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Moisture Stress Effects on the Relative Water Content, Stomatal Resistance, Transpiration Rate and the Yield of Mungbean at Different Growth Stages

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Abstract

The effects of soil moisture stress on the relative water content (RWC), stomatal resistance (RS), transpiration rate (TR) and the yield of mungbean (cv. MI-6) were studied during the vegetative, flowering and pod-filling stages in the greenhouse of the Department of Botany, Eastern University, Sri Lanka. This experiment was layed out in a Completely Randomized Design (CRD) with four treatments and five replications. Moisture stress was imposed for a period of 12 days by withholding water completely at once. The control plants were regularly watered to field capacity at four days interval. The RWC, RS and TR were measured on the 12^{th} day from the commencement of the stress at different growth stages. The RS was significantly ($P<0.05$) higher and the RWC and TR were significantly ($P<0.05$) lower in the stressed treatments than the control irrespective of the stages of growth. The increase in RS was due to stomatal closure resulted in decreased TR values. Decreased water content of the stressed plants would have reduced the RWC values. The relationship between RS and TR was assessed for the stressed and the control treatments on the 12^{th} day from the commencement of the

stress during the flowering stage. Large variations in the TR values for a narrow range of low RS values in the control treatment indicated the stress avoidance features of mungbean under well-watered condition. Persistence of low TR values for a wide range of high RS values in the water stressed treatment indicated the stress tolerance features of mungbean under water deficit situation. Hence, mungbean show stress avoidance and stress tolerance features under two different situations. Moisture stress significantly ($P<0.05$) reduced the yield of mungbean and the reduction was highest when the stress was imposed during the flowering stage. The next highest yield reduction was observed when the stress was given during the vegetative stage of the plants. The plants when subjected to moisture stress during the flowering stage would have lost large number of flowers. In addition, the size of the newly produced flowers also would have reduced. All these events would have contributed for the highest yield reduction during the flowering stage of these plants. A significantly high correlation ($r = 0.42^*$) was found between the RWC and the yield of mungbean. This indicated that the reduction in yield was attributed to reduced water content in these plants.

1.0 Introduction

Mungbean (*Vigna radiata* (L.) Wilezek) is one of the important pulse crops grown and consumed in Sri Lanka. Mungbean is a well known source of vegetable protein, some essential minerals and vitamins for human body. Its short duration, low water requirement, wide adaptability to fit into different crop rotations and varying cropping patterns can contribute to sustainability in increasing the farm productivity per unit area [1]. Moisture deficit limits the productivity of mungbean in Sri Lanka. The stress condition had the highest effect on the yield of mungbean; consequently, the current yield is significantly below the potential yield [2]. In order to face water deficit in coming years, research is needed to improve the water use efficiency of crops and identify drought tolerant varieties. Hence, research on plant responses to water deficit is vital.

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* Statistically significant at 5% level

Relative water content (RWC), stomatal resistance (RS) and transpiration rate (TR) are the indicators of plant response to water deficit. It has been shown that drought resistant crop varieties had higher RWC than susceptible ones under moisture stress condition [3]. Thus, RWC is a significant physiological parameter to evaluate plant responses to water deficit and it has close correlation with yield. TR is closely related to the amount of dry matter production [4]; whereas, RS is a key factor in controlling both photosynthesis and transpiration [5]. The objectives of this study were to assess the effects of moisture stress on RWC, RS, TR and yield of mungbean var. 'MI-6' at different stages of growth and to identify the most critical stage/s of growth of mungbean to moisture stress.

2.0 Materials and Methods

2.1 Location

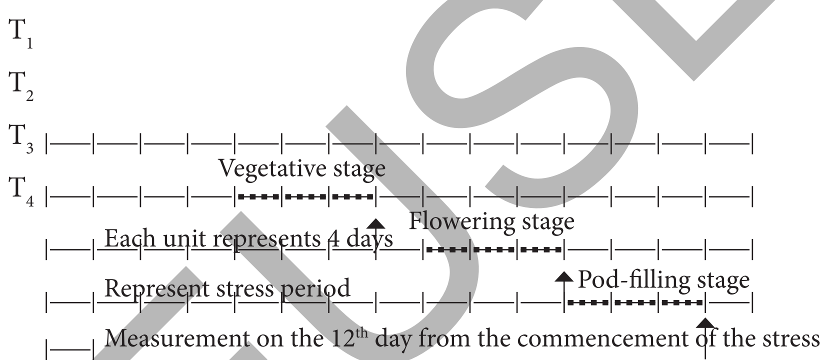
This experiment was conducted in the greenhouse of the Department of Botany, Eastern University, Sri Lanka from May to July 2006. The Altitude of the site is about 100m above mean sea level and it comes under the Agro-ecological zone of Low Country Dry Zone. The annual mean temperature varies from $28^{o}C$ to $32^{o}C$ and the humidity ranges from 60% to 90%. The mean annual rainfall of the district ranges from 1800mm to 2100mm. The average temperature and the relative humidity inside the green house were $34 \pm 1.2\ ^{o}C$ and 53 ± 1.8 % respectively during the experimental period.

