

Full Length Research Paper

# Soybean response to nitrogen fertilizer under water deficit conditions

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Accepted 23 December, 2010

In order to determine the effect of water deficit and nitrogen fertilizer application on growth indices, yield and yield component of three soybean (*Glycine Max* L. Merr) genotypes a split plot factorial experiment based on randomized complete block with three replications was carried out. Soybean genotypes (Williams, K1410 and HS95-4118) were subjected to three irrigation regimes (90, 120 and 150 evaporation from class A lysimeter) and three levels of nitrogen fertilizer (30, 60 and 90 kg N/ha). The results showed that, water deficit significantly decreased 1000 grain weight, yield, total dry mater and harvest index. The highest seed per pod (2.59) and maximum 1000 grain weight were obtained from HS95-4118 from Williams's genotypes, respectively. Although, the HS95-4118 genotype had the highest seed yield (499.89 g/m<sup>2</sup>). Increasing nitrogen application rates up to 90 kg N /ha increased pod per plant, grain per pod, 1000 grain weight, yield and total dry mater significantly. Maximum harvest index (33.8) obtained from 60 kg N /ha.

**Key words:** Growth indices, nitrogen, soybean, water deficit.

## INTRODUCTION

Environmental stresses which reduce plant performance will also reduce biological nitrogen fixation. Under such stresses, legumes responses to mineral nutrition especially nitrogen supplying. Poor nitrogen status in the field can be easily improved by inoculation, fertilization, irrigation and other practical management. Soybean supplies part of its nitrogen demand from nitrogen fixation. This legume may fix up to 250 lbs of nitrogen per acre and are not usually fertilized by nitrogen, but if the nitrogen is applied, the rate is low (10 to 15 lbs per acre) and the plant literally slows or shuts down the nitrogen fixation process (Lindemann and Glover, 2003).

Drought stress frequently limits growth, yield and N-accumulation in plant production (Heatherly and Spurlock, 1999; Pandey et al., 2000; Deblonde and Ledent, 2001; De Costa and Shanmugathan, 2002). Increasing soil water deficiency correlated with reduction in dry matter accumulation (Lopez et al., 1996a, b; Lazcano-Ferrat and Lovatt, 1999; Grieu et al., 2001). Drought stress decreases water uptake and nutrient flux and translocation. Therefore, transport allocation and metabolism of nitrogen may serve as adequate indicators for stresses and may help to understand how plants cope with a wide variety of suboptimal environmental conditions (Gotz and Herzog, 2000). The ability of nitrogen uptake differs between soybean cultivars (Gotz and Herzog, 2002). Drought stress at early stage of pod development due to increasing the rate of pod abortion significantly decreases final grain yield (Liu et al., 2003). Drought may also affect nitrogen status and metabolism in plants (Lawlor and Cornic, 2002). Numerous studies have demonstrated that, symbiotic nitrogen fixation was highly sensitive to drought. Reduction in nitrogen fixation has been assumed as associated with decreases in carbohydrate

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**Abbreviations:** LAR, Leaf area ratio; LAI, leaf /ground area; SLA, specific leaf area; NAR, net assimilation rate; CGR, Crop growth rate; LDM, leaf dry matter; RGR, relative growth rate; LAR, leaf area ratio; LWR, leaf weight rate; GDD, growing degree days.

supply to the nodules (Serraj et al., 1997, 1999). Symbiotic nitrogen fixation is highly sensitive to drought which results in decreased N accumulation and yield of legume crops (Serraj et al., 1999). The aim of this study was to investigate the responses of three-soybean genotypes to mineral nitrogen application under water deficit condition.

## MATERIALS AND METHODS

This experiment was carried out in Agricultural Research Station of Moghan, Ardabil province, Iran, in 2007. The research station is located in 39°N and 47°E longitude and has 32 m altitude. The soil type is tiosols with clay loam texture with pH 7.8 and electrical conductivity (EC) was one mmhoscm<sup>-1</sup>. Three genotypes of soybean (Williams, K1410, and HS95-4118) exposed to three irrigation schedules (90, 120 and 150 mm evaporation from class A lysimeter) and three nitrogen fertilizer rates as 30, 60 and 90 kg N/ha as urea. The experimental design was a split plot factorial based on randomized complete block, with three replications. Seeds of soybean genotypes were sown at 60 × 4 cm inter and intra row spacing, respectively. Seeds were inoculated with *Bradyrhizobium japonicum* at sowing time. To determine plant growth indices such as dry matter accumulation and leaf area index and plant sampling were taken periodically (24 days after sowing). Leaf area was measured using an automatic leaf area meter (Ci-202 USA). Plant parts after separating were dried at 75°C until reached steady weight. Growth indices were calculated as follow as: LAI, leaf /ground area; leaf area ratio (LAR, cm<sup>2</sup> g<sup>-1</sup>); specific leaf area (SLA, cm<sup>2</sup>g<sup>-1</sup>); net assimilation rate (NAR, g cm<sup>2</sup>d<sup>-1</sup>) (Hunt, 1982).

In order to determine yield components, five plants were cut from central rows of each plot at maturity. Data were subjected to analysis of variance by a statistical software (SAS) and means were separated by least significant difference at  $p = 0.05$  level (LSD 0.05).

## RESULTS

### Growth parameters

During growth season, leaf area index was increased in all irrigation schedules. Maximum leaf areas (5.5, 5.4 and 4.6) were obtained from 90, 120 and 150 mm evaporation, respectively, at 801.6, growing degree days (GDD). Maximum LAI for K1410, Williams and Hs95-4118 genotypes were 5.7, 5.6 and 5.1, respectively, which was obtained at 1018.8 GDD. Increases in nitrogen application raised LAI and the amounts of this trait was at 887.0 GDD for 30, 60 and 90 Kg Nha<sup>-1</sup> were 4.8, 5.3 and 5.5, respectively (Figures 1, 2 and 3c).

