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**RESPONSE OF TWO RED BEAN (PHASEOLUS VULGARIS L.)
CULTIVARS TO CONTROLLED WATER DEFICIT STRESS DURING
POST-FLOWERING GROWTH STAGE**

SUMMARY

A pot experiment conducted to evaluate the response of two red bean cultivars, Sayad and Derakhshan, to water deficit stress during post-flowering growth stage and recovery potential of plants after stress. Treatments were included regular irrigation or control, water deficit during flowering stage, water deficit during pod formation and water deficit during pod filling period. Results showed that plant height had positive effects on yield of cultivars so that, the tall cultivar, 'Sayad', had higher yields. Stress application during flowering stage showed the highest negative impact on plant height and subsequently yield. The longest and the higher number of pods as well as the greatest number of seeds in pods were recorded in control treatment in 'Sayad'. Stress application during pod formation resulted in the minimum amount of all studied traits in both cultivars. Stress encountered during seed filling period had the least effect on number and length of pods and seed/pod. But 100 seeds weight significantly decreased. The highest amount for 100 seeds weight was record in control plants in 'Derakhshan'. Under all treatments, 'Sayad' had higher biologic and seed yield compared to 'Derakhshan'. The least amount of yield was recorded during stress application in pod formation and flowering period for 'Sayad' and 'Derakhshan' respectively. Harvest index of 'Sayad' was more affect by stress application. Data related to photosynthetic rate showed that during stress application, 'Derakhshan' owned rapid decline in photosynthesis. Beyond stress alleviation and onset of irrigation, recovery potential of 'Sayad' was higher than 'Derakhshan' and this cultivar was able to rapidly restore the photosynthesis rate of stress faced plants near control ones. In total, stress had lower impacts on photosynthetic rate of 'Sayad' cultivar.

Keywords: Common bean, water stress, yield, yield components, photosynthetic rate

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important food crop grown under rain-fed conditions in Iran where drought is a major limiting factor for production. Common bean is a cosmopolitan food crop containing 22-25% protein and 56-58% carbohydrate (Majnoon Hosseini *et al.*, 2008). Drought stress remains the main restraint for common bean production nearly in all developing countries (Fageria *et al.*, 2006). It is reported, 60% of common bean production commonly occurs under water deficit conditions and areas (Majnoon Hosseini *et al.*, 2008). About 60% of common beans produced world-wide are grown in regions subjected to water stress, making drought after diseases the second largest contributor to the yield reduction in this specie. This is one of the main reasons why in bean the average yield has remained for long time at low level ($< 900 \text{ kg ha}^{-1}$) (Martinez *et al.*, 2007). Meanwhile, bean has limited drought tolerance potential (Fageria *et al.*, 2006). Water deficit impact is dependent upon severity and time course of stress as well as plant growth stage (Desclaux and Roumet, 1996). In bean plants, water deficiency encountered during flowering and seed filling period led to the constant yield loss and early ripening (O'Tool and chang, 1979). Furthermore, low water availability during flowering period adversely affected yield of plant (Robins and Doming, 1959). It seems that flowers abscission and young pods shedding are the main reasons for yield reduction under drought situations (Stoker, 1974). In bean cultivars, drought stress damage on yield components rely on plant growth stage and stress time course encountered (Fageria *et al.*, 2006). Plants own some intrinsic mechanisms to withstand drought conditions and for retaining of high water potential and/or avoidance of tissues water loss. Several studies conducted on cowpea revealed that beyond stress alleviation, plants had the potential to retain the normal leaf water potential during only three hours (Subramanian and Maheswari, 1992). However, this recovery potential greatly depends on cultivar and plant growing environmental conditions. Imam *et al.*, (2010) concluded that plant height, number of leaves, leaf area, number of pods, pod dry weight and total dry weight of both cultivars responded significantly to water stress conditions. Water stress also reduced stem height and reduced leaf area. Furthermore, it reduced pod dry weight in both cultivars and in 50 and 25% water stress levels, all plant pods of both cultivars were aborted. Also drought stress resulted in the reduction of the number of grain in the pod and the length of the plant (Zadehbagheri *et al.*, 2012). Our previous studies demonstrated that low water availability during vegetative growth period had the minimum adverse effect on yield potential of bean plants. At the same time water deficit conditions during flowering and pod formation period had the most deteriorative effects on plants yield components (Shekari, 2001; Shekari, 2006).