2.2 Agronomic Practices

The experiment was conducted using polyethylene bags. Black polyethylene sheets (500gauge) were used to prepare the bags. Potting mixture was added in the ratio of sand: red soil: cow dung 1:1:1. The mungbean seeds (250g) were treated with Captan ($1g\ L^{-1}$) before dibbling in the bags. Three seeds were dibbled in each bag. Only the vigorous seedling was allowed to grow and the rest were removed 10 days after the emergence. The plants were arranged 30 cm × 10 cm apart [1]. Weeding was done manually at 10 days interval. Watering was done daily in the morning until germination and water was applied to Field Capacity at 4 days interval two week after sowing. The fertilizer was incorporated with the potting mixture before sowing as the basal dressing at the rate of Urea (35kg ha^{-1}), Triple Super Phosphate (100 kg ha^{-1}) and Muriate of Potash (75 kg ha^{-1}). Urea (30 kg ha^{-1}) was applied as the top dressing 30 days after sowing during the flowering stage [1].

2.3 Experimental Design

The treatments are shown in Fig. 1. They were arranged in a Completely Randomized Design (CRD) with 5 replications for each treatments. A number of 120 plants were used for the experiment and they were divided into 4 treatments. Moisture stress was applied by withholding water completely at once. The control plants were watered to Field Capacity at four days interval.



T_1 = Control – Regular watering to Field Capacity at 4 days interval

T_2 = 12 days moisture stress during the vegetative stage 16 days after sowing

T_3 = 12 days moisture stress during the flowering stage 32 days after sowing

T_4 = 12 days moisture stress during the pod-filling stage 44 days after sowing

Fig. 1: The diagrammatic representation of the manner in which moisture stress was imposed at different growth stages of mungbean

2.4 Treatment structure

The treatments were defined as follows: Treatment-1 (T_1) served as the control. Moisture stress for a period of 12 days was imposed for treatment-2 (T_2) during the vegetative stage. Treatment-3 (T_3), experienced moisture stress for a period of 12 days during the flowering stage. Moisture stress was applied for treatment-4 (T_4), for the same period of time during the pod-filling stage.

2.5 Measurements

The leaves which were matured most recently, i.e., the 3rd or 4th leaf from the apex was selected for the determination of physiological characteristics. The RWC, RS and TR were measured on the 12th day from the commencement of each stress cycle during the vegetative, flowering and pod-filling stages. The RS and TR were measured by a Portable Steady State Porometer (Model LI-1600, LICOR Inc, USA). The RS and TR were measured for the stressed, control and re-watered plants between 10.00 a.m. to noon when the Photosynthetically Active Radiation (PAR) was above the saturation PAR of 1500 $\mu\ Es^{-1}m^{-2}$.

A number of ten leaves representing ten plants were randomly collected from each treatment for the determination of RWC. Leaf samples were kept inside the polyethylene bags to avoid evaporation. Similar sized discs (1 cm diameter) were obtained from the leaves using a cork borer and their fresh weights (FW) were recorded soon after collection. These discs were placed over night in a beaker containing distilled water to obtain turgid weight (TW). This beaker was placed inside a refrigerator during darkness to avoid physiological deterioration of leaf cells. The leaf discs were blotted with filter paper to dryness and their TW was recorded. The leaf discs were then placed in an oven at 60 ^{0}C for 24 hours and their dry weights (DW) were recorded. The RWC was estimated using the following equation:

$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

The matured pods were harvested in two pickings. The first picking was done on the 60th day after sowing and the second one was made on the 70th day after sowing. The pods were collected from different treatments and were pooled together. All the measured data collected from the experimental plants were subjected to statistical analysis.