Crop growth rate (CGR) was increased in early growth stages and reached maximum rate of 0.007, 0.006 and 0.004 (g plant<sup>-1</sup> GDD<sup>-1</sup>) at 564.4 GDD for 90, 120 and 150 mm evaporation, respectively and subsequently after 1130, 1018.8 and 911 GDD, decreased to zero. These results showed that, increases in water stress severity from 90 to 150 mm evaporation, reduced CGR by 50 percent approximately. Also, water deficit enhanced reduction rate of CGR in 150 mm treatment at 219 GDD

was earlier than 90 mm evaporation. Maximum CGR for K1410, Williams and Hs95-4118 genotypes were 0.0085, 0.0076 and 0.0082 g plant<sup>-1</sup> GDD<sup>-1</sup> which was obtained in 564.4 growing degree days (GDD). Maximum CGR for nitrogen levels was 0.006, 0.007 and 0.008 g plant<sup>-1</sup> GDD<sup>-1</sup> and was obtained at 669 GDD (Figures 1, 2 and 3g).

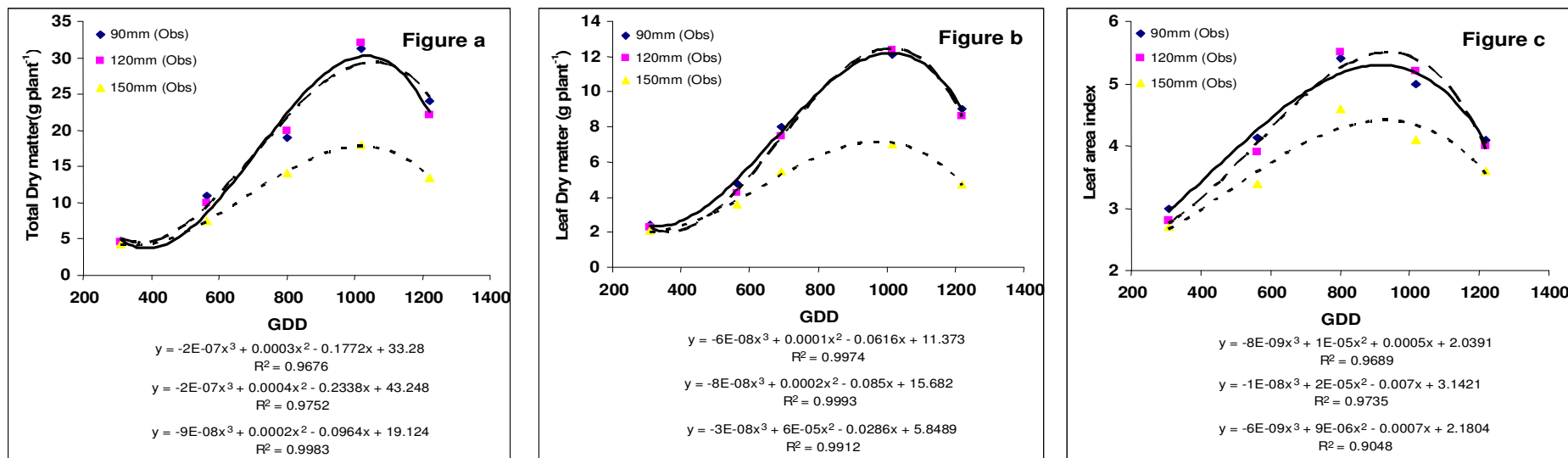
Net assimilation rate (NAR) in all treatments was decreased with ageing of plant foliage. Maximum NAR in 90, 120 and 150 mm evaporation treatments was 0.007, 0.006 and 0.005 g plant<sup>-1</sup> GDD<sup>-1</sup>, respectively. In the end of growth stages in 90 mm evaporation, NAR was decreased to 0.001 g plant<sup>-1</sup> GDD<sup>-1</sup> after 1018.8 GDD and then, become negative. Nevertheless, in 150 mm evaporation it decreased to 0.0008 g plant<sup>-1</sup> GDD<sup>-1</sup> after 801.6 GDD and then it was negative. These results revealed that, in severe stress condition, the amount of NAR reduction rate is higher than 90 mm treatment. Maximum NAR for Williams, K1410 and Hs95-4118 genotypes at the end of growth stage was 0.0065, 0.0070 and 0.0075 g plant<sup>-1</sup> GDD<sup>-1</sup>, respectively and it was decreased to 0.0008, 0.0013 and 0.0012 g plant<sup>-1</sup> GDD<sup>-1</sup>. The maximum NAR for 30, 60 and 90 Kg ha<sup>-1</sup> nitrogen levels was 0.0042, 0.0048 and 0.0050 g plant<sup>-1</sup> GDD<sup>-1</sup>, respectively, at the end of growth stage which was decreased to -0.0003, 0.001 and 0.0012 g plant<sup>-1</sup> GDD<sup>-1</sup> (Figures 1 to 3f). Results indicated that, nitrogen application ameliorates reduction rate of NAR and in all plant growth stage 60 kg Nha<sup>-1</sup> had higher rate of NAR relative to 30 kg N/ha.

