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Full Length Research Paper

Effect of leaf area on dry matter production in aerated mung bean seed

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An area of plant science that is still unexplored is how leaf area affects crop dry matter production due to aeration of seed in osmoticum. In view of this, an experiment was conducted at Agricultural Research Farm of NWFP Agricultural University, Peshawar, Pakistan in the summer of 2003 and repeated in 2004. The seeds of two mung bean cultivars (NM-92 and NM- 98) were primed, some for 6 h and others for 12 h in using either distilled water (0MPa osmotic potential) or Polyethylene glycol-8000 (PEG) solution having -0.2, -0.5 and -1.2 MPa osmotic potential. A control treatment (dried seeds) was also included in the experiment. The primed seed were dried back, till the weight become constant and were store for sowing at 25°C. Data was collected on mung bean leaf area, dry matter production and growth parameters at different growth stages. Seed moisture content at maturity stage was also determined. There was no significant difference in leaf area for the different cultivars and seed treatment duration also did not lead to a significant difference in leaf area. However, seed priming techniques significantly affected the measured parameters. Dried seed had developed lower leaf area and dry matter compared to primed seeds. An exponential linear model of leaf area and total dry matter revealed that dry matter production was linearly related to leaf area (r^2 = 77.23). The linear relationship between the leaf area and dry matter hold true our hypothesis and thus we concluded that beside environmental and genetical factors, the dry matter production is a function of leaf area in aerated seed of mung bean crop in semi-arid areas like North western Pakistan.

Key words: Mung bean, priming, growth, dry matter and leaf area.

INTRODUCTION

Leaf area *(L)* of a crop is an important variable in models for predicting crop growth and dry matter production, quantifying crop- weed competition, or modeling heat, energy and water exchanges in the plant soil-atmosphere continuum. Leaf area and vertical *L* profile influence the interception and utilization of solar radiation of crop and consequently, the dry matter production (Boote et al., 1998). Penning de Vries et al. (1989) predicted leaf area from leaf biomass using the parameter specific leaf area, assuming that leaf area is limited only by assimilate or carbon supply. The daily increase in *L* (ΔL) is calculated *first as a function of temperature up to a specific L and then in proportion to the increment of leaf biomass* (Goudriaan and Van Laar, 1994). Van-Delden et al. (2001) evaluated this two-phase approach and found that it described field-observed *L* better than when based solely on carbon supply or temperature.

Variable rainfalls in early summer leading to poor crop establishment, coupled with periods of high temperature are major production limitations of mung bean dry matter production in Peshawar, Pakistan. The growing season is limited by variable rainfall and temperature at both ends. Extreme soil temperature which causes drought by evaporating soil moisture at planting, coupled with low rainfall, reduce the germination and early vigor. In addition to these the relatively low temperature during the latter part of grain filling limits dry matter potential. Priming, a technology that enhances early emergence and stand establishment would enable the crop capture more soil moisture, nutrients and solar radiation and thus increase dry matter production. Rapid and uniform field

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emergence is an essential pre-requisite to reach the yield potential, quality and ultimately obtain profit from annual crops.

Priming, which is a pre-sowing hydration treatment with the objectives to allow water uptake and germination metabolism to proceed to a point just short of radical extension (Bradford, 1986), has been used to enhance seed germination response and subsequently dry matter production. Heydecker and Gibbins (1973) defined seed priming as a pre-sowing treatment in osmotic solution that allows seeds to imbibe water to proceed to the first stage of germination but prevents radical protrusion through the seed coat. It is seen as a viable technology to enhance rapid and uniform emergence, high vigor and better yields mostly in vegetable and flower species (Dearman et al., 1987; Parera and Cantliffe, 1994; Bruggink et al., 1999), some field crops (Basra et al., 1988; Hartz and Caprile, 1995; Harris et al., 1999; Chiu et al., 2002) and in soybean (Khalil et al., 2001).

Seed priming has been a common seed treatment to reduce the time between seed sowing and seedling emergence and the synchronization of emergence (Parera and Cantliffe, 1994). Priming of seed prior to sowing has a key role in improving the crop growth during seedling emergence and consequently affects the crop leaf area. Establishment of primed seed is more successful if rain fell or if the soil dried out slowly after sowing. Consequently, both stand count and dry matter are directly proportional to the rate at which seedlings emerge (Harris, 1996).

