The physiological response of mungbean (*Vigna radiata*) to water deficit stress and *Meloidogyne javanica* infection

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Abstract

The physiological response of mungbean (*Vigna radiata* (L.) R. Wilczek) to drought stress and root-knot nematode infection was studied under greenhouse conditions at King Saud University, Saudi Arabia. A randomized complete block Design (RCBD) with factorial arrangement having three replications was used. Treatments included three water deficit (80%, 40% and 20% of field capacity), two mungbean genotypes (Kawmay-1 and VC2010) and two root-knot nematodem, *Meloidogyne javanica* (Treub) Chitwood, infection levels (non-infected and infected @ 15000 egg/pot). Results showed that water deficit stress and *M. javanica* infection significantly hampered most of the studied parameters, except shoot water content (SWC). There were highly significant differences in stomatal conductance, shoot dry weight and leaf area among the tested mungbean genotypes. A significant positive correlation among chlorophyll (a and b) contents, stomatal conductance (SC), leaf area (LA) and shoot dry weight (SDW) was recorded. The outcome of the study also revealed that maximum water deficit stress has adversely affected all parameters except SWC, regardless of the genotype or nematode infection status. Similarly *M. javanica* infection adversely affected the growth and physiological processes of mungbean plants. Moreover, drought and *M. javanica* infection had synergistic adverse effects on the growth and physiology of mungbean plants. Results also showed that VC 2010 genotype surpassed kawmay-1 in most of the studied characteristics which

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could be used for the development of drought as well as nematode resistant genotypes in future breeding programs.
Keywords: drought stress, Meloidogyne javanica, mungbean, stomatal conductivity, leaf area, root-knot nematode, shoot water content.

1 Introduction

Drought stress causes reduction of plant water content [1], diminished leaf water potential and turgor loss, closure of stomata and decrease cell enlargement and growth [2, 3]. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of the plant [4, 5]. Water stress also inhibits cell enlargement and division. It reduces plant growth by affecting various physiological and biochemical processes such as photosynthesis, respiration, translocation, ion uptake, carbohydrates accumulation, nutrients metabolism and hormonal expression [6]. Water deficit is frequently the primary limiting factor for crop production [7]. However, the stress response depends upon the intensity, rate and duration of exposure as well as the plant growth stage [8]. Drought problems for agricultural crops are worsening with the rapid expansion of water stressed areas of the world. Limited water supplies and increasing demand sectors impose innovative and efficient water employment in agriculture.

Root-knot nematodes (*Meloidogyne* spp.) are obligate endoparasites, complete greater part of their life cycle inside the hosted plant and parthenogenetically reproduced by mitotic divisions [9]. More than 2,000 plant species are attacked by *Meloidogyne* spp. These nematodes are responsible for $ 157 billion which represent more than 50% of the overall damage caused by nematodes in agricultural crops annually. They are widely distributed in warmer, tropical and subtropical regions wherever the climate is suitable for reproduction [10, 11]. Formation of root galls by these nematodes increases wilting, and reduces growth, nutrient and water uptake, resulting in mineral deficiency and low yield. Among the various *Meloidogyne* ssp. *M. javanica* is a potential threat to pulse crops [12]. Host plant biochemical and physiological activities of susceptible plants are hampered by *M. javanica* infection [13]. In *Vigna radiata*, 23–49% yield losses have been reported by *M. javanica* infection [14].

Mungbean, *V. radiata*, is originated from South Asia, and is being cultivated in the short rainy season in Southern Asia [15]. Now it has also been introduced in South East Asia, Austronesia, Africa, China and South America [16]. Attempts to grow mungbean under Saudi Arabian conditions have been made by the crop production group at the College of Food and Agriculture Sciences, King Saud University. Preliminary results showed that it can be successfully grown under Saudi Arabia conditions as a summer legume crop [17]. Among the favourable characteristics of growing mungbean are: short-term growth, nitrogen fixation capability, soil reinforcement and prevention of soil erosion [18, 19]. Mungbean seed is a rich source of protein (23.86%), carbohydrates (62.62%), minerals (K, P, Mg, Ca), vitamins (C and A) and dietary fiber [20]. It enhances human body immunity, lowers the cholesterol and protects against diabetes [21].

