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Growth Indices of Kimiya Cultivar of Lentil in Response to Drought Stress at Flowering and Pod Filling Stages under Greenhouse Conditions

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ABSTRACT

Drought stress is one of the most important environmental factors which can limit the lentil production. To evaluate the effect of drought stress at flowering and pod filling stages on growth indices of lentil cultivar Kimiya (a new high yielding lentil cultivar for moderate cold and semi warm climate of Iran), an experiment was conducted at the research greenhouse of Higher Educational Complex of Saravan in completely randomized design with four replications. The drought stress was applied by withholding irrigation until the soil moisture reached to 20% of field capacity at stages of flowering and pod filling. In this experiment, we measured grain yield per plant, growth indices including plant leaf area (PLA), relative growth rate (RGR), plant growth rate (PGR) and net assimilation rate (NAR). Results showed that the flowering stage in lentil plant is more sensitive to drought as exhibited significant decline in PLA, RGR, PGR and NAR. PGR declined at the end of growing season when vegetative growth stop, senescence of leaves, the reduction of NAR and the allocation of assimilate to the seeds.

Key words: Allocation, Leaf area, Moisture, Vegetative.

INTRODUCTION

Lentil is an annual food legume highly valued in the world (Ghanem *et al*. 2015). It has great significance in cereal-based cropping systems because of its nitrogen fixing ability, the high protein, high micronutrients seeds for human diet and its straw for animal feed. Lentil also plays an important role in sustaining soil fertility by symbiotic nitrogen fixation because there are major economic limitations in the use of commercial nitrogen fertilizers (Kumar *et al*. 2016).

The sowing time of lentil varies according to the coldness of the winter. Autumn is the best time for lentil cultivation areas with a temperate winter. Spring is also very commons in with cold winters and Highlands (Oweis *et al*. 2005). Since the amount and distribution of rainfall is variable in autumn and spring, the occurrence of drought stress at all stages of vegetative and reproductive growth is likely (Kashiwagi *et al*. 2006). Intermittent drought stress occurs due to the intermittent interruption of autumn rainfall and terminal drought stress occurs due to the early stopping of spring rainfall. In Mediterranean areas, lentil is grown in autumn or winter, so reproductive growth stages are affected by drought stress. However, the occurrence of drought stress led to decrease lentil yield (Gangali and Nezami, 2008). Farooq *et al*. (2016) showed that during the pod filling stage, drought stress reduced vegetative growth and the LAI, thereby decreasing the number of flowering nodes and produced flowers and causing flowers and seeds to abort which in turn, led to a 70% reduction in grain yield.

On the other hands, the evaluation of the growth indices is very important to determine the extent of them in the final yield. Quantitative growth analysis is a useful method to justify and interpret the plant's response to the various environmental conditions that the plant encounters during its growth. This method provides a better understanding of how the photo-synthetic assimilates are allocated to different organs and also accumulation of dry matter produced during the plant growth season (Prasad *et al*. 2008). The major indices in plant growth analysis include: crop growth rate (CGR), relative growth rate (RGR), net assimilation rate (NAR), leaf area index (LAI). LAI is a determinant factor in the inter-change of water and energy. The main effect of low drought stress is declining the number of leaves, speed of development and the size of the leaves. In severe drought stress, leaf developmental rate may decrease and leaf growth may be stopped. Also, drought stress affects leaf area index by decreasing the initiation of new leaves (Xu and Zhou, 2008). Reduction of LAI under drought stress is

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indeed a stress tolerance strategy (Parsa *et al*. 2012). Ghanbari *et al.* (2014) showed that in bean (*Phaseolus vulgaris* L.) under drought stress conditions, CGR decreased as a result of stopping cell development and reducing carbon allocation. Niari Khamssi *et al*. (2007) concluded that drought stress in the lentil decreased NAR and RGR. There was a positive correlation between water consumption and NAR.

Lentil is a forgotten crop in Saravan region by farmers. Therefore, recognizing the susceptibility of phenological stages particularly the reproductive stages of lentil to drought stress and its subsequent growth stages after providing the required moisture can be effective in agronomic methods to reduce the effects of drought stress on grain yield. However, the objective of this research was evaluation of the effect of drought stress on growth indices of Kimiya as a predominant lentil cultivar at flowering and pod filling stages under greenhouse condition.