The rate of dry matter accumulation is a function of leaf area of a crop, because the light interception is mainly associated with leaf area. In fact, the effect of priming on leaf area of a canopy growth of agricultural crops is well understood. The withdrawal of nutrients due to enhanced early and post emergence growth seems to be inevitable so as to support seed filling and may meet the high nitrogen demand required to build seed proteins (Sinclair and de Wit, 1975). If the nutrients are withdrawn by the leaves of a crop at latter stage, this may cause leaf senescence and crop *L* declines accordingly, a phenomenon described by Sinclair and de Wit (1975) as 'self destruction'.

The objective of this paper is to provide a quantitative framework for determining the *L* in relation to primed seed and its effect on the dry matter production. It will cover whole picture of the *L* and priming seed relationship, covering canopies in the early, fully developed and in-between transitional phases of development and subsequently on the growth dynamics of mung bean crop not done.

METHODS AND MATERIALS

Experimental location

An experiment was carried out in the summers of 2003 and 2004 at the Agricultural Research Farm, NWFP Agricultural University Peshawar, Pakistan (17°, 35´ N and 35°, 41´ W). The soil of the experimental farm was a silt clay loam, well drained, fine textured soil. The experimental site has warm to hot, semi -arid subtropical continental climate with a mean annual rainfall of about 360 mm. The soil is deficient in nitrogen (N) and phosphorus (P), but has adequate potassium (K) with a pH of 8.2 and organic matter content less than 1% (Khan et al., 2008)

Materials and treatments

The seeds of two mung bean cultivars NM-92 and NM-98, obtained from Agricultural Research station NWFP Agricultural University Peshawar and ARI Rataculachi Dera Ismail Khan (DIK) NWFP, respectively were primed for 6 and 12 h in three levels of polyethylene glycol-8000 (PEG) that is, 100, 200, 300 g PEG liter $^{-1}$ distilled water and distilled water of osmotic potential equivalent to -0.2, -0.5, - 1.1 and 0 MPa, respectively (Michel, 1983). Two control treatments were un-soaked seeds for each cultivar. A total of 20 treatments (2 varieties × 2 seed treatment durations × 5 priming treatments) were evaluated in a randomized complete block design with 4 replications.

The concentration used for treatments were based on previous research work. Except for the control treatment, 18 g (> 360 seeds) of each cultivar for all these 3 levels of PEG and distilled water alone were primed in 200 ml solution of PEG for 6 and 12 h in conical flasks. An aquarium pump was used for continuous oxygen supply. The primed seeds were then allowed to air-dry at room temperature (25°C) till constant weight.

Planting was done on 17th May in 2003 and 29th May in 2004. A total of 360 seeds of each cultivar for each treatment including control were sown in plot of 3 × 1.8 m size with 3 m length and rows 30 cm apart. After germination, manual thinning was done in order to maintain a 10 cm interplant distance in all 6 rows of each plot. Nitrogen and P fertilizers as starter dose at the rate of 20 kg N ha^{-1} as Urea (46% N) and 60 kg P_2O_5 ha^{-1} as single super phosphate (18% P_2O_5) were applied during seed bed preparation. Weeds were controlled manually as well as by application of chemical herbicides (Premixtra). Irrigation was done based on past history and crop demand, that is, approximately applied at 15 days interval mostly for the whole period of the experiment that is, about 80 days.

Observations and measurements

Daily rainfall and temperature data were obtained from the weather station Peshawar, which is a short distance from the experimental site, and summarized in Figure 1. Sampling of whole plants (above ground) from 0.5 m long rows in each plot was made at 15 days interval starting after 30 days of sowing until crops matured physiologically. The plants were separated into leaf lamina, stem and pods (only in last growth sampling). Total leaf area of all the leaves lamina, after removal from the plants, was recorded using leaf area meter (LI-COR model 3100A). The components were oven dried at 65°C till constant weight (24±1hours). All components of plants including leaves lamina, stems, and pods were separately weighed with the help of balance (A&D GR120-EC 120g x 0.1mg). The method of growth analysis used in this trial involved the calculation of various mean rate changes in plant weights (W_2 and W_1) observed at two sampling periods, that is, t_2 and t_1 (Radford, 1967) as follows:

$$AGR = \frac{W_2 - W_1}{t_2 - t_1}$$

Absolute growth rate (AGR),

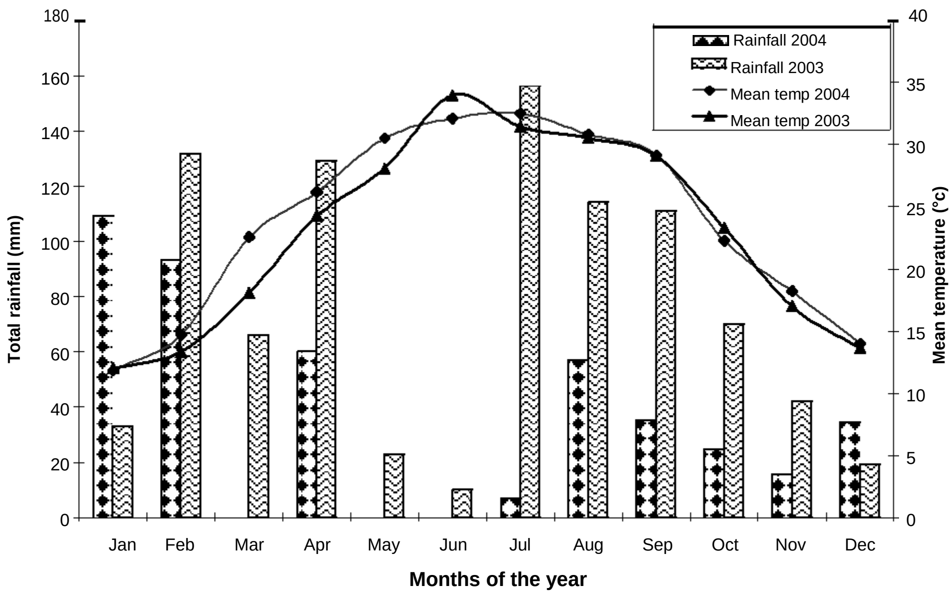

Figure 1. Total precipitation (mm) and mean air temperature (°C) at the experiment site in Peshawar during 2003 and 2004.

Crop growth rate (CGR), $$CGR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{1}{GA}$$

Relative growth rate (RGR), $$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Net assimilation rate (NAR), $$NAR = \frac{W_2 - W_1}{t_2 - t_1} \times leaf.area$$

Seed moisture content was calculated as:

$$\text{Moisture \%} = \frac{\text{fresh weight of the seed - dried weight of the seed}}{\text{dried weight of the seed}}$$

Statistical analysis

The experimental data was subject to the analysis of variance appropriate to RCB design using GenState Release 8.1 (GenState, 2005). Treatments mean were separated by standard error of means (Gomaze and Gomaze, 1980). Regression analysis was carried out to determine the relationship between leaf area and dry matter production.

RESULTS

The mean values of temperature and rainfall during the crop cycle are shown in Figure 1. Mean air temperature was 31.5°C, however the rainfall was below 30 mm during emergence and early crop growth. In spite of these harsh climatic conditions, the crop emergence was successful, due to seed priming technique. Past history of the region revealed a drought and warm climatic conditions, similar to the current growing session Khan et al. (2008). During the latter growth stage, that is, after pod initiation, the severe rainfall might have affected the crop growth performance.

On average, the plant took 40 and 45 days to get to the vegetative stage and had fixed reproductive period in 29 and 32 days, in NM-92 and NM-98 variety, respectively.

Dry matter production

Effects of mung bean cultivars

No significant difference in leaf area, stem and total dry matter production, at any stage of crop cycle, was attained by mung bean cultivars (Figure 2), suggesting that cultivars performed in similar fashion for dry matter accumulation. Maximum leaf lamina dry weights (27 g) were reached at (45 days) and thereafter reduction was observed. Stem (Figure 2b) and total (Figure 2c) dry matter were adequately described by a linear exponential functions, across the growth stages (r^2 = 0.97 for stem and 0.99 for total dry matter production across the two cultivars). The leaf dry matter continued to increase more rapidly till flowering (reproductive stage) compared to stem dry matter.