Other parameters such as LDM (Leaf dry matter); RGR, (Relative growth rate); LAR, (Leaf area ratio); LWR, (Leaf weight rate) showed similar trends and at 90 mm evaporation, K1410 cultivar and 90 Kg ha<sup>-1</sup> nitrogen level had higher value for these traits in comparison to other treatments, (Figures 1 to 3b, d, e, i), also, in all growth stage LDM and LWR for 150 mm which was lower than other water stress treatments. Higher value for in SAL was obtained at 150 mm evaporation for Hs95-4118 cultivar, although, this trait has different trend with other parameters, but different level of nitrogen did not show any considerable difference for SAL (Figures 1, 2, and 3h). Other parameters such as LDM, RGR, LAR (Leaf area relative) and LWR showed similar trend, although, for SAL the Hs95-4118 cultivar was superior (Figures 1, 2, 3b, d, e, and i).

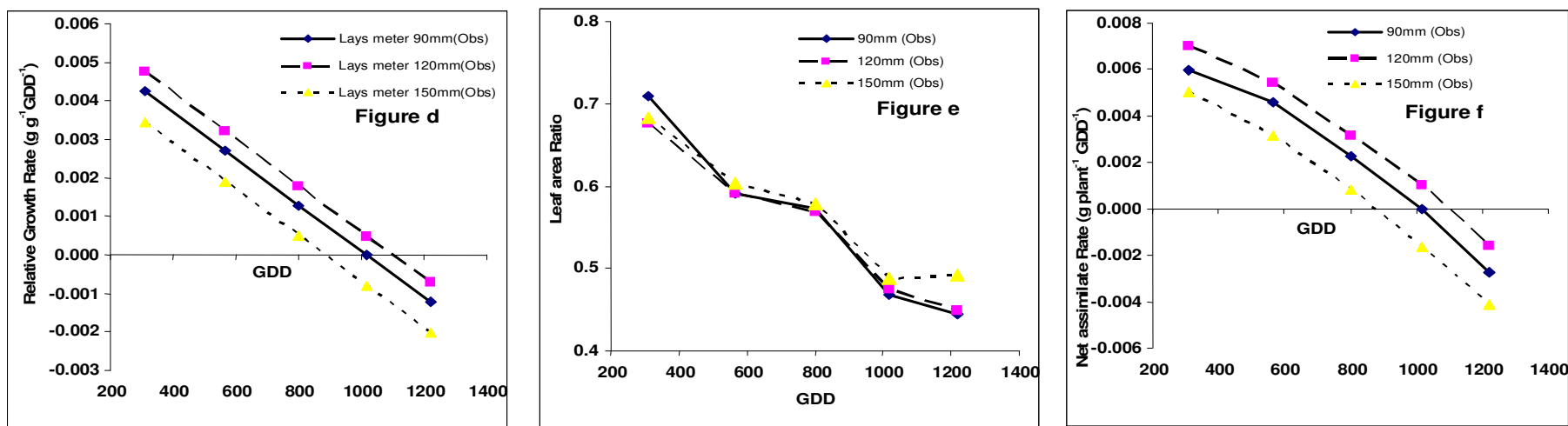
### Yield and yield component

#### Yield and water stress

Water stress, decreased total dry matter significantly ( $\alpha = 0.5$ ), (Table 1), so it decreased 43.04% at 150 mm evaporation in comparison to 90 and 120 mm. The results showed that, irrigation schedules had significant effect on seed yield ( $\alpha = 0.5$ ), and maximum and minimum seed yield (575.51 and 397.64 g m<sup>-2</sup>) was obtained over 90 and 150 mm evaporation, respectively. Pod and seed



**Figure 1.** Growth parameters in soybean (*Glycine Max L. Merr*) genotypes under water stress treatments.(Figure 1a) TDM, (Total dry matter); (Figure 1b) LDM, (Leaf dry matter); (Figure 1c) LAI, (Leaf area index); (Figure 1d) RGR, (Relative growth rate); (Figure 1e) LAR, (Leaf area relative); (Figure 1f) NAR, (Net assimilate rate); (Figure 1g) CGR, (Crop growth rate); (Figure 1h) SAL, (Special leaf area); (Figure 1i) LWR, (Leaf weight relative).