MATERIALS AND METHODS

To assess the effect of drought stress at flowering and pod filling stages of lentil, an experiment was conducted in the greenhouse at Higher Educational Complex of Saravan in a completely randomized design with four replications in pots containing 5 kg of soil with a diameter of 22 cm and a height of 18 cm. The lentil cultivar was Kimiya (a new high yielding lentil cultivar for moderate cold and semi warm climate of Iran) and planting depth was 5 cm and also, plant density was three plants in each pot.

The amount of water in the dry soil was measured in base of the field capacity. To determine the amount of water in each pot, we first placed 4 kg of soil inside the oven at 103°C and weighed after 48h. Then, the dried soil was poured in a pot and water slowly added to reach the saturation point and after full withdrawal of water, the pot was weighed. After subtracting the weight of the pot and the dry soil, the amount of water stored in the field capacity was determined. After that, 56 pots were prepared with 5 kg of soil in each pot. Twenty eight pots were considered for control treatment. Total of 16 and 12 pots were considered respectively for flowering and pod filling stages. At flowering stage, drought imposition was applied 32 days after the emergence when 50% of the plants entered flowering stage. Furthermore, in pod filling stage, drought imposition was applied 44 days after the emergence when 50% of the plants had pods. The drought imposition period for each phenological stage was initiated by stopping irrigation and until the water soil reached up to 20% of the field capacity that coincided with the emergence of stress symptoms, including the closure of leaflets and the withering of the terminal buds. At this time, a number of pots were destroyed in order to sample and measure the traits and the rest of the stressed pots were irrigated (4). Four samplings were performed for drought stress in the flowering stage and three samplings at the pod filling stage. Moreover, sampling before water stress imposition in each phenological stages was considered the same with control treatment while sampling was done for the control treatment at each phenological stage. Pots were irrigated every three days until the soil water reached 70% field capacity. Water level was equally maintained among the pots of different plant biomass weight. In the other words, during each sampling, the average weight of the plants in each drought stress treatments were subtracted from the initial weight of the pots and then the pots reached field capacity. The leaf area index was calculated via leaf area meter device. Samples were placed in an oven at 72°C for 48 hours and then weighted. At the last sampling, grain yield per plant was measured.

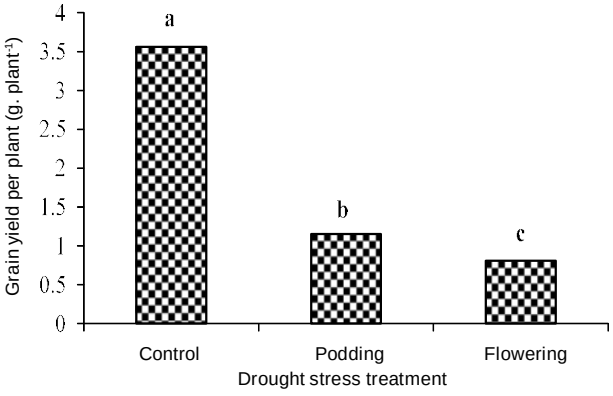

Fig 1: The effects of drought stress at flowering and pod-filling stages on grain yield per plant.

Growth indices were calculated based on plant and due to the constant temperature in greenhouse conditions, calendar days after emergence was used instead of the growth degree day. To determine the leaf area per plant, plant growth rate, relative growth rate, net assimilation rate, the equations 1, 2, 3 and 4 were used, respectively.

PLA= [(LA 2 + LA 1) /2]1/plant) (1)
PGR= (W2-W1)/(T2-T1)plant (2)
RGR= (Ln W2- Ln W1)/ (T2-T1) (3)
NAR =
[(LnW2.LnW1)/(T2-T1)] [(LnLA2-LnLA1)/(LA2-LA1)] (4)

In the equations above, LA was the leaf area (cm2), W was dry weight of the plant (g), T was sampling time. Analysis was done using SAS v 9.1.2 (SAS, 2003) software. Duncan test was used at the 5% probability level to compare the meanings. Excel software was also used to draw charts.

RESULTS AND DISCUSSION

Grain yield per plant

The effect of drought stress at flowering and pod filling stages on grain yield per plant was significant (Table 1). The highest

Table 1: Analysis of variance for yield per plant of lentil in drought stress conditions at flowering and pod-filling stages.