Plant tolerability to various abiotic and biotic stresses at cellular as well as at plant level is a complex [22]. This complexity is due to interactions among various physiological, molecular, metabolic and morphological phenomena affecting growth and development under stress factors. Therefore 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 to drought-nematode coupled stress under Saudi Arabian conditions.

2 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) during 2012–2013.

2.1 Plant material and experimental design

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 minutes, washed with 0.1M $MgSO_4$ solution thrice and dried in open air. These seeds were sown in 30 cm plastic pots filled with soil-peat moss, 2:1 mixture, and sterilized by steam under pressure at 126°C for 30 seconds. Experiment was consisted of 36 pots arranged in a Randomized Complete Block Design (RCBD) in a factorial arrangement with three replicates for each treatment [23]. The studied factors included; plant cultivars (Kawmy-1 and VC-2010), Irrigation (80, 40 and 20% of water capacity) and nematode inoculation (inoculated and non-inoculated). Thus, the experiment has 12 treatments ($2 \times 3 \times 2$). Pots were irrigated and fertilized as needed till the end of the experiment, 45 days after nematode inoculation.

2.2 Drought stress induction

Three weeks after sowing plants were exposed to the drought stress induction. Three irrigation levels (80%, 40% and 20% of field capacity) were used. All pots were irrigated weekly according to the treatments given above.

2.3 Root-knot nematode inoculum

M. javanica population was derived from single egg mass cultures maintained on tomato, *Solanum lycopersicum* L. plants in the greenhouse. After 60 days of nematode inoculation, eggs were extracted from tomato roots using 0.5% sodium hypochlorite (NaOCl) [24–26]. After four weeks from sowing, half of the mungbean pots were inoculated with root-knot nematodes @ 15000 egg/pot while the other half were kept non-inoculated as control.

2.4 Observations

After 45 days of nematode inoculation, data were recorded on plant growth, physiology and nematode bioassays. Chlorophyll a and b contents were determined according to the technique described by Metzner e*t al*. [27]. Stomatal conductance was measured using Leaf Porometer, Model: SC-1 (Decagon Devices USA). Leaf area was recorded with a portable Leaf Area Meter, Model: LI 3000C (LI-COR). Shoots and roots were harvested, washed, measured and weighed immediately. These shoots and roots were then oven dried at 72°C until weight constant, and the dry weight was determined.

2.5 Statistical analysis

Analysis of Variance (ANOVA) was performed by Statistical Analysis System [28] software. Means were compared by Fishers LSD analysis at 5% probability.

3 Results and discussion

Photosynthetic pigments undoubtedly are valuable representatives of plants under stress conditions. Irrigation, root-knot nematode infection I*N were found to be highly significant for chlorophyll a (Chl a) contents of mungbean. However, genotype, and all other possible first order as well as second interactions were found non-significant for this trait as illustrated in Table 1. Gradual increase in drought stress level (80% < 40% < 20%) have adversely hampered the Chlorophyll a contents. *M. Javanica* infection resulted a clear reduction in chlorophyll a contents under all drought stress levels and genotypes. Results revealed that nematode stress has reinforced the damaged caused by drought stress (Fig. 1).