**Figure 1.** Continued.

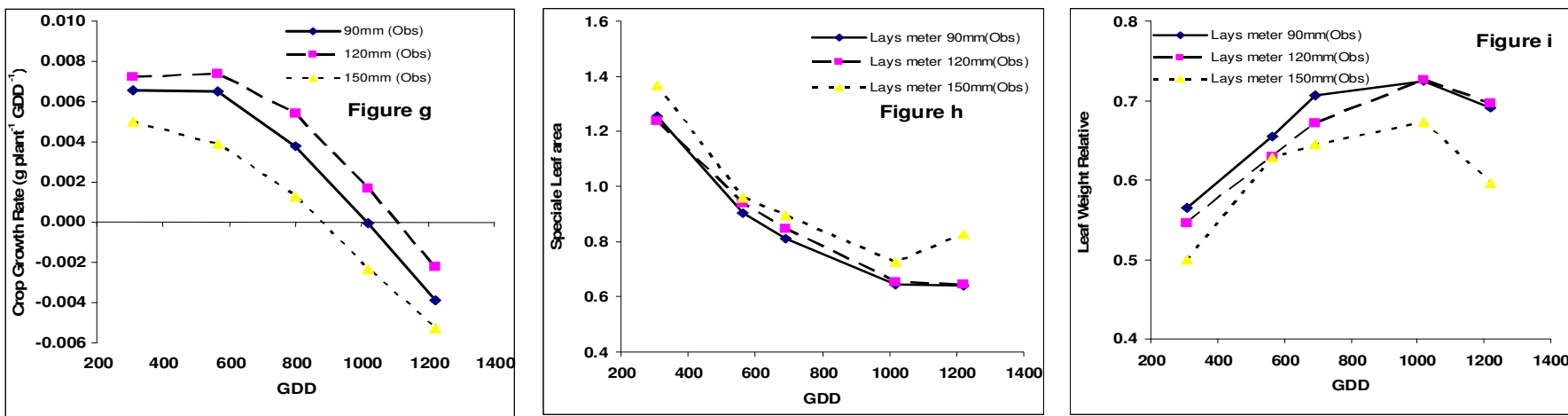
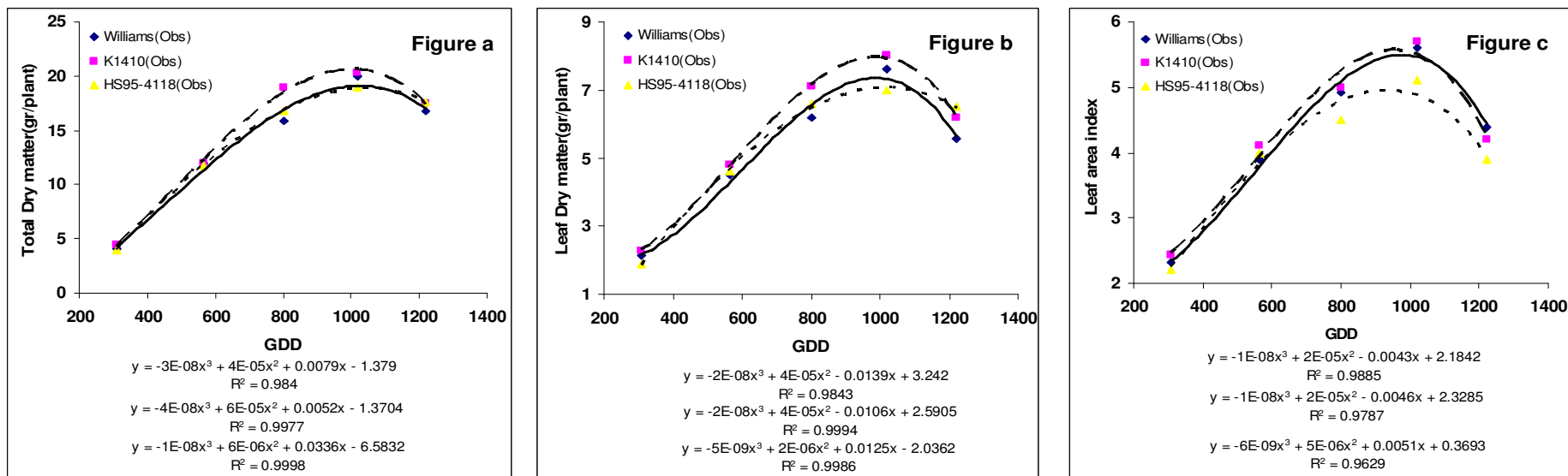


Figure 1. Continued.



**Figure 2.** Growth parameters in soybean (*Glycine Max L. Merr*) genotypes under water stress treatments.(Figure 2a) TDM, (Total dry matter); (Figure 2b) LDM(Leaf dry matter); (Figure 2c) LAI, (Leaf area index); (Figure 