S. O. V	df	Yield per plant
Treatment	3	5250**
Error	9	500**
CV		8.2

**: Significant at α = 0.01.

and lowest grain yield per plant was observed with control and drought stressed plants at flowering stage with 3.5 and 0.8 g. plant^{-1}, respectively (Fig 1). Drought stress at flowering stage decreased the flowering period, number of flowers and grain yield per plant, because at flowering stage, the lentil had active vegetative growth and drought stress would decrease the crop growth rate and this trend did not compensate in later stages (Amiri Deh Ahmadi, *et al*. 2010a).

The highest grain yield of lentil was obtained under the full irrigation conditions. Thus, the avoidance of drought stress after flowering stage is essential (Zyaie *et al*. 2012; Nezami *et al*. 2011). Moreover, Amiri *et al*. (2015) reported that under optimal soil moisture conditions, lentil had a rapid vegetative growth at the beginning of flowering stage increasing the reproductive growth period and photosynthesis. On the other hands, the result of high photosynthesis was the formation of more flowers in the plant that affected the formation of fertile pods and finally grain production. As lentil has an indeterminate growth habit and growth continues after the first flowering, the imposition of water deficits during flowering and podding also reduces leaf area and foliage dry matter, resulting in reduction of grain yield (Karimzadeh *et al*. 2017; Shrestha *et al*. 2006).

Plant Leaf Area (PLA)

The results showed that few days after the emergence, the leaf area increased in all treatments. At the end of growing season, the control treatment had the highest leaf area, so that after 25 to 71 days after emergence, this trend was remained almost constant afterwards (Fig 2). The lowest amount of leaf area was obtained for drought stress at flowering and pod filling stages, respectively. This could be due to drought stress in this stage led to senescence leaves and also accelerated the aging of plant.

The results also indicated that development of leaves decreased after plant was exposed to drought stress, and this reduction trend continued until irrigation. Finally, when the speed of senescenced leaves was more than the speed of development of new leaves, leaf area and photosynthetic production reduced. Higher leaf area index in chickpea in non-stress conditions is also confirmed by other researchers (Goldani and Rezvani, 2007; Shabiri *et al*. 2007). By reducing the soil water content, leaf area index showed a decreasing trend. The leaf area index is one of the most important growth indices which have significant correlation with biological and grain yield (Singh, 1997). However, the optimum leaf area index (LAI) is an important factor for grain yield. If the LAI reached an optimum level within a shorter time (Fig 2), a higher grain yield could be achieved. The slow development of the leaf area could weaken development of the canopy, decreasing radiation absorption and the growth rate (Darzi-Naftchali *et al*. 2017).

Drought influences several plant processes, from the cell to the canopy. Cell biochemistry, division and expansion are all very sensitive to water deficit condition. Consequently, the vegetative growth rate of crop under water stress may

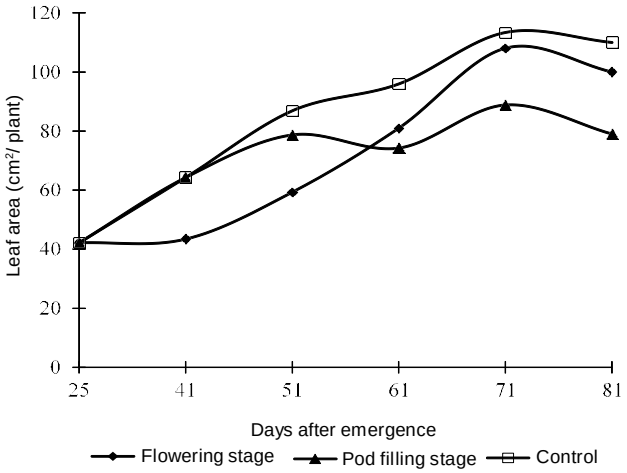

Fig 2: The effect of drought stress at flowering and pod filling stages on leaf area at days after emergence.