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

Sources of variations	df	Mean squares					
		Chl a	Chl b	SC	SDW	SWC	LA
		µg/g	µg/g	µ mol/m^2/sec	g/plant	%	Cm^2/plant
Genotype (G)	1	32100.6^{NS}	1002.7^{NS}	971.4**	0.11**	0.44^{NS}	104.7**
Irrigation (I)	2	4613564.5**	292269**	1668.0**	0.38**	9.69^{NS}	2743.4**
Nematode (N)	1	3462700.6**	95069.4**	1013.4**	0.223**	1.00^{NS}	196.9**
G*I	2	150.7^{NS}	4386.11^{NS}	80.8*	0.0098**	4.69^{NS}	5.3**
G*N	1	30334.0^{NS}	69.44^{NS}	1013.4**	0.001^{NS}	18.77^{NS}	0.5^{NS}
I*N	2	501992.4**	1886.11^{NS}	40.4^{NS}	0.0099**	1.75^{NS}	2.5*
G*I*N	2	12234.0^{NS}	536.11^{NS}	37.0^{NS}	0.0001^{NS}	5.86^{NS}	1.8^{NS}

*, and **:F-test significant at $P \leq 0.05$, and $P \leq 0.01$, respectively. NS, not significant.

Chlorophyll b (Chl b) contents have shown an almost similar trend to Chlorophyll a under deficit water stress conditions and *M. javanica* infection (Table 1), whereas genotypes and the interactions did not significantly differ. Highest water deficit level (20%) has maximally reduced the chlorophyll b

contents (Figure 2). Similar results have been reported by Allahmoradi *et al*. [29], Lalinia *et al*. [30] and Thalooth *et al*. [31] that drought stress significantly reduced the chlorophyll contents in mungbean. However, according to Naresh *et al*. [32], root-knot nematode reduced photosynthetic pigments in mungbean, and that was supported by Akhtar *et al*. [33] and Ahmed *et al*. [34].

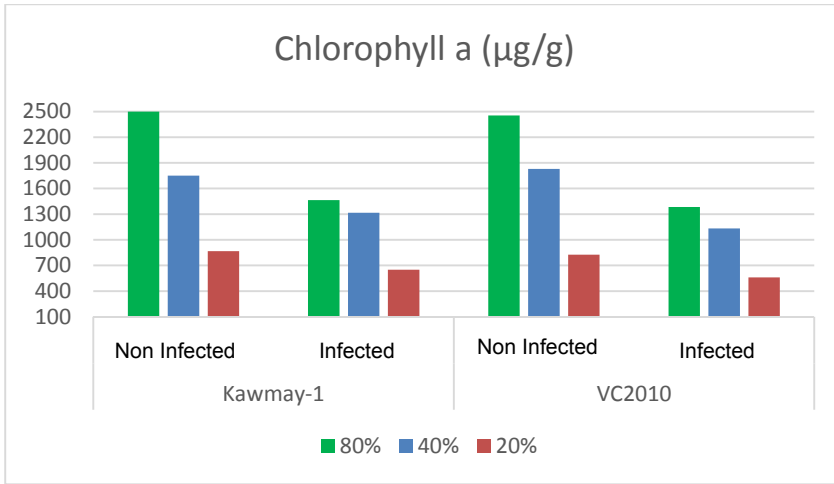

Figure 1: Chlorophyll a contents affected by water deficit and *M. javanica* infection.