3.0 Results and Discussion

3.1 Relative Water Content (RWC)

It was found that there were significant differences ($P<0.05$) in the RWC values of plants between the stressed and the control treatments during the vegetative, flowering and pod-filling stages (Table 1). In the treatments where the stress cycle was experienced by plants during the above growth stages, the RWC on the 12th day from the commencement of the stress was significantly lower ($P<0.05$) than the control values. Moisture stress reduced the RWC of mungbean. As soil water content decreases, plant water content also decreases followed by a reduction in the leaf water content. Hence, water loss from the stressed plants decreased the RWC of mungbean leaves. In the regularly watered plants, leaves were turgid therefore, RWC remained high.

Table 1: The effects of soil moisture stress on the Relative Water Content (RWC) of mungbean at different growth stages

Treatments	**Relative Water Content (%)**		
	Vegetative	**Flowering**	**Pod-filling**
T_1	83.93 a	84.03 a	81.65 a
T_2	**59.67 b**	82.12 b	81.78 a
T_3	83.41 a	**54.03 c**	82.00 a
T_4	83.38 a	83.68 a	**50.15 b**

(Values in the same column followed by the same letter do not differ significantly ($P<0.05$). Values are the means of 20 plants in 5 replicates.)

When comparing the growth stages, in the treatment where the moisture stress was experienced by plants during the vegetative stage (T_2), RWC showed the highest value compared to the other treatments. Plants when young do not possess well differentiated tissues and cells are mostly filled with water. Hence, water filled tissues would have existed in large numbers during the vegetative stage. As such, this stage showed the highest RWC. The RWC was lowest in the treatment where moisture stress was experienced by plants during the pod-filling stage (T_4). This may be because, in the matured plant tissues, cells are highly differentiated and hence, water content would have been very much low. Matured plants when subjected to moisture stress, would have accumulated high amounts of secondary metabolites. In order to keep osmotic balance, specific types of organic molecules such as soluble sugars, betaines, polyols, proline etc. are accumulated in the cytoplasm [6]. The rate of accumulation of compatible solutes would have increased with maturity. This also would have contributed for the occurrence of low RWC during the pod-filling stage.

3.2 Stomatal Resistance (RS)

It was observed that there were significant differences ($P<0.05$) in the RS values of plants between the stressed and the control treatments during the vegetative, flowering and pod-filling stages of the crop (Table 2). In the treatments where the stress cycle was experienced by plants during the above growth stages, the RS on the 12th day from the commencement of the stress was significantly higher ($P<0.05$) than the control values. Moisture stress increased the RS of plants. Changes in the soil water status result in changes in root water status. Signals from drying root cells are transmitted to leaf cells and cause increase in RS in the guard cells. Stomatal closure, which results from soil water depletion, is mediated by changes in root water status through effects on the flow of signal from root to shoot [7].

Table 2: The effects of soil moisture deficit stress on the Stomatal Resistance (RS) of mungbean at different growth stages

Treatments	**Stomatal resistance (s cm^{-1})**		
	Vegetative	**Flowering**	**Pod-filling**
T_1	0.91 b	0.98 b	1.02 b
T_2	**10.98 a**	0.99 b	1.05 b
T_3	0.89 b	**14.01 a**	1.02 b
T_4	0.88 b	0.98 b	**14.80 a**

(Values in the same column followed by the same letter do not differ significantly (($P<0.05$). Values are the means of 20 plants in 5 replicates.)

Stomatal closure due to low leaf water status is also based on the changes in turgor balance between guard cells and surrounding epidermal cells. The degree of opening of stomatal pore depends on the turgor of guard cells. When they are turgid, the pore between them is large and RS becomes low. When they lose turgor, the pore decreases in size and RS would increase. Changes in guard cell turgor were attributed to changes in plant water status. The turgor difference occurs because of differential loss of water as a result of difference in osmotic potential between these two types of cells [8]. For stomata to open substantially, osmotic potential must be much more negative and solutes much more concentrated in guard cells. Conversely, for stomata to close as a consequence of water stress, the turgor difference and hence, solute difference between guard cells and subsidiary cells must be markedly reduced. Hence, stress apparently triggers the efflux of solutes from guard cells. Starch is implicated as a potential source of solute in stomatal opening. Reduction in leaf starch is a general phenomenon with water stress [9]. It is because of reduced level of carbon fixation by leaves.

Among the three growth stages, the pod-filling stage showed the highest RS value. In matured plant tissues, water content would be lower than that of the young ones. During the pod-filling stage, need for water is very much high, because ample amount of water is needed for photosynthesis, translocation of assimilates for the reproductive organs and for the formation of pods. Hence, in order to conserve the available water under stressed conditions, stomata would have exerted greater RS during the pod-filling stage. Age is another factor in determining the RS values of plants. As these plants were aged during the pod-filling stage, they showed high RS values than the plants of the younger ages. This phenomenon also would have contributed for the increase in RS during the pod-filling stage.