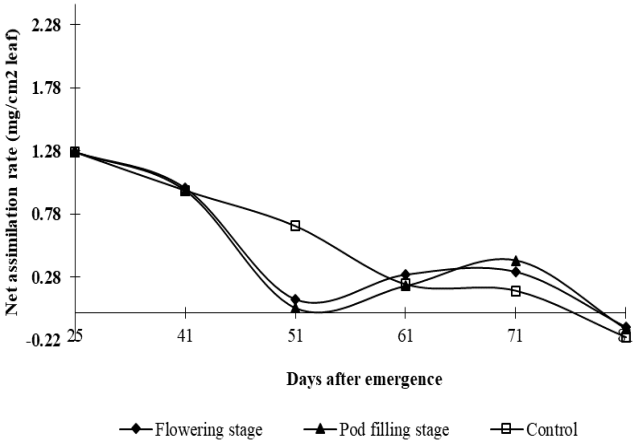

Fig 3: The effect of drought stress at flowering and pod filling stages on net assimilation rate at days after emergence.

be severely restricted, resulting in reduced total dry matter (TDM) and smaller leaf area. Reduction in both leaf area and dry matter production have been reported in many crops including legumes, such as groundnut [Vigna subterranea] (Mwale *et al*. 2007), cowpea [*V. unguiculata*] (Anyia and Herzog, 2004) and chickpea [*Cicer arietinum*] (Purushottam *et al*. 2018).

Net Assimilation Rate (NAR)

Curve of NAR is presented in Fig 3 over growing season. Control treatment decreased with steady slope. NAR is an estimation of the average photosynthetic intensity of leaves in a plant canopy and its maximum was at the time of exposure to sunlight. NAR occurred when the lentil is at early stages of growth and leaves are so large that are not available in the shade (Ghanbari *et al*. 2014).

The drought stress at flowering and pod filling stages caused a dramatic decrease in NAR because the lentil in the reproductive stage had an active growth and drought stress caused decreasing trend of NAR. At the end of growing season, NAR in all treatments increased a bit due to falling leaves and low shading of leaves on each other.

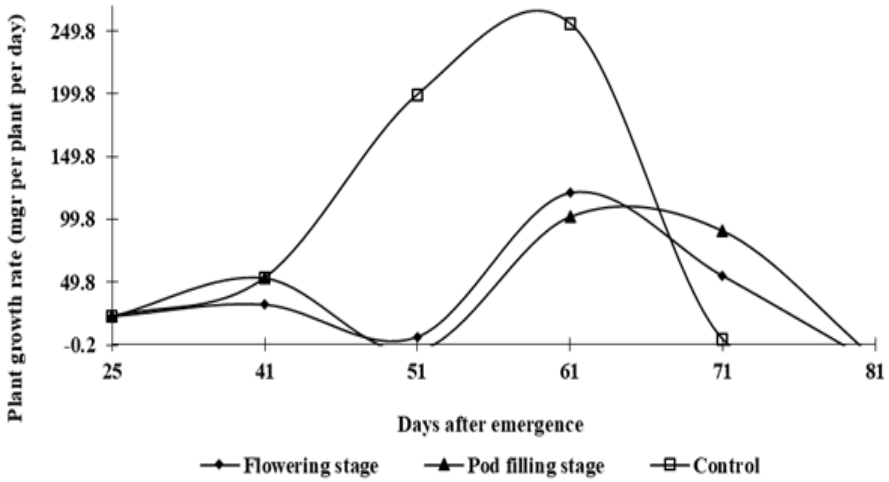

Fig 4: The effect of drought stress at flowering and pod filling stages on plant growth rate at days after emergence.

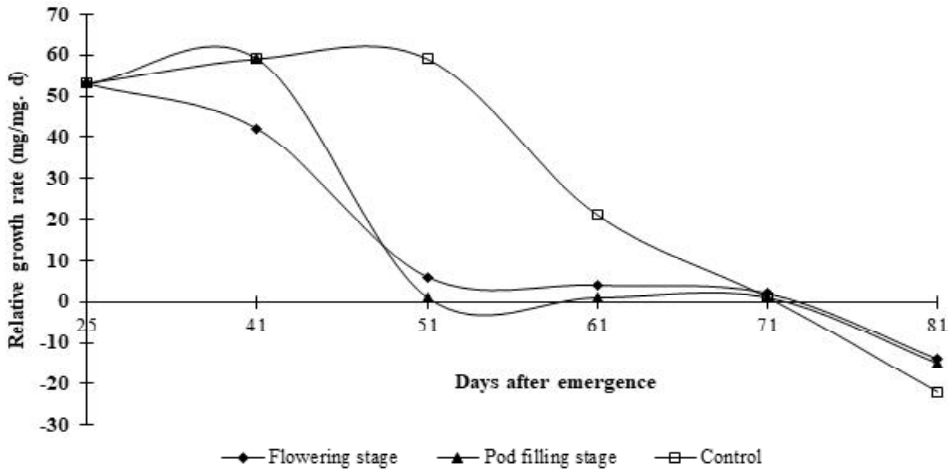

Fig 5: The effect of drought stress at flowering and pod filling stages on relative growth rate at days after emergence.