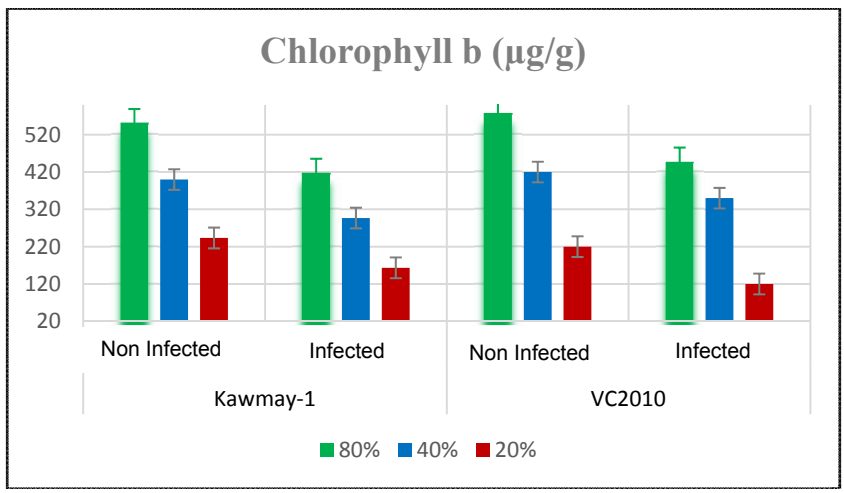

Figure 2: Chlorophyll b contents affected by water deficit and *M. javanica* infection.

Stomatal conductance (SC) is an important and frequently varying plant parameter under both biotic and abiotic stresses and respond immediately to stress. It regulates a number of physiological as well as biochemical process simultaneously e.g., carbon assimilation rate, radiation absorption, transpiration, biomass production, plant water contents, yield and yield components. SC was high significantly affected by genotype, drought stress, *M. javanica* infection and G*N while G*I stood significant whereas I*N and three way interaction (G*I*N) were recorded non-significant for this parameter as explained in Table 1. Mungbean genotype; VC2010 performed better under both stresses; nematode infection and drought states as compared to Kawmay-1. It was recorded remarkably resistant under both stress conditions as well as their couple stress (I*N) for SC. First two water deficit levels (80% and 40%) did not show a valuable difference for SC in both genotypes and *M. javanica* infection but highest drought stress (20%) has consistently abridged the SC value. Proline accumulation and K+ balance facilitate stomatal closure under drought stress conditions as reported by Naresh *et al*. [32], Kaya *et al*. [2] and Jaleel *et al*. [4] for mungbean summer crop [35]. At the same time nematode infection resulted significant reduction in SC (Figure 3).

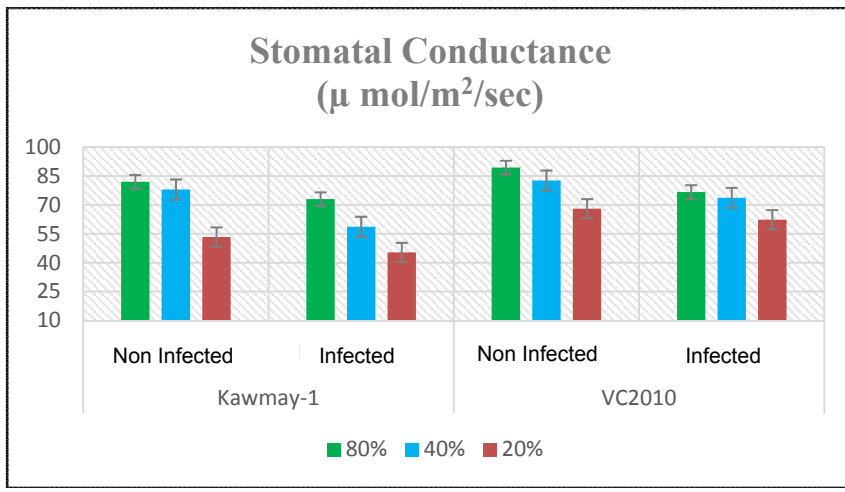

Figure 3: Stomatal conductance affected by water deficit and *M. javanica* infection.

Shoot dry weight (SDW) is an outcome of a number of physiological changes, process and metabolic factors. Results evidences cleared that genotype, drought stress, *M. javanica* infection, G*I and I*N significantly affected SDW. However, G*N and G*I*N were recorded non-significant as presented in Table 1. Drought stress has hampered total dry mass accumulation in mungbean [2, 36, 37]. VC2010 genotype has highly valuable resistivity against nematode stress as well as drought stress. Teresa *et al*. [35] have also claimed that genotypic variations are present within *Vigna radiate* against water deficit stress.

Within each genotype, nematode infection has decreased SDW and that may be as a result of poor root structure, reduced ion and water uptake as compared to control (Figure 4), similar results have also been conveyed by Khan *et al*. [38].