The aim of the present experiment was to evaluate the response of two bean plants to water deficit conditions during post flowering periods as well as recovery potential of plants beyond stress alleviation.

MATERIAL AND METHODS

Seeds of two red bean cultivars; 'Sayad' and 'Derakhshan' were afforded from Zanjan Legume Crops Research Station, Iran. Seeds were surface sterilized with 5% sodium hypochlorite for 2 minutes. Then, they were rinsed twice with distilled water. 5 Seeds were planted per pot (25 cm diameter and 45 cm height) filled with 10 kg common field soil at 3 cm depth. After germination and emergence, they were thinned to 3 plants per each pot. Table 1 shows the soil characteristics employed in the experiment.

Table 1. Physicochemical characteristics of soil used at the present experiment

Texture	Loam
Organic matter (%)	1.11
Total N (%)	0.005
Available P (ppm)	47.8
Available K (ppm)	748
pH	1.57
Soil bulk density (g/cm ³)	7.4
EC (Ds/m)	1.51
Saturation present	25

Field capacity of soil was determined by pressure plate set. Pots water content was maintained at FC until stress treatments application. Experiment was carried out in a greenhouse with ambient light of 800-1000 μ mole m⁻² s⁻¹, temperature and relative humidity, 20-30 °C and 50-70% respectively. Water deficit treatments were applied on plants based on weighing method until 50% of FC of soil containing in pots. Treatments were included:

C: regular irrigation or control.

F: water deficit during flowering stage when the first flower buds were observed on the plants.

P: water deficit during pod formation when the first pod was emerged from fertile flower, and Pf: water deficiency during pod filling period whenever pods attained 3-4 mm in diameter.

Experiment was arranged as factorial based on RCBD with four replications.

Pod numbers, seed number per pod and seeds weight were measured at the end of experiment. Leaf area was recorded with a leaf Area Meter (VM 900E/K, UK) beyond stress application. Dry weight of plants was measured beyond drying of weight fresh material in an air-forced oven at 70°C for 48 hrs. Net photosynthesis rate (μ mole CO₂ m⁻² s⁻¹) in middle leaflet of leaf before terminal end of stem measured with a portable photosynthesis meter set (IRGA, LCA4, England).

Data were subjected to analysis of variance by MSTATC software. Mean comparisons were carried out by Duncan's multiple range test at $P \leq 1\%$ and $P \leq 5\%$. Graphs were drawn by Microsoft office Excel

RESULTS AND DISCUSSION

The results showed that treatments had significant effects on all studied traits. Only interactive effect of cultivar and water deficit stress was not significant on plant height. 'Sayad' (41cm) had higher plant height compared to 'Derakhshan' (34 cm) (Figure. 1).