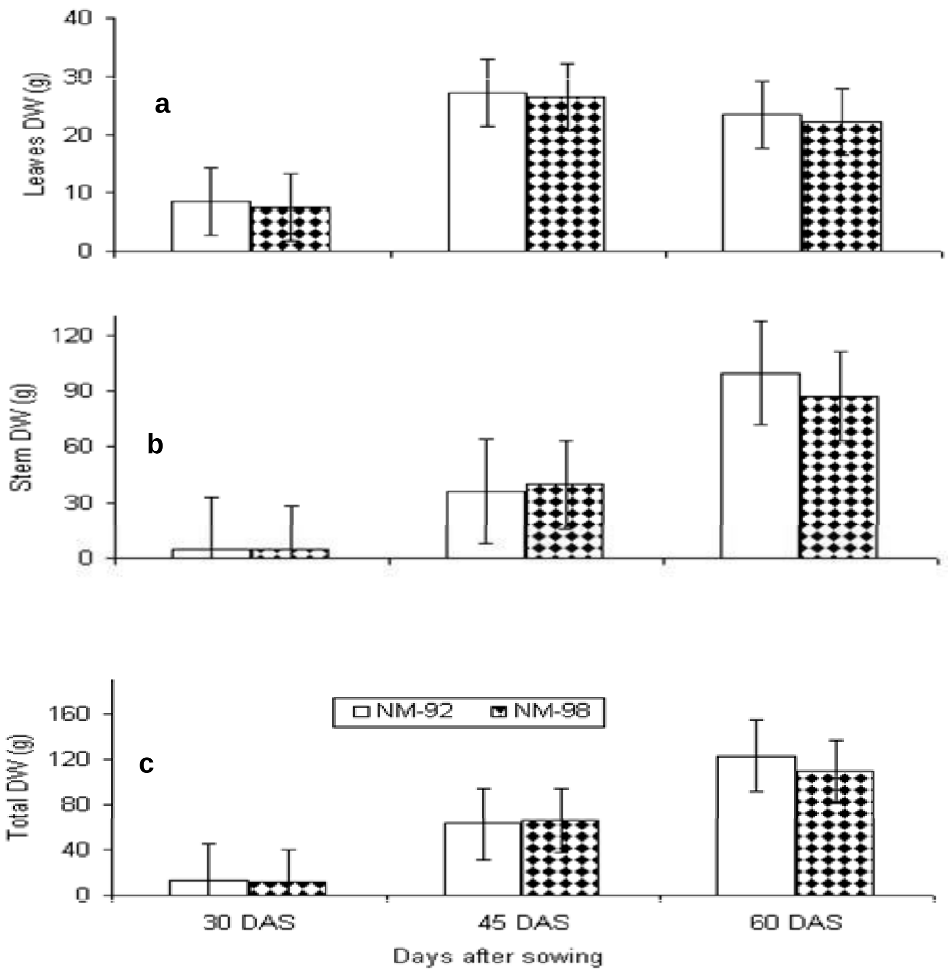

Figure 2. Production of dry matter as affected by cultivars used. a) leaves dry matter production, b) stem dry matter production; and c) total dry matter production. The vertical bars are the standard error of means.

Effects of seed treatment durations

Seed treatment duration, had little or no effect on either leaf, stem or total above ground dry matter production (Figures 3 a, b and c). The effect of priming duration would be the main factor affecting rate and end point of imbibition when primed in water, but would have less effect when osmotic solutions are used as priming source.

Effects of priming treatments

Priming the seed had affected dry matter accumulation during the different phases of the mung bean growth (Figure 4). Leaf dry matter production (Figure 4a) showed a quadratic exponential response that is, higher at 45 days after sowing and then decreased with the passage of time.

In contrast to leaf dry matter production, stem (Figure 4b) or total dry matter production showed a linear increase. More than 80% of total dry matter (Figure 4c) was accumulated in the last stage of mung bean growth. Setting of pods had exerted a significant effect on the amount of total dry matter production.

Seeds primed in PEG solution of -0.2 and -0.5 or only hydro-primed seeds accumulated significantly higher stem dry matter (Figure 4b) and total dry matter (Figure 4c) as compared to control seed or seed primed in higher osmotic potential (-1.2MPa) of PEG solution.

Effect of mung bean cultivar, seed treatment duration, and priming treatments on plant growth

Absolute growth rate, the dry matter production per day as shown in (Table 1), was higher (0.8 g day^{-1}) after flowering for mung bean cultivar NM-92, in the same

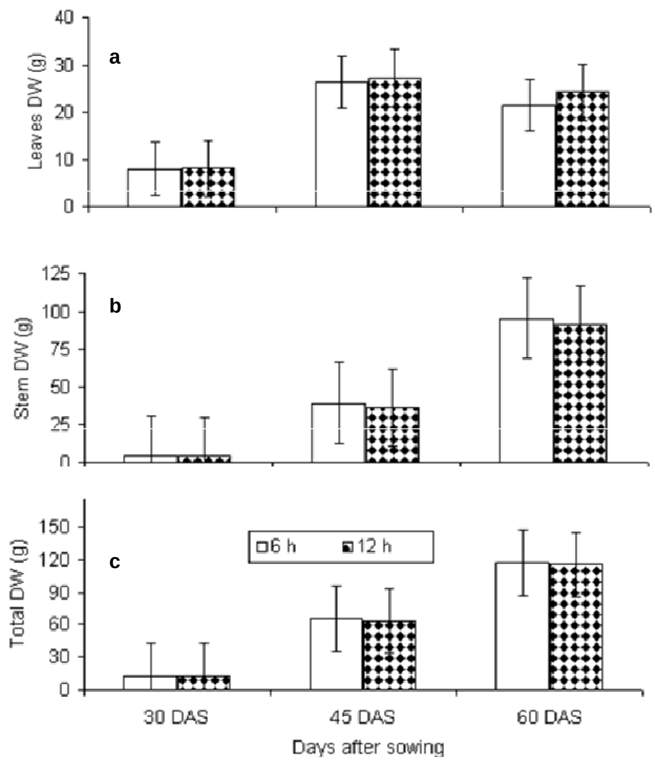

Figure 3. Production of dry matter as affected by seed treatment duration a) leaves dry matter production, b) stem dry matter production, and c) total dry matter production. The vertical bars are the standard error of means.