3.3 Transpiration Rate (TR)

It was found that there were significant differences ($P<0.05$) in the TR values of plants between the stressed and the control treatments during the vegetative, flowering and pod filling stages (Table 3). In the treatments where the stress cycles were experienced by plants during the above growth stages, the TR values on the 12th day from the commencement of the stress were significantly lower (P<0.05) than the control treatments. Moisture stress reduced the TR of mungbean. Reduction in TR would have been due to increased RS. There was an increase in RS when the stress was imposed during the three growth stages. Reduced water content during the stress period also would have caused reduction in the TR values of these plants. Stomatal movement was found to exert a large controlling influence on the TR. The plants stressed during the vegetative and flowering stages showed higher TR values than those during the pod-filling stage. This may be due to low RS values observed in the vegetative and flowering stages than the pod-filling stage.

Table 3: The effects of moisture deficit stress on the Transpiration Rate (TR) of mungbean at different growth stages

Treatments	Transpiration Rate (μg cm^{-2} s^{-1})		
	Vegetative	**Flowering**	**Pod-filling**
T_1	33.0 a	31.0 a	27.7 a
T_2	**11.2 b**	30.0 a	26.5 a
T_3	34.6 a	**10.5 b**	25.2 a
T_4	32.9 a	31.1 a	**9.9 b**

(Values in the same column followed by the same letter do not differ significantly (P<0.05). Values are the means of 20 plants in 5 replicates.)

3.4 The Relationship Between Stomatal Resistance (RS) and Transpiration Rate (TR)

The relationship between RS and TR was assessed for the control and stressed treatments during the flowering stage of mungbean by plotting graphs using the RS and TR values obtained from the experimental plants. These measurements were done on the 12^{th} day from the commencement of the stress. It was found that the relationship was negative for the control and stress treatments (Fig. 2). The RS values increased with a decrease in TR values. Stomatal closure is accompanied by reduced transpiration rate. The TR values of the control plants were highly responsive to a narrow range of low RS values. This shows moisture conserving ability of mungbean and it is a drought avoiding mechanism of these plants. The TR values were very low during the moisture stress treatment. They showed low sensitivity to a wide range of high RS indicating the low responsiveness of TR to RS under water stress situation. From the above observation it could be stated that this variety of mungbean shows stress tolerance and stress avoidance features under two different situations. The stress tolerance features could be used for crop improvement programmes in the dry zone of Sri Lanka where severe water shortage occurs for a substantial period of time.

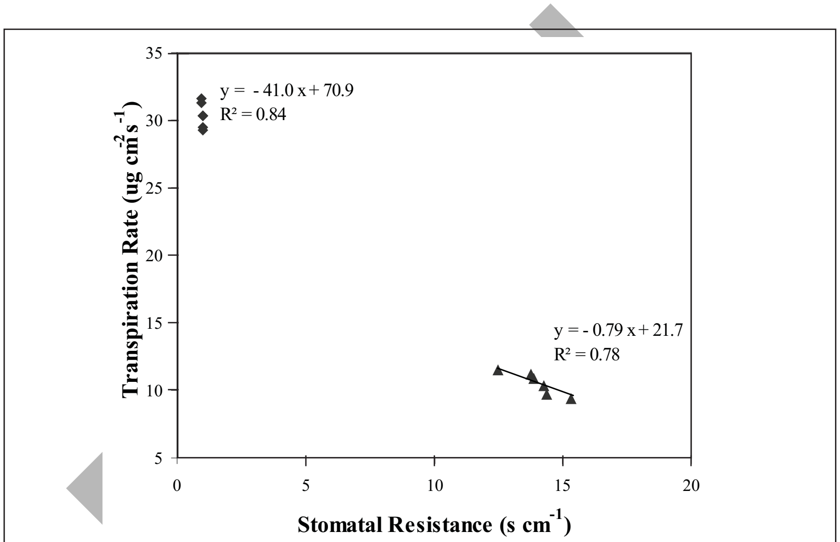


Fig. 2: The relationship between the Stomatal Resistance (RS) and Transpiration Rate (TR) of mungbean var. 'MI-6' during the flowering stage [◆-Control plants, ▲-Stressed plants]