2d) RGR, (Relative growth rate); (Figure 2e) LAR, (Leaf area relative); (Figure 2f) NAR, (Net assimilate rate); (Figure 2g) CGR, (Crop growth rate); (Figure 2h) SAL, (Special leaf area); (Figure 2i) LWR, (Leaf weight relative).

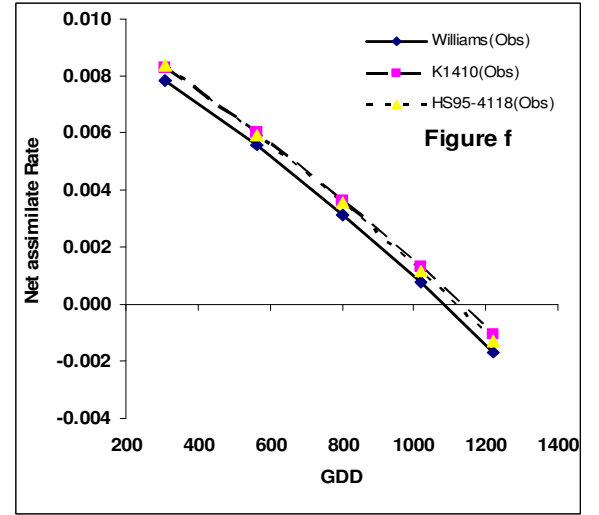
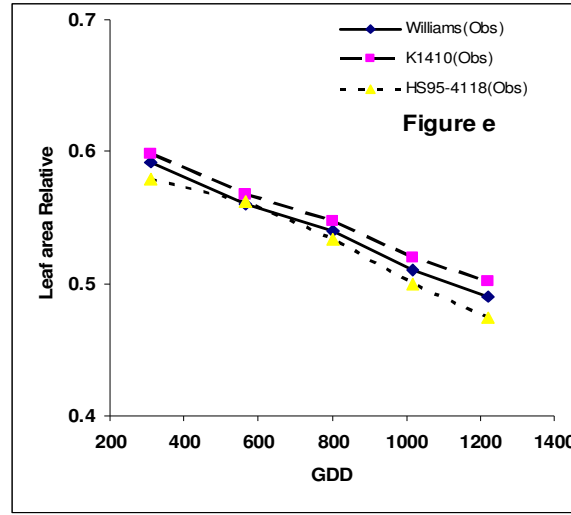
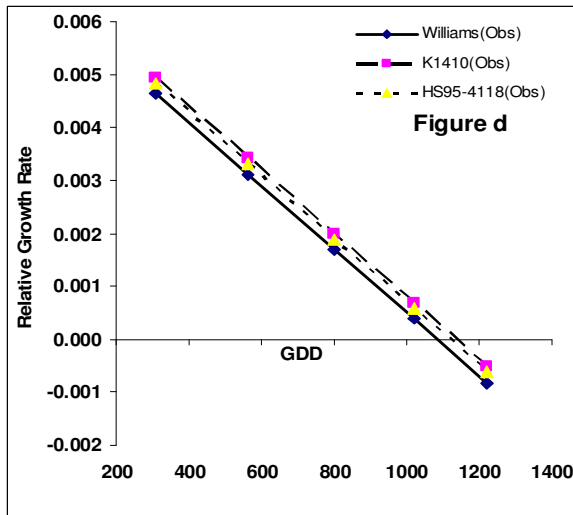