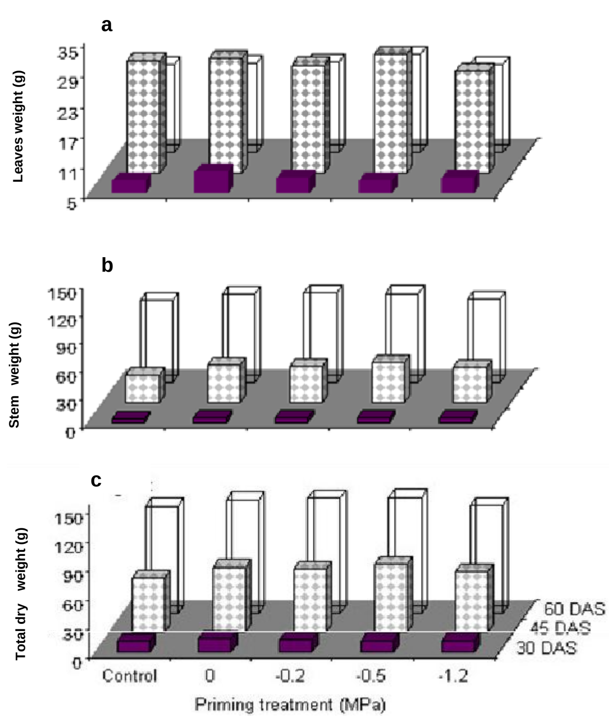

Figure 4. Production of dry matter as affected by different priming treatments a) leaves dry matter production, b) stem dry matter production, and c) total dry matter production.

Table 1. Growth analysis of mung bean in relation to two different cultivar of mung bean.

Studied variable	Mung bean varieties		S.E (57df). Sig
	NM-92	NM-98	
Before flowering			
AGR (g day^{-1})	0.67	0.72	0.023^{NS}
CGR (g day^{-1} m^{-2})	4.44	4.81	0.152^{NS}
RGR (mg g^{-1} Dry matter)	0.07	0.02	0.348^{NS}
NAR (mg cm^{-2})	50.21	60.46	4.01*
After flowering			
AGR (g day^{-1})	0.80	0.58	0.058^{NS}
CGR (g day^{-1} m^{-2})	5.33	3.84	0.389*
RGR (mg g^{-1} Dry matter)	8.86	6.56	2.83^{NS}
NAR (mg cm^{-2})	15.29	10.98	1.24^{NS}
Moisture (%) of the grains	4.59	6.66	0.299**

S.E = Standard error of means. *Significant at 5% level of probability **Significant at 1% level of probability. NS = Non-Significant.

Table 2. Growth analysis of mung bean as affected by the seed treatment duration.

Studied variable	Seed treatment duration		S.E (57df).Sig
	6 h	12 h	
Before flowering			
AGR (g day^{-1})	0.71	0.67	0.023^{NS}
CGR (g day^{-1} m^{-2})	4.76	4.50	0.152^{NS}
RGR (mg g^{-1} Dry matter)	0.07	0.02	0.348^{NS}
NAR (mg cm^{-2})	59.69	50.99	2.83*
After flowering			
AGR (g day^{-1})	0.68	0.70	0.058**
CGR (g day^{-1} m^{-2})	4.53	4.64	0.389^{NS}
RGR (mg g^{-1} Dry matter)	7.41	8.00	0.587*
NAR (mg cm^{-2})	12.82	13.45	1.24*
Moisture (%) of the grains	6.20	5.06	0.299**

S.E = Standard error of means.* Significant at 5% level of probability. **Significant at 1% level of probability. NS = Non-Significant.