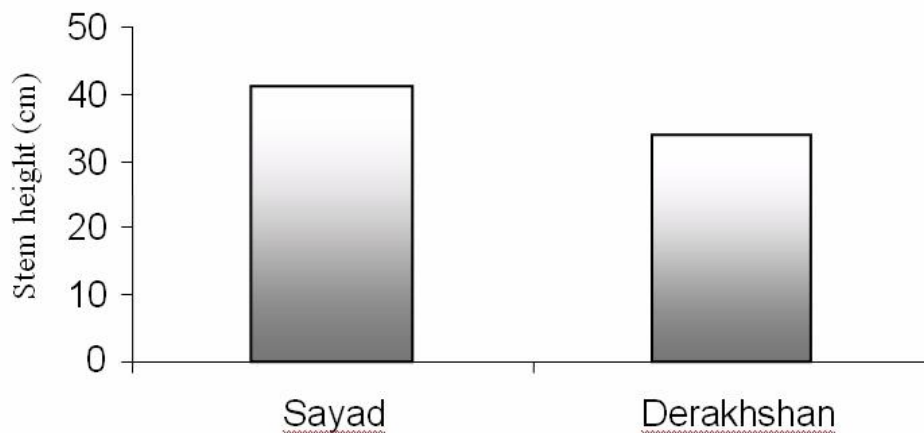


Figure 1. Cultivar differences in stem height of two red beans under water deficit.

Control plants (without stress treatment) had the highest plant length. In contrast, the lowest recorded data for plant height belonged to plants stress faced at flowering stage. Meanwhile, stress application during pod formation and seed filling period caused moderate plant height (Figure 2). In this experiment, data showed that water deficit stress encountered during flowering period had more deteriorative effect on plant height compared to other growth phases. There is evidence that flowering time-course is the most critical growth phase of plants affected by adverse growing conditions and environments (Martínez *et al.*, 2007). Grzesiak *et al.*, (1989) reported that plant height was dependent on node number of stem and internodes distance. Any growth retardant by stress conditions led to reduced internodes growth, expansion, and finally reduced height of plant. In a study on pinto bean, Shekari (2001) and Shekari (2006) reported that drought stress had the most negative effect on plant height during flowering period. Nielsen and Nelson (1998) reported same results on other bean cultivars as well.

There was significant and positive relationship between plant height and yield (Table 3) ($r = 0.758$). This means that any increase in plant height or situations encourage stems growth and extension led to increased yield and productivity of plants. This is due to increased number of nodes and/or auxiliary shoots and subsequently higher number of pods and finally yields improvement.

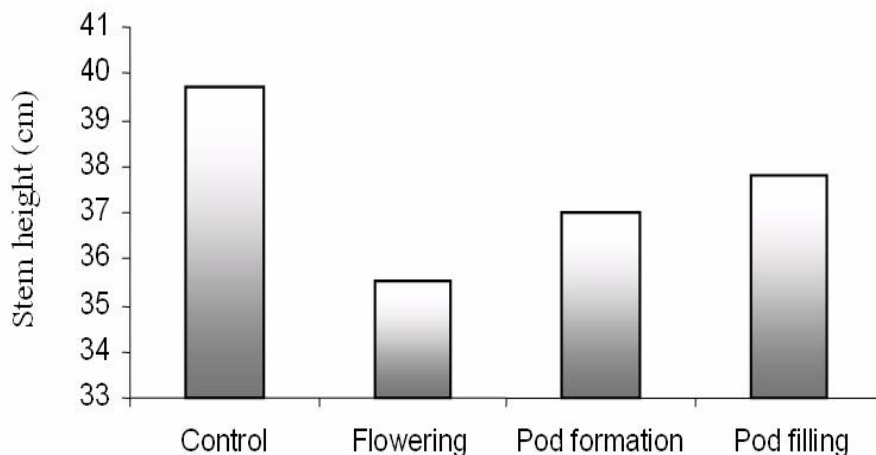


Figure 2. Effect of water deficit on stem height at different growth stages.