3.5 Yield

It was observed that there were significant differences ($P<0.05$) in the yield of mungbean between treatments (Fig. 3). The highest yield reduction was observed when the stress was imposed during the flowering stage of mungbean. The plants stressed during the vegetative stage showed the next highest yield reduction. During the flowering stage, ample water is required for cell divisions and for the development of reproductive structures. Limitation of water at this stage not only reduces flower formation but also increases flower shedding. The plants when subjected to moisture stress during the flowering stage, shedded large number of flowers. Moreover, the size of the newly produced flowers also were reduced. Decreased number of new flowers also appeared during the stress period. There is a positive relationship between the number of flowers persisted in a plant and the final yield. Reduction in flower number, reduces the amount of final yield. This may be the reason for the highest reduction in yield when the stress was imposed during the flowering stage of these plants. Legumes are drought sensitive crops particularly during the flowering stage [10]. Stress during the vegetative stage would have reduced the amounts of photosynthates due to reduction in leaf area. This inturn would have reduced the translocation of photosynthates into the reproductive structures. This may be the reason for the next highest yield reduction during the vegetative stage.

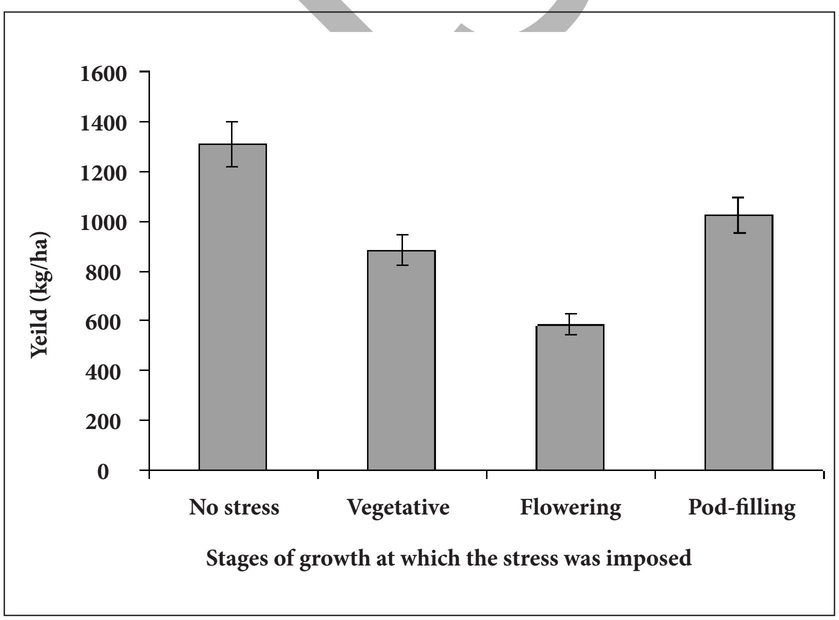


Fig. 3: The effects of moisture stress at different growth stages on the yield of mungbean var. 'MI-6'

In this study, a significantly high correlation was observed between the RWC and the yield (r = 0.42*) than the RS (r = -0.38*) and TR (r = 0.27*). RWC is the appropriate measure of plant water status in terms of physiological consequence of cellular water deficit [11]. RWC is positively correlated with the amount of flower formation in the plants. Maintenance of high RWC is important to maintain high turgor. Turgor maintenance is important for the differentiation of flower primordia and flower initiation. Reduction in RWC during the flowering stage would have reduced the flower formation and increased the flower dropping. When the plants were exposed to moisture stress at the flowering stage, a severe flower drop was noticed. This limitation resulted in low pod set which reduced the number of pods per plant and ultimately reduced the final yield. Hence, a high correlation was observed between the RWC and the yield. Strong correlation might be found between the yield and the RWC if plants were stressed, thus, generating wide range of RWC values [12].

4.0 Conclusions

This experiment determined the extent to what, RWC, RS, TR and yield were affected when mungbean plants were subjected to moisture stress at different growth stages. The flowering stage was found to be the most critical growth stage and water stress during this stage showed the highest yield reduction. Severe flower drop was noticed when the stress was imposed during this stage. A highest correlation was found between the RWC and the yield. Reduction in RWC reduces cell wall turgor and affects normal plant processes, thereby affecting the ultimate yield. This study also revealed the stress avoidance and stress tolerance features of mungbean under two different situations. The stress tolerance characteristics could be used for crop improvement programmes in the dry zone of Sri Lanka.

* Statistically significant at 5% level.

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