period than for NM-98 (0.58 g day^{-1}). However, the differences were not statistically significant. Before flowering the opposite was observed. This may imply that NM-98 cultivar is late maturing. Significantly higher CGR (5.33 g day^{-1} m^{-2}) was achieved with mung bean cultivar NM-92 after the pods initiation as compared to (3.84 g day^{-1} m^{-2}) cultivar NM-98 (Table 1). Crop growth rate before flowering was not significantly different for the cultivars. The RGR, significantly increased from 50.21 mg g^{-1}DM for NM-92 to 60.46 mg g^{-1}DM for NM- 98, before the start of reproductive stage. Moreover, a reduction from 15.29 to 10.98 mg g^{-1}DM was recorded for cultivar NM-92 and NM-98 respectively, after the start of flowering, respectively (Table 1). This was however not significant. Higher NAR was recorded for cultivar NM-98 as compared to NM-92 both before and after flowering. Before the (reproductive stage), the NAR production by NM- 98 was significantly higher (60.46 mg $day^{-1}cm^{-2}$) than was obtained for NM-92(50.29 mg $day^{-1}cm^{-2}$) . The higher NAR and delayed maturity, had resulted in significantly higher moisture in NM-98 (6.66 %) as compared to NM-92 (4.59%).

Seed treatment duration has significant effects on NAR and seed moisture content before the reproductive period (Table 2). A significantly higher NAR (59.69 mg day^{-1} cm^{-2}) was recorded for 6 h seed treatment duration as

Table 3. Growth analysis of mung bean as affected by different osmo-primed and hydro-priming treatments.

Studied variable	Priming treatment ($_o$ in MPa)					S.E (57df).Sig
	Control	0	-0.2	-0.5	-1.2	
Before flowering						
AGR (g day^{-1})	0.60	0.71	0.70	0.78	0.67	0.036*
CGR (g day^{-1} m^{-2})	4.01	4.75	4.66	5.23	4.48	0.240*
RGR (mg g^{-1} Dry matter)	0.14	0.02	0.02	0.02	0.02	0.055 NS
NAR (mg cm^{-2})	44.50	52.26	53.73	66.72	59.48	4.48*
After flowering						
AGR (g day^{-1})	0.73	0.67	0.73	0.66	0.66	0.092 NS
CGR (g day^{-1} m^{-2})	4.86	4.46	4.84	4.39	4.37	0.614 NS
RGR (mg g^{-1} Dry matter)	9.06	7.32	8.07	6.65	7.43	0.929 NS
NAR (mg cm^{-2})	13.97	12.40	14.22	11.74	13.35	1.77 NS
Moisture (%) of the grains	4.38	7.17	4.63	6.17	5.79	0.492**

S.E = Standard error of means. *Significant at 5% level of probability. **Significant at 1% level of probability. NS = Non-Significant. $_o$ = Osmotic potential. MPa = Mega Pascal.

compared to that produced with 12 h (50.99 mg $day^{-1}cm^{-2}$). The moisture percentage was higher for 6 h treatment (6.2%), than 12 h seed treatment (5.06 %).

The plants from primed seed had a greater growth rate and CGR as compared to the control (Table 3) before the start of reproductive stage. The relative growth rate was not statistically different for the same period. Primed seed in PEG solution either in -0.2 or - 0.5 MPa osmotic potential had a better AGR, CGR, and NAR, before the start of reproductive stage, as compared to control (dried seed) or seed primed in -1.5 MPa osmotic potential of PEG solution, but these results were also not different from the hydro-primed seed treatment. In case of moisture content of the seed more moisture (7.17%) was recorded for hydro-primed seed as compared to dried seed (4.38%).

Relationships between leaf area and dry matter

Individual analyses of leaf area of each factor are presented in (Figure 5). Both cultivars attained similar leaf area at each growth stage. Leaf area increased significantly from one growth stage to the other. Similar results were obtained for seed treatment duration (Figure 5a). Seed primed either in PEG solution of -0.2 or -0.5 MPa osmotic potential, or in only water of 0 MPa osmotic potential performed significantly better in attaining the average leaf area, when compared to the dried seed or seed primed in the solution of PEG of higher concentration, that is, -1.2 MPa osmotic potential at each crop growth stage. The increment in leaf area occurred when crop proceeded from one growth stage to another (Figure 5b). No significant interaction was found in cultivar-duration-priming treatments, and thus main factors influencing the leaf area, might be either due to the vigorous growth of plant or the priming technique.

Total mung bean productivity either primed or not, was linearly related to average leaf area present in the mung bean over the season, however the linearity was higher in primed seed than non primed seed (Figure 6). Furthermore, the regression between leaf area and dry matter was significant, when the data was pooled for both priming techniques, duration and years for both mung bean cultivars. Therefore, mung bean cultivars seed, after priming, when planted presented a high variability. This allows us to explain the amount of dry matter formed inclusively from leaf area development for each plant. The total variations caused by leaf area to total dry matter production were 75.22%. The negative regression slope if extended suggests an incredible reduction in dry matter production at the lower leaf area (Figure 6).