Table 3 shows that there was direct relationship between plant height with pod number, seeds number per pod, pod length, biological yield and harvest index. Contrarily, plant height and one hundred seeds weight had negative relationship. This means that any increase in pod number and seed number per pod resulted in reduced mean weight of individual seed owing to increased sinks for assimilates partitioning. In both cultivars, control plants had the highest number and the greatest pods as well. 'Sayad' had the superior amounts for those traits compared to 'Derakhshan'. Water deficit treatment during pod formation stage resulted in the lowest amounts for pod number and length (Table 2). Increase in pod length accompanies the higher number of seeds per pod and vice versa. A positive and significant correlation ($r = 0.74$) was found between pod length and seed number per pod (Table 3). The lowest number of pods in all stress treatments was recorded during flowering and pod formation growth stages. It seems that flowers abscission, newly formed pods abortion as well as reduced flowering period are the main reasons for reduced pod number per plant. Martínez *et al.*, (2007) concluded that yield component that was more affected by the water stress treatments was the number of pods per plant. The reduction in this parameter was particularly important in cultivars Arroz Tuscola, and Barbucho where a 72 and 56% of reduction were observed, respectively. During the flowering and pod set periods, water stress exacerbation of the abortion of these organs is well documented (Graham and Ranalli, 1997; Ter'an and Singh, 2002). The results obtained from the present experiment are in well accordance with the findings of Nielsen and Nelson (1998) and Mouhouche *et al.*, (1998). Since, pod number per plant is one of the main yield components of bean plants, 'Sayad', with higher pod number attained the higher seed yield (Table 2).

Table 2. Mean comparison of measured characters of two red beans under water deficit conditions at different growth stages.

Cultivar	Stage	Pod length (cm)	Pod number	Seed / pod	100 seed weight (g)	Biological yield (g/m ²)	Seed yield (g/m ²)	HI
Sayad	Control	12.73	18.7	6.67	34.37	1111	548.0	49.51
	Flowering	8.8	12.73	5.23	24.63	918.6	380.8	41.45
	Pod formation	6.13	9.83	3.7	18.57	959.3	253.1	26.35
	Pod filling	9.2	14.67	5.47	25.87	1101	403.7	36.67
Derakhshan	Control	9.67	11.33	3.8	39.43	892.5	242.6	27.12
	Flowering	8.13	9.033	3.00	32.47	749.7	198.2	26.42
	Pod formation	7.7	8.87	3.13	31.37	930.0	218.6	23.47
	Pod filling	8.77	10.47	3.43	35.00	876.0	236.6	27.01
LSD(%5)		0.91	0.854	0.59	4.78	61.11	37.83	4.34

Table 3 shows that pod number had a strong correlation with yield ($r = 0.963$) compared with pod length ($r = 0.859$). In both cultivars water limitation during different growth phases led to lessened seed number per pod. The least amount for seed number per pod was recorded in plants stress faced during pod formation period. Stress encountered during flowering and pod-filling period had less negative effects regarding this trait. During pod filling period especially in lower nodes of plants drought stress had no significant effect on seeds number per pod owing to former development of pods and seeds. At the same time, young and newly formed pods affected by water deficit stress. Meanwhile, water deficit during pod formation period caused the greatest number of seed abortion in pods and in consequence the least number of seeds in both studied cultivars. Furthermore, water stress encountered during flowering period induced flower abscission and diminished fertile flowers as well as seed/pod. Same results on the negative effects of water deficit stress on seed/pod have been reported by Nielsen and Nelson (1998) and Shekari (2006). 100 seeds weight influenced significantly by cultivar effect. The highest amount for this trait belonged to 'Derakhshan' (39.4 g) under control condition. There was no significant difference between control plants and stress affected ones during pod filling period regarding above-mentioned trait (Table 2).

The least amount for 100 seeds weight belonged to 'Sayad' during water deficit stress at pod formation stage. It seems that, reduction in seed filling time-course and concomitant decrease in photo assimilates partitioning toward seeds is principle reasons for 100 seeds weight reduction during pod formation period. Furthermore, taking into account the related trait showed that water stress during any reproductive stage of growth had deteriorative effects on 100 seeds weight of both cultivars. Correlation coefficients revealed that 100 seeds weight had negative and non-significant relationship with nearly most of the traits.

Negative relationship between 100 seeds weight and seed number per pod is due to increased sink numbers and consequently reduced availability of seeds to assimilates which goes to diminished weight. Our findings are in conformity with the reports of Shekari (2006) and Ney *et al.* (1994). In contrast, Grzesiak *et al.* (1989) noted that drought stress dose not affected 100 seeds weight of been plants.