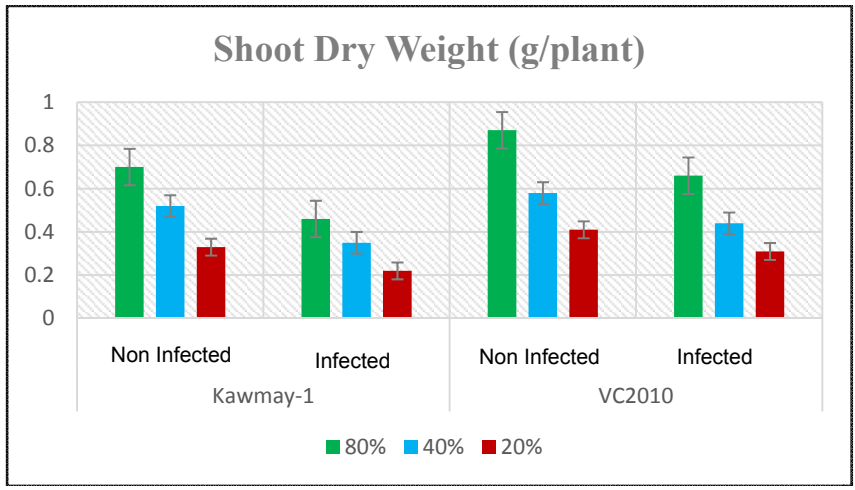

Figure 4: Shoot dry weight affected by water deficit and *M. javanica* infection.

Shoot water contents (SWC) is a complex and net result of many plant regulations and adaptabilities. Under any biotic or abiotic stress homeostatic mechanisms of plants maximize its effort to survive, so maintenance of SWC is one of the priorities. In the present study no significant differences have been recorded for all sources of variations (as shown in Table 1). In addition, no particular drift has been seen for SWC but it has a dramatically high value under highest water deficit stress (Figure 5). However, Moradi *et al*. [39] argued the significant reduction in SWC under drought stress. Ocampo and Robles [40] stated genotypic differences while, Abad *et al*. [11] reported nematode infection was significantly limiting factor for SWC in mungbean.

Genotypic differences, drought stress levels, *M. javanica* infection, G*I and I*N were observed highly significant in dimensioning leaf area (LA) of mungbean plants while G*N and G*I*N were noted non-significant (Table 1). LA is an integrated, visual and fundamentally true representative of stressed plants either under limiting biotic factors or abiotic. Figure 6 clearly summarized the valuable differences of genotypes; VC2010 comparatively performed better than Kawmay-1 in both nematode infection and control. Although not too much high but significantly *M. javanica* infection has reduced LA within each water regime. Within both genotype as well as infection status, highest drought stress (20%) fashioned noteworthy difference in LA as compared to other two irrigation levels (Figure 6). This might be the resulted that 20% irrigation imposed such a high stress which crossed the maximum homeostatic ability of mungbean plants [41, 42].