DISCUSSION

The leave lamina dry weight behave in sigmoid manner, as maximum values were reached at (45 days), and thereafter decrease, which might be associated to the translocation of assimilates to the grains, the senescence and the leaf fall. This may be presumed that early growth, before the start of reproductive stage, would increase leaf area, resulting in higher interception of sun light and ultimately more dry matter. The stem and total dry matter accumulation showed a linear function, which could be associated to the continuous building of dry matter. Optimum photosynthetic activity during growing season having optimum temperature for mung bean cultivars (Malik, 1994) had no significant effects on total dry matter production. But across the growth stages, the

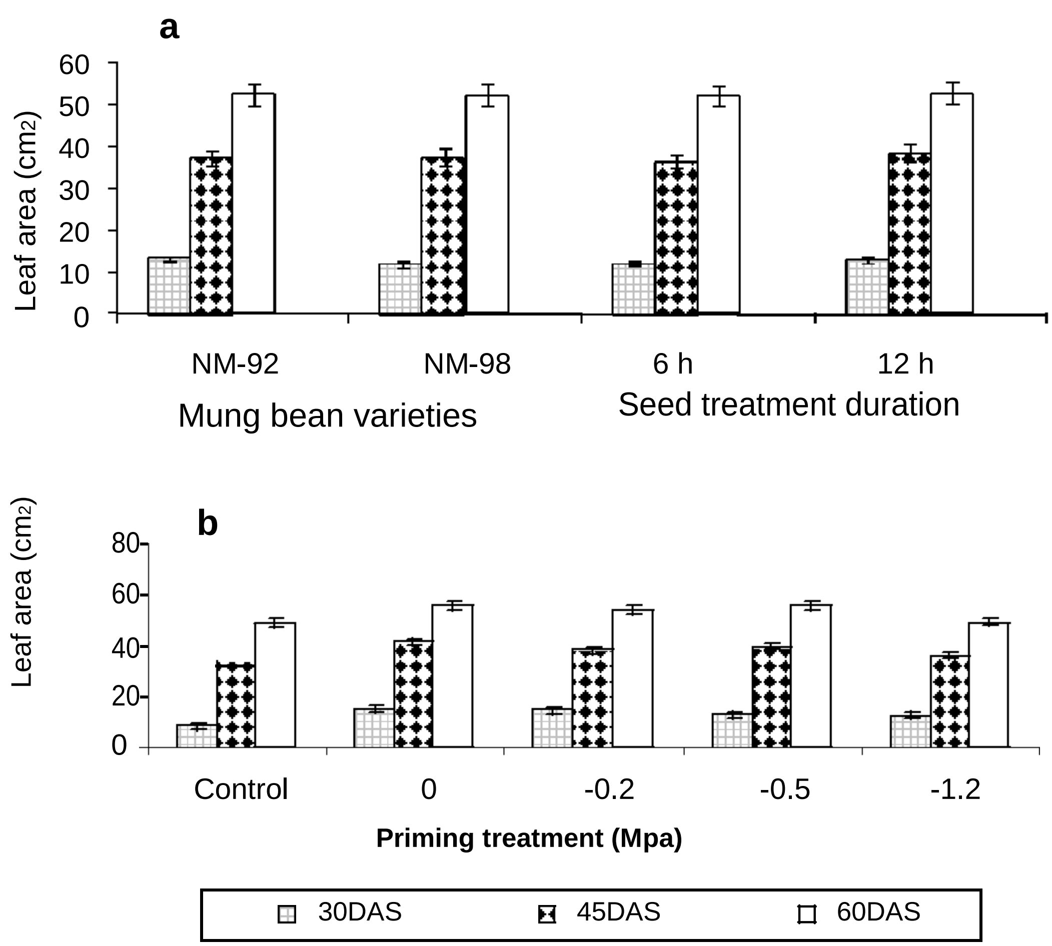

Figure 5. Leaf area (cm^2) of mung bean as affected by cultivars, seed treatment duration, and priming treatments.