Mean comparisons showed that the highest amounts of seed and biological yield were full filled in the control plants of both cultivars. 'Sayad' had about two-fold higher seed and biological yield compared to 'Derakhshan' (Table 2). In the present experiment, drought stress during any growth stage reduced the yield of plants. In this case, the most sensitive growth stage for 'Sayad' and 'Derakhshan' were pod formation and flowering period, respectively (Table 2). In general, 'Sayad' was more susceptible to drought stress during all growth stage rather than 'Derakhshan'. This is evidenced by this fact that, harvest index of 'Sayad' during normal irrigation was about 50% but it decreased to 26% beyond water deficit stress at pod formation period. This realizes that biological yield was more stable than seed yield and the effects of water deficit treatments on seed yield were stronger than on biological yield. Under all conditions and treatments, 'Derakhshan' had lower yield relative to 'Sayad'. However, former cultivar had relative stability in its yield. So that, water deficit stress had little effects on yield reduction of 'Derakhshan' compared to control ones.

Seed yield and biological yield had a significant and positive correlation with together ($r = 0.95$). This demonstrates that any condition or cultivar could accumulate more dry matter, may produce higher seed yield.

The results showed that under all stress treatments 'Sayad' had higher photosynthetic rate compared to 'Derakhshan' (Figure 3-5). Stress application at the flowering stage was longer (12 days) than two other stages. During this growth stage, net photosynthetic rate of 'Sayad' showed declining pattern. Its initial recorded rate was $3 \mu \text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. However, in the last measurement at the end of stress time course its amount was about half of initial rate. In addition, during remaining stages of growth, photosynthesis potential of stress faced plants of this cultivar had no significant difference with its control counterparts. In contrast, photosynthetic rate reduction of 'Derakhshan' was slower beyond stress application. At the same time, after irrigation and water supply, recovery potential of those plants was very low and stress encountered plants had lower photosynthetic rate compared to control ones. Same trend was recorded in plants under stress at other two growth stages but with some differences. Photosynthesis rate in pod formation period was higher than flowering period. However, its rate showed decreasing trend from flowering till pod filling period.

Increased demand for photo-assimilates and elevated photosynthesis potential of individual leaves could be as the principle reasons for intensified photosynthesis potential of plants during pod formation period. Contrarily, it is possible that leaf senescence and related low photosynthesis potential of leaves

are the chief factors for declined CO₂ fixation capability of plants during pod filling period.

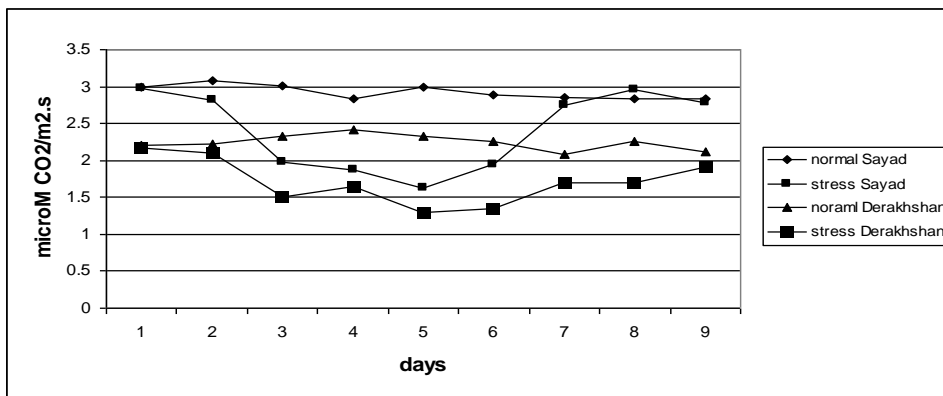


Figure 3. Changes in photosynthetic rates during flowering in two red bean cultivars under water deficit conditions.