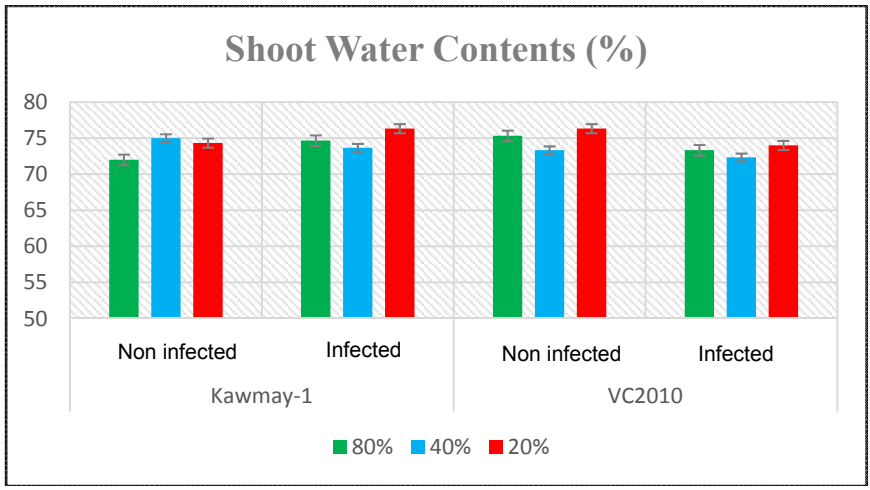

Figure 5: Shoot water contents affected by water deficit and *M. javanica* infection.

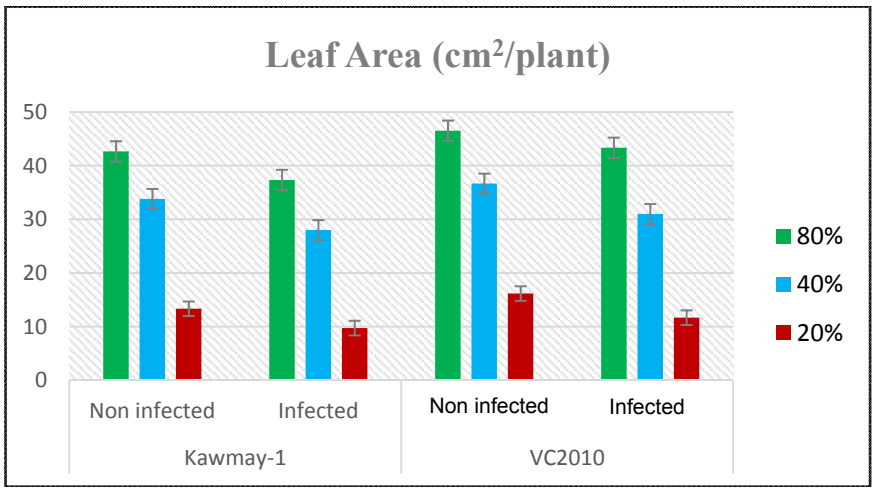

Figure 6: Leaf area affected by water deficit and *M. javanica* infection.

Plant response to unusual stresses is a complex of a multidisciplinary networked correlation among different plant processes and genetic combinations simultaneously. In the present study an overall correlation among observed parameters under drought and root knot nematode stresses have been summarized in Table 2 for two mungbean genotypes. Results revealed a positive and highly significant correlation among chlorophyll contents (a and b), SC, SRW and LA whereas SWC sustained a non-significant correlations with all other mentioned parameters.

Table 2: Correlation among studied parameters of mungbean genotypes under water deficit stress and *Meloidogyne javanica* infection.

Parameters	Chl a	SC	Chl b	SDW	SWC
SC	0.7529**				
Chl b	0.9363**	0.7520**			
SDW	0.8763**	0.8673**	0.8821**		
SWC	-0.1186^{NS}	-0.0923^{NS}	-0.1576^{NS}	-0.2232^{NS}	
LA	0.9092**	0.8174**	0.9409**	0.8877**	-0.1685^{NS}

*, and ** significant at $P \leq 0.05$, and $P \leq 0.01$, respectively. NS, not significant.

4 Conclusion

The present study revealed that mungbean can be successfully cultivated in the Saudi Arabian climate; however, deficit irrigation and *M. javanica* could be the serious limiting factors. Mungbean chlorophyll contents a and b, stomatal conductance, leaf area and shoot dry mass were significantly reduced by both stress conditions whereas, shoot water contents have not shown any alteration. Although drought-nematode coupled stress have reinforced the damage caused individually by the both stresses but genotypic differences were valuable to cope with drought and *M. javanica* stresses. A highly significant positive correlation among studied parameters were recorded except shoot water contents which stood non-significant in correlation with all other parameters.

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