}
I*T	2	40.4^{NS}	0.001**	50199**	1886.1^{NS}	60.3**	1509.8**	2.216**	0.2003*
G*I*T	2	37.0^{NS}	0.0001^{NS}	12234^{NS}	536.1^{NS}	51.2*	30.3^{NS}	0.001^{NS}	0.0053^{NS}

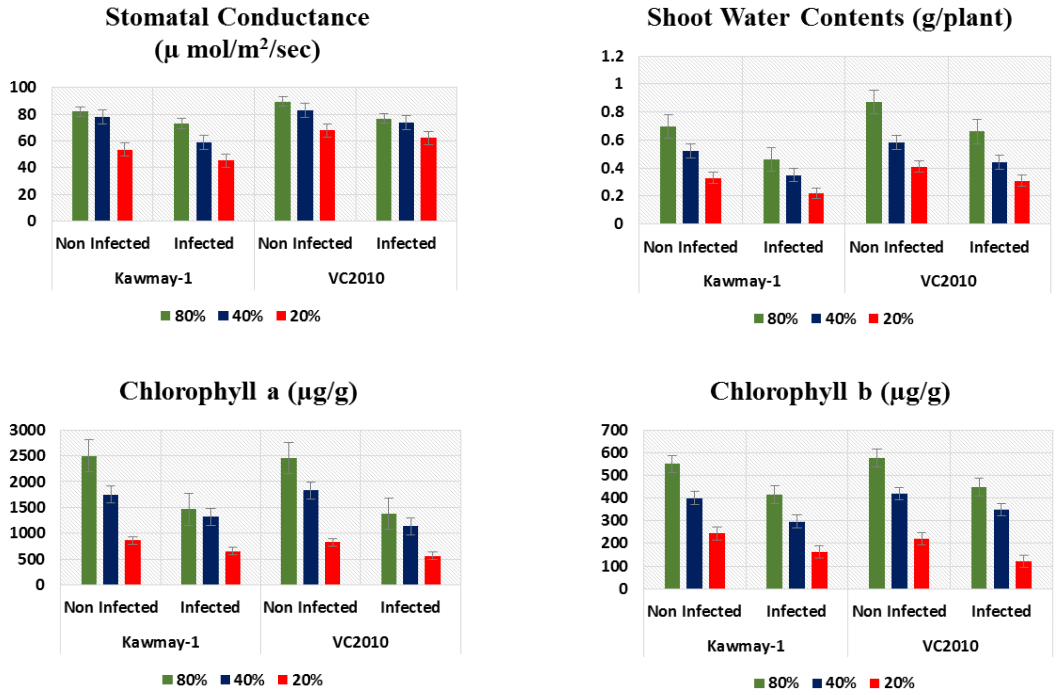

Figure 1. Physiological parameters of mungbean subjected to water deficit and *Meloidogyne javanica* infection.

Table 2. Antioxidant enzymatic activity of mungbean subjected to water deficit and *M. javanica* infection.

Genotype	Irrigation (%)	Nematode Infection	Catalase (CAT) (µ mol/mg/min)	Superoxide dismutase (SOD) (µ mol/mg/min)	Ascorbate Oxidase (APX) (µ mol/mg/min)	Polyphenol oxidase (PPO) (µ mol/mg/min)
Kawmay-1	80	Non-Infected	26.40±0.90	125.0±5.00	2.80±0.60	4.63±0.55
		Infected	18.40±2.00	119.3±1.52	2.23±0.15	3.90±0.30
	40	Non-Infected	58.60±1.80	270.0±5.00	6.83±0.35	7.33±0.45
		Infected	42.83±2.95	226.0±6.00	5.50±0.10	6.60±0.10
	20	Non-Infected	37.53±2.95	234.0±4.00	11.2±0.85	8.10±0.40
		Infected	29.23±0.95	200.0±12.0	8.93±0.35	6.93±0.15
VC2010	80	Non-Infected	31.50±0.80	138.3±6.50	3.23±0.55	4.90±0.30
		Infected	29.03±1.45	122.0±6.00	2.63±0.45	4.16±0.06
	40	Non-Infected	70.93±1.25	300.0±15.0	6.70±0.10	7.30±0.10
		Infected	60.20±5.90	238.0±2.00	5.30±0.40	6.73±0.35
	20	Non-Infected	56.40±4.20	261.3±5.50	11.4±0.10	8.83±0.06
		Infected	39.13±6.45	204.0±6.00	9.10±0.10	7.73±0.06

species (ROS) production. Reactive oxygen species accumulation induces oxidative damage of membrane lipids, nucleic acids, and proteins (Mitller, 2002).The response of antioxidant systems to drought stress has been widely studied in plants (Hernandez et al., 2001, 2010, Mittova et al., 2003, Gomez et al., 2004, BenAmor et al., 2006). In general, it is well accepted that plants with high levels of activity of the antioxidant systems, both constitutive and induced, have greater resistance to oxidative damage. Therefore, antioxidant enzymatic activity is known as a good mechanism can be used to protect plant from environmental stresses such as drought, heat, salinity and diseases infection (Sharma et al., 2000, Ahmed et al., 2002 and Hernandez et al., 2010). Our result indicated that CAT and SOD were recorded as being effected by nematode infection (Table 2). However, the enzymatic activity increased when irrigation level reduced from 80 to 40 but a reduction for 20% irrigation was noticed. APX and PPO were also found significantly reduced by nematode stress. Irrigation deficiency stress increased the APX and PPO activity as irrigation reduced from 80 to 20%, with the highest values recorded in 20%. This means that, under high stress, plants may increase the enzymatic activity to minimize the effect of oxidative damage by ROS produced under environmental stress. However, CV2010 mungbean genotype stood high for all four enzyme's activity recorded in this study, which indicated its tolerability to biotic and abiotic stresses. This finding may help for selection mungbean genotypes under arid climate of Saudi Arabia. Ahmed et al. (2002) reported similar results in a research conducted on mungbean plant.

Conclusions

The results illustrated that water deficit (Abiotic stress) may seriously damage the physiological activity and enzymatic performance in mungbean. Root-knot nematode (Biotic stress) may also significantly reduce the plant performance under particular conditions. However, it is considered that the present results confirm the relevance of induction of the antioxidant system to protect the plant against the oxidative damage under environmental stresses.

Also, genotypic differences for tolerance against biotic and abiotic stresses could be the potential inputs for variety development and agronomic practices. Further extensive research is necessary in order to understand and obtain deeper insights on the mechanism stress damage and biochemical changes behind physiological alterations.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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