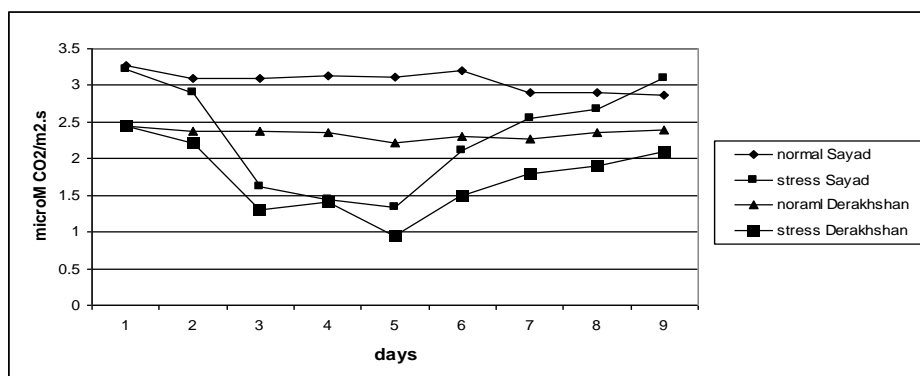


Figure 4. Changes in photosynthetic rates during pod formation in two red bean cultivars under water deficit conditions.

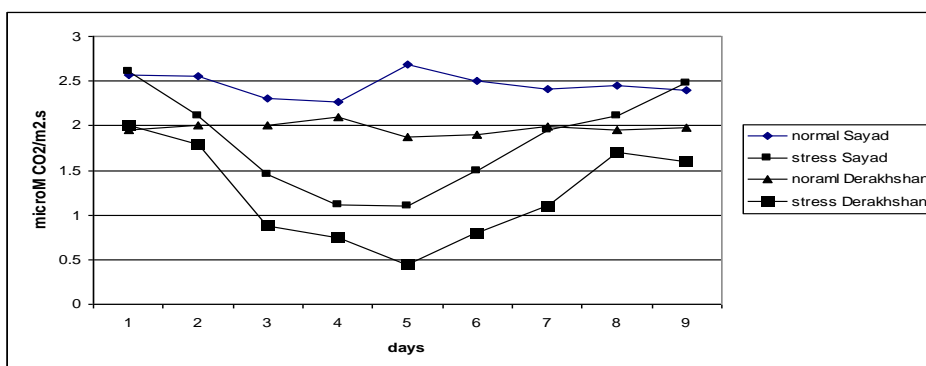


Figure 5. Changes in photosynthetic rates during pod filling in two red bean cultivars under water deficit conditions.

According to photosynthetic rate and as mentioned above, recovery potential of 'Sayad' was higher than 'Derakhshan' beyond stress alleviation. It is reported the photosynthesis restricts by stomatal and non-stomatal resistances. Net CO₂ assimilation rate via photosynthesis is the initial step in biomass production². The limitation effects of water stress initially implicate by stomatal resistance (Hopkina, 1999 and Yordanov *et al.*, 2001). There is evidence that, growth inhibition due to stomatal closure is one of the first responses of plant to drought stress (Klamkowski and Treder, 2006). Net assimilation rate (NAR) is the critical physiological and biochemical component of plants to water deficit stress as well as the key regulatory step in drought tolerance management (Kruger *et al.*, 1995). Molnar *et al.* (2004) reported that elevated net photosynthesis rate and maintenance of photosynthesis potential in cultivated wheat's under drought conditions were of main strategies and breeding goals for better acclimatization to stress situations. Furthermore, reduced photosynthesis rate and enhanced leaf senescence by water deficit stress during seed filling period were the principle constituents of wheat seed yield reduction under stress conditions (Kobata, 1992).

Figures 3, 4 and 5 displays that with progress in growing period and towards late season, photosynthesis rate of both cultivars showed reducing pattern. So that photosynthesis rate in early flowering stage was $3 \mu \text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ reduced to 2.4 and $1.6 \mu \text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$ during pod filling period (in control plants and stress plants respectively).