Rezaeyanzadeh (2008) concluded that if NAR was prevented from occurring drought stress at flowering stage with supple -mental irrigation, it would increase and the slope of NAR rate smoothly decreased. Since the onset of flowering stage were coincided with a high rate of photosynthesis and plant growth, the irrigation at this stage improved the photosynthetic activity and thus increased the NAR.

According to findings of Amiri *et al*. (2010b), the amount of NAR did not remain constant over time, and as the age of plant increased, a declining trend observed in growth and development and this trend was also accelerated in an inappropriate environment and drought stress conditions. The new leaves are also overlapped on each other which led to decrease dry weight per unit leaf area.

Plant Growth Rate (PGR): In all treatments, plant growth rate initially increased and then decreased and finally, the trend became negative at the end of growing season. This could be due to high absorption of solar radiation along with the increase in the leaf area during the growing season which led to high rate of dry matter accumulation in lentil. PGR increased in the control treatment with a steady slope up to 61 days after the emergence and subsequently decreased (Fig 4). It seems that increasing LAI under non-stress conditions leads to high radiation intake, which ultimately increases photo-synthesis and subsequently rate of plant growth. PGR decreased under drought stress due to reduction in leaf area, amount of photosynthesis and occurrence of leaf senescence.

Karimi *et al*. (2009) concluded that PGR decreased due to high respiration and low photosynthesis in drought stress condition. Drought stress at flowering and pod filling stages caused a sharp decline in PGR up to 51 days after emergence and then after this time up to 61 days after emergence, an amount of increase in this treatment was as much as the half of the control and at the end of growing season had decreasing trend. On the other hands, the yellowing and falling of leaves that occurred under drought stress period did not recover.

According the curves of PGR and leaf area, the maximum PGR was obtained before the occurrence of maximum leaf area. When PGR reached its maximum, leaf area still had an increasing trend. PGR was directly dependent on the leaf area and NAR. However, with the progress of time, NAR had a low trend which can decrease the PGR. At the end of growing season, PGR declined due to lack of new vegetative growth, senescence of leaves, reduction of NAR, and allocation of assimilate to the seeds.

Relative Growth Rate (RGR)

RGR was decreased with aging of the plant (Fig 5), because the parts added to the plant were structural tissues that were not metabolically active and did not have a role in photosynthesis (Karimi and Siddique, 1991). At the beginning of the growing season, the amount of RGR is boosted due to greater light penetration, less leaf shading and higher NAR.

The drought stress in the flowering and pod filling stages reduced the RGR by 51 days after emergence and then remained constant. Drought stress in these stages reduced the leaf area and did not compensate it until the end of the growth period (Fig 2) and also the exacerbation of leaf loss caused a decrease trend in the RGR. However, at the end of the growing season, the RGR had negative trend due to physiological ripening and high respiration of seeds as well as aging and leaf loss and reduction of the current photosynthesis of canopy (Fathi *et al*. 2011). Parsa *et al*. (2012) reported that supplemental irrigation at the flowering stage increased the RGR and also decreased the slope of the curve. This reduction is related to shading and aging of lower leaves of the lentil canopy.

CONCLUSION

The results showed that flowering stage was the most sensitive stage of lentil growth to drought stress. which at this critical stage decreased the flowering period, number of flowers, pale and leaf loss, resulting in decreasing photosynthetic dry matter to support the seed development and finally, decreasing the growth indices such as PLA, RGR, PGR, and NAR. Thus, it is vital to avoid drought stress at the early flowering stage because the lentil plant is active and has the highest rate of photosynthesis and plant growth. The irrigation at this stage boosted photosynthetic activity and consequently increased the growth indices and lentil grain yield.

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