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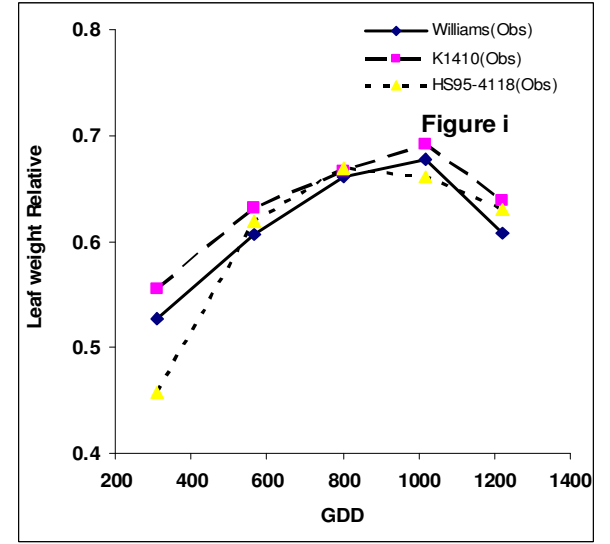
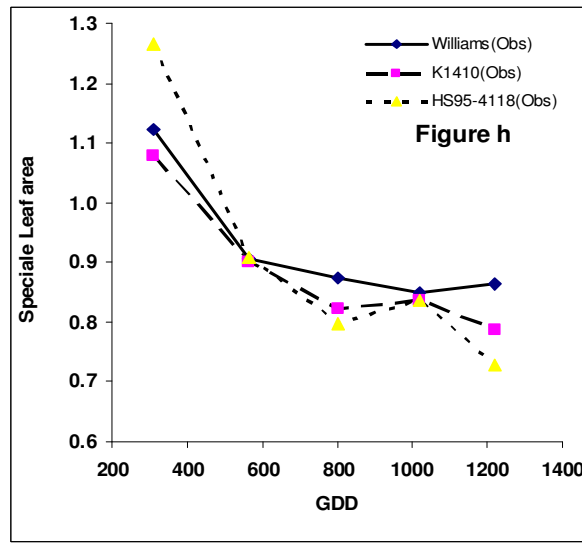
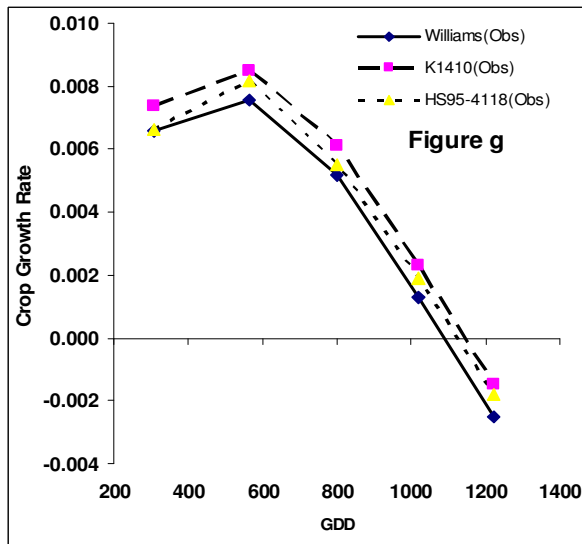
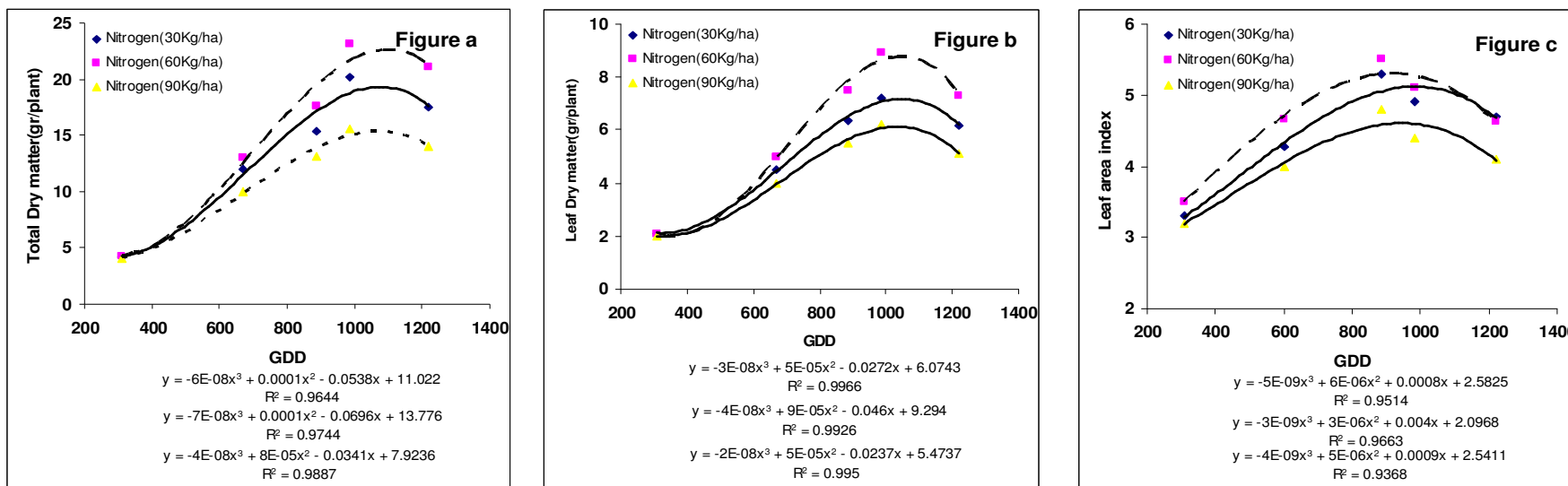
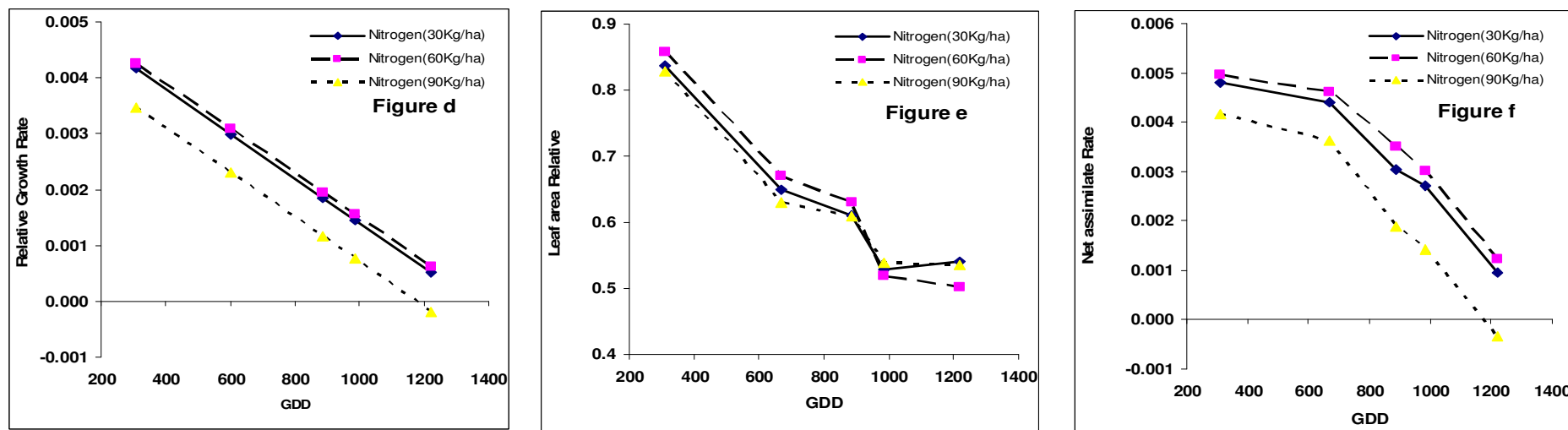