Figures 3-5 show that by any progress in stress time, photosynthesis rate was greatly more declined. Anyia and Herzog (2004) reported that prolonged drought stress on cowpea reduced its photosynthesis, transpiration and stomatal conductance but increased CO₂ internal concentration in leaves. They state that this might be the result of leaflets senescence. In addition, decreased assimilation rate was greatly due to stomatal closure. Meanwhile, non stomatal factors may have instant effects on CO₂ assimilation. It is possible that, diminished recovery potential in 'Derakhshan' beyond irrigation might be due to damage on photosynthesis apparatus.

CONCLUSIONS

Overall, data showed that drought stress had great impact on number and length of pods, seed number per pod, 100 seeds weight and finally yield of plants. It seems that pod number and seed number per pod had higher effects on yield than one hundred seeds weight. Higher harvest index reduction of 'Sayad' from 49.5% in control to 26.4% during stress at pod formation period showed that 'Sayad' was more susceptible to drought stress in contrast to 'Derakhshan'. Furthermore, photosynthesis data showed that CO₂ assimilation as well as post-stress recovery potential of 'Sayad' was higher than 'Derakhshan' (figs. 3-5). In total, it seems that in order to selection and breeding of red bean cultivars for drought tolerance, tall cultivars with higher pods and seeds per pods as well as adaptability to mechanized harvest, are superior to other cultivars..

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REAGOVANJE DVIJE SORTE PASULJA (*PHASEOLUS VULGARIS* L.) NA KONTROLISANI DEFICIT VODE U FAZI RASTA NAKON CVJETANJA

SAŽETAK

Laboratorijski eksperiment sproveden sa ciljem da se procijeni reakcija dvije sorte pasulja, Sayad i Derakhshan, u uslovima deficita vode tokom rasta nakon cvjetanja i potencijal oporavka biljaka nakon stresne situacije izazvane sušom. Tretmani su podrazumijevali redovno navodnjavanje ili kontrolu, deficit vode tokom cvjetanja, deficit vode u toku formiranja mahuna i deficit vode tokom perioda rasta zrna mahune. Rezultati su pokazali da je visina biljke imala pozitivan uticaj na prinos sorti, tako da je visoka sorta Sayad imala veće prinose. Stresna situacija u fazi cvjetanja je imala najveći negativan uticaj na visinu biljke i kasnije na prinos. Najdužu mahunu i najveći broj mahuna, kao i najveći broj zrna u mahunama zabilježen je kod sorte Sayad. Stresna situacija tokom formiranja mahuna rezultirao minimalnim iznosom svih ispitivanih osobina u obje sorte. Stresna situacija izazvana tokom rasta sjemena je imala najmanji uticaj na broj i dužinu mahuna i broj zrna u mahuni. Ali masa 100 zrna je značajno smanjena. Najveći iznos za masu 100 zrna je zabilježen kod kontrolne sorte Derakhshan. U svim tretmanima, Sayad imao veću biološki i sjemenski prinos u odnosu na Derakhshan. Najmanja količina prinosa zabilježena je tokom stresnog perioda u formiranju mahune i tokom cvjetanja i kod sorte Sayad i sorte Derakhshan. Stresna situacija je mnogo više uticala na indeks žetve kod sorte Sayad. Podaci u vezi sa stopom fotosinteze pokazali su da je kod sorte Derakhshan do naglog pada fotosinteze u stresnoj situaciji. Osim veće otpornosti na stres i deficit vode, sorta Sayad je pokazala veći potencijal za oporavak nego sorta Derakhshan i ta sorta je bila u stanju da brzo povрати stopu fotosinteze. Generalno, stresna situacija je slabije uticala na stopu fotosinteze kod sorte Sayad.

Ključne riječi: pasulj, deficit vode, prinos, komponente prinosa, stopa fotosinteze