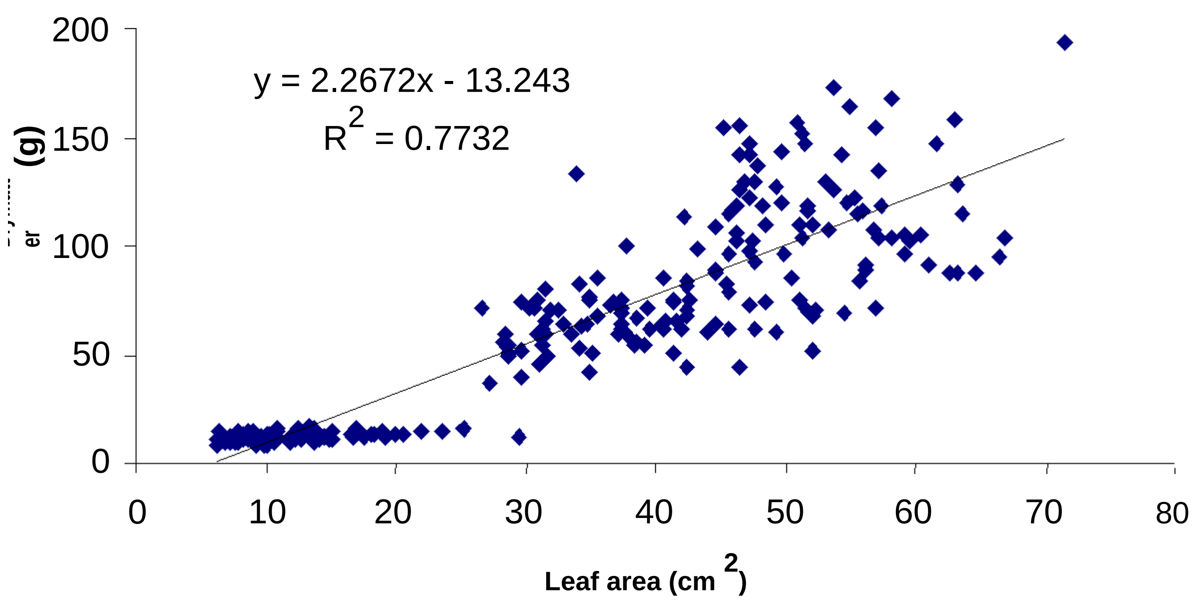

Figure 6. Relationship between dry matter production (g) and leaf area (cm^2), the values are average of three growth stages, four replications, two varieties, two seed treatment duration and five priming treatments.

difference in total dry matter production would be due the photo-thermal response of mung bean cultivars (Ellis et al., 1994).

The higher dry matter during the later stage from the primed treatments may indicate that most of the energy intake was used to develop grain. The faster dry matter accumulation after the reproductive stage was the result of increase in dry matter of stem and pods, whose continuous gain occurred as a result of plant height, number of tillers and internodes (Surya et al., 2004). During seed priming the rapid imbibition occurs which disrupt cell membrane and cause localized cells in cotyledon and embryonic axis of seed (Powell and Mathews, 1978) and is known to provide oxygen (Dlocknina et al., 2003) and thus the seedling could experience a less systematic resistance, which ultimately would result in more dry matter production compared to non primed seed.

Non significant differences for cultivars for AGR may be assorted to the indeterminate nature of mung bean. The unusual behaviors in mung bean cultivars for CGR were due to unknown reasons. The higher NAR, and delayed maturity, had resulted in significantly higher moisture (6.66 %) in NM- 98 as compared to (4.59%) in NM-92. With the findings of the present study, this suggests that any benefits of on-farm seed priming on mung bean are indirect growth, rather than direct changes in relative growth rate. Seedlings from primed seed experience due to different soil physical environment compared to non-primed seed (Murungu et al., 2004). Post-emergence seedling relative growth rates did not differ among either in cultivar, whether or not. The changes in early growth rates due to priming would be attributed to the low moisture content in the soil or due to minimum rainfall, which was sustained by primed plants, as against the latter heavy rainfall. The adverse effect of excessive rainfall during latter growth stage, was not sustained by the primed seed, would be the reason for non-significant results of growth parameters.

The similar varietals performance for leaf area and their relation to the dry matter production, at each growth stage could be associated to the genetic make up of the cultivars. The strong carbohydrates sink development during the later stages of growth continued the vegetative growth of the crop. Distribution of leaf area between lateral and priming shoots appears to be under genetic control, and priming further modified this trait in mung bean. No significant interaction was found in cultivar-duration-priming treatments interaction, and thus main factors influencing the leaf area, was either the vigorous growth of plant or the influence experienced by the plants due to priming technique. The high variability could be due to morphological difference between mung bean or difference in the development of structural organs. As such, inducing a primping technique would modify photosynthetic efficiency, as the varied increment in leaf area, produced significantly increased in dry matter production.

Conclusion

It is concluded from the study that dry matter production is direct function of the leaf area, which is further affected by priming technique. Greater the leaf area due to priming had developed greater dry matter into plant tissue as a result of vigorous plant growth and photosynthetic ability.

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