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**Figure 3.** Growth parameters in soybean (*Glycine Max L. Merr*) genotypes under water stress treatments.(Figure 3a) TDM, (Total dry matter); (Figure 3b) LDM, (Leaf dry matter); (Figure 3c) LAI, (Leaf area index); (Figure 3d) RGR, (Relative growth rate); (Figure 3e) LAR, (Leaf area relative); (Figure 3f) NAR, (Net assimilate rate); (Figure 3g) CGR, (Crop growth rate); (Figure 3h) SAL, (Special leaf area); (Figure 3i) LWR, (Leaf weight relative).



**Figure 3.** Continued.

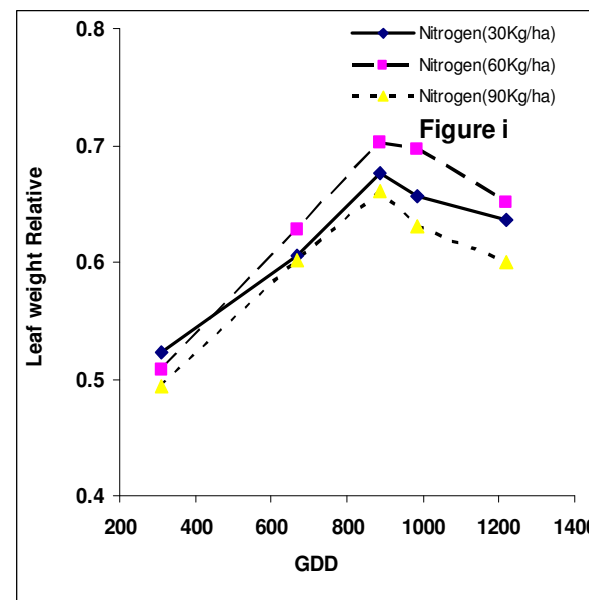
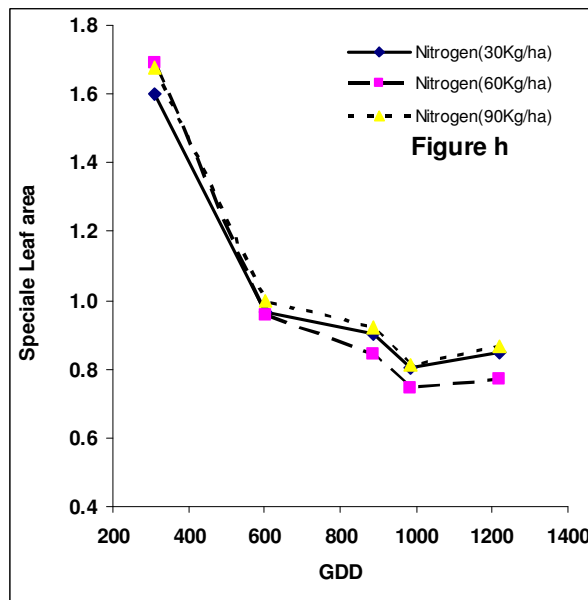
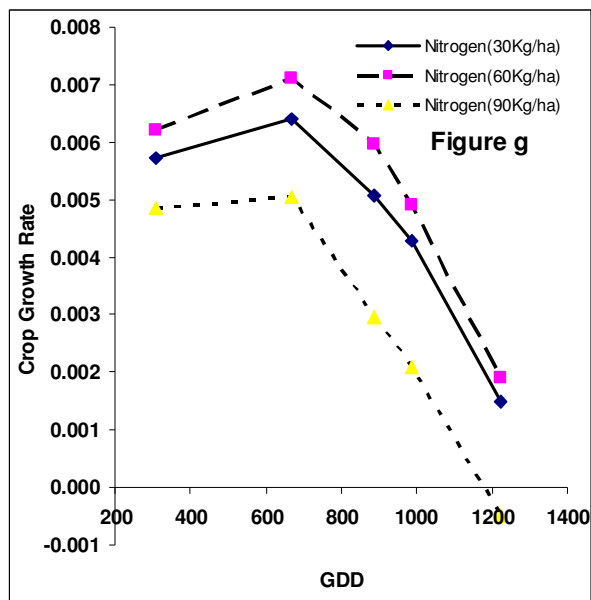


Figure 3. Continued.

numbers were increased over the 90 mm evaporation (29.81 and 2.61, respectively), whereas these values was 24.7 and 2.39 for 150 mm evaporation. One thousand seed weight was decreased with increasing water stress. The maximum and minimum (176.74 and 156.81, respectively) 1000- grain weight was obtained from 90 and 150 mm evaporation. The highest harvest index was obtained as over 90 mm evaporation (Table 2).

**Yield and soybean cultivars**

There was no significant difference between cultivars in total dry matter and pod number (Table 2), but seed number per pod in Hs95-4118 cultivar was higher than others. The maximum 1000- seed

weight obtained from Williams and the minimum values belongs to K1410 cultivars (179.22 and 161.51) (Table 2).

**Yield and nitrogen fertilizer**

The highest dry matter (2013.45 g/m<sup>2</sup>) obtained from 60 Kg N/ ha. Total dry matter increased by improving mine-ral nitrogen application until 60 Kg N /ha, but in higher amount of nitrogen application no increase in dry matter production (Table 3). The highest seed yield was obtained from 60 Kg ha<sup>-1</sup> level of nitrogen fertilizer (663.45 g/m<sup>2</sup>). Pod per plant and seed numbers per pod (35.11 and 2.73, respectively), were increased over 60 Kg N/ ha, whereas there was no significant difference between 30 and 90 Kg/ ha of nitrogen fertilizer.

The maximum 1000- seed weight (187.96 gr) was also obtained from 60 Kg N/ ha (Table 3).

**Correlation**

There was significant positive correlation between harvest index and seed yield (Table 4). Basavaraja et al (2005) and Iqbal et al (2010) also reported similar findings in different soybean genotypes. Plant height had significant positive correlation with seed yield (Table 4). The result is in agreement with Faisal et al., (2007) who observed significant and positive correlation of plant height with seed yield. The highest positive correlation was observed in number of pods per plant (r = 0.935\*\*) with seed yield. Liu et al (2005) reported that pod per plant is higher in soybean

**Table 1.** Effect of water stress on yield and yield components of soybean.

Treatment	Pod per plant	Seed per pod	1000- seed weight(g)	Seed yield (g/m <sup>2</sup> )	Plant height(cm)	Dry matter (g/m <sup>2</sup> )	Harvest index (%)
90 ET	29.81 a	2.61 a	176.74 a	575.51 a	81.21 a	1823.40 a	32.66 a
120 ET	28.37 ab	2.48 ab	173.44 a	488.72 a	79.19 a	1809.37 a	26.66 b
150 ET	24.70 b	2.39 b	156.81 b	397.64 b	63.84 b	1432.89 b	27.81 b

**Table 2.** Differences between soybean cultivars in yield and yield components.

Treatment	Pod per plant	Seed per pod	1000- seed weight(g)	Seed yield (g/m <sup>2</sup> )	Plant height(cm)	Dry matter (g/m <sup>2</sup> )	Harvest index (%)
Williams	26.96 a	2.43 b	179.22 a	484.42 a	76.34 a	1646.42 a	29.18 a
K1410	26.81 a	2.47 b	161.51 b	477.55 a	70.12 a	1757.67 a	27.70 a
Hs95-4118	29.11 a	2.59 a	166.25 ab	499.89 a	77.78 a	1661.57 a	30.25 a

**Table 3.** Effect of nitrogen fertilizer levels on yield and yield components of soybean.

Treatment	Pod per plant	Seed per pod	1000- seed weight(g)	Seed yield (g/m <sup>2</sup> )	Plant height(cm)	Dry matter (g/m <sup>2</sup> )	Harvest index (%)
30 kg N/ha	24.07 b	2.41 b	165.37 b	410.64 b	73.94 a	1579.40 b	26.22 b
60 kg N/ha	35.11 a	2.73 a	187.96 a	663.45 a	77.16 a	2013.45 a	33.85 a
90 kg N/ha	23.70 b	2.34 b	153.66 b	387.77 b	73.15 a	1472.80 b	27.07 b

**Table 4.** Correlation between yield components in soybean cultivars.

Parameter	Pod per plant	Seed per pod	1000- seed weight(g)	Seed yield (g/m <sup>2</sup> )	Plant height(cm)	Dry matter (g/m <sup>2</sup> )
Harvest index (%)	0.791**	0.655**	0.55**	0.826**	0.175	0.451*
Dry matter (g/m <sup>2</sup> )	0.788**	0.664**	0.637**	0.868**	0.61**	
Plant height (cm)	0.394	0.388	0.561**	0.477*		
Seed yield (g/m <sup>2</sup> )	0.935**	0.773**	0.735**			
1000- seed weight (g)	0.688**	0.467*				
Seed per pod	0.743**					

genotypes with high productivity characteristic. Significantly positive correlations were also observed for seed per pod and seed yield, it is expected that the genotypes with higher seed per pod would have high seed yield. A significant positive correlation was observed between 1000-seed weight and seed yield. Bangar et al (2003) have reported significant positive correlation of 1000-seed weight with seed yield characters in soybean.

## DISCUSSION

Total dry matter decreased significantly with increases in water stress severity. This trait decreased to 43.04% at 150 mm evaporation in comparison to 90 and 120 mm. Increasing water deficit reduced leaf area index (LAI).

Increases in dry matter accumulation were obtained until 60 Kgha<sup>-1</sup> nitrogen applications, which can be attributed to nutritional status of soil in experimental. Total dry matter of 60 Kg N ha<sup>-1</sup> was 36.7 and 27.48% more than 90 and 30 Kgha<sup>-1</sup>. Inhibitory effect of water deficit on plant growth was reported by other researchers (Heatherly and Spurlock, 1999; Pandey et al., 2000; Deblonde and Ledent, 2001; De Costa and Shanmugathan, 2002). Also, correlation between soil water deficit and dry matter reduction has been reported previously, (Lopez et al., 1996a, b; Lazcano-Ferrat and Lovatt, 1999; Grieu et al., 2001). Although in normal condition, soybean can fix up to 250 lbs of nitro-gen per acre and applying nitrogenous fertilizers slows or shuts down the nitrogen fixation process (Lindemann and Glover, 2003), but in water deficit condition, some disorders such as decreasing



nodule turgor pressure and  $O_2$  diffusing into the nodules, decreases photosynthesis due to lowering leaf growth and enhancing leaf abscission which led to photo-assimilates restriction to nodules and significant reduction in  $N_2$  fixation can be observed. Other researchers have argued that, host(plant) mitochondria have lower than bacteroidal affinity to oxygen ( $K_m(O_2) = 50$  to  $100$  nM which indicates that, even small changes in the concentration of free oxygen could critically affect their capacity to ATP production (Millar et al., 1995). Limitation of oxygen diffusion and decreased nodule respiration could occur under water deficit, salinity or flooding stress and have an inhibitory effect on nitrogen fixation. Due to carbon restriction or metabolic limitations, as well as other environmental factors involved in mechanism in order to enhance resistance of mid-cortex cell layer to oxygen diffusion (Minchin, 1997; Serraj et al., 1999) and which could further decrease hypoxic conditions in bacteroids surrounding (Kuzma et al., 1999).

Our results about increases in LAI, total dry matter and so many yield components by mineral N application can be explained by prior physiological reasons. In such conditions, transport, allocation and metabolism of nitrogen may serve as adequate indicators for stresses (Gotz and Herzog, 2000), while the ability of soybean cultivars for N absorption is differ (Gotz and Herzog, 2002).

Irrigation schedules had significant effects on seed yield and components. Reduction in yield components (1000- seed weight, pod per plant and seed per pod), harvest index and biomass toward increases in water stress severity causes yield reduction. Pod number per plant, seed per pod, 1000- seed weight, seed yield, biomass and harvest index was higher in  $60 \text{ Kg N ha}^{-1}$  application. Growth limitation, yield and N-accumulation of plants in water deficit condition have been reported previously (Heatherly and Spurlock, 1999; Pandey et al., 2000; Deblonde and Ledent, 2001; De Costa and Shanmugathan, 2002). Liu et al. (2003) reported that, drought stress occurrence at early stage of pod development significantly increased the rate of pod abortion thus, decreasing final seed yield. Amelioration of drought effects on plant growth and yield by nitrogen application revealed that, nitrogen fixation of soybean was affected by irrigation schedules. Other researchers have mentioned that, drought may affect nitrogen status and metabolism in plants and both processes interact with each other (Lawlor and Cornic, 2002). Numerous studies have demonstrated that, symbiotic nitrogen fixation was highly sensitive to drought and decrease in nitrogen fixation has been assumed associated with decreasing carbohydrate supply to the nodules (Serraj et al., 1997, 1999). Our results showed that, water deficit had an adverse effect on soybean growth and yield by affecting some growth indices and yield components which are partly due to restriction of nitrogen fixation and metabolism. In other words, mineral nitrogen application improved growth and yield of soybean under water deficit condition and  $60 \text{ Kg}$

$\text{N ha}^{-1}$  was adequate in this experiment.

## ACKNOWLEDGEMENT

We thank all those who helped us in this research, especially Moghan Agricultural Research center Iran.

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