

ORIGINAL ARTICLE

**Effects of Water Deficit and Chitosan Spraying on
Osmotic Adjustment and Soluble Protein of Cultivars
Castor Bean (*Ricinus communis* L.)**

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The present study was aimed investigating the effect of water deficit and chitosan spraying on osmotic adjustment and soluble protein of cultivars castor bean under field condition. experiment was carried out as a split factorial based on randomized complete block design with three replications. The results showed that water deficit caused increase a significant ($P < 0.05$) in the concentration of sugars and proline content in the leaves of castor bean. The most amount of total soluble sugars obtain of Levels (D2: Water deficit in beginning of flowering stage, D3: Water deficit in beginning of seedling stage, 0.042%) and minimum amount related to treatment control (D1: complete Irrigation, 0.014%) and maximum proline content related to (D3: water deficit in beginning seedling stage) and minimum proline content related to (D1: complete Irrigation). Also water Deficit caused decrease a significant ($P < 0.05$) in Protein content. The mean comparison shows that maximum amount Protein related to (D1: complete Irrigation, 26.79%) and the minimum amount Protein obtain from (D2: Water deficit in beginning of Flowering stage, 21.04%). also a had cultivars between different a significant ($P < 0.01$) of total soluble sugars. Chitosan spraying no had a significant in osmotic Adjustment and soluble protein. The accumulation of the osmolytes can help the castor bean plant to maintain the cell turgor and the structural integrity of membranes. castor bean herb is drought tolerant, the experimental our, cultivars between no had a significant different of proline and protein content. But, cultivars between had a significant different of total soluble sugars, the result show that cultivar Ahvaz local the most amount of total soluble sugars. therefore suggested that Ahvaz Local cultivar in water deficit condition rate of other cultivar toleranter, we can be with attention Damghan Climate condition, there Cultivate Ahvaz local cultivar.

Key words: Castor bean / chitosan / Proline / Soluble Protein / Total Soluble Sugars

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Castor bean (*Ricinus communis* L.) is an important oilseed crop which produces an oil rich in ricinoleic acid, commonly over 80%. This property makes the castor bean a promising candidate for

bio fuel production as well as other industrial applications (Li *et al.*, 2010). Among the environmental stresses, drought stress is one of the most adverse factors of plant growth and

productivity, More than 70% of Total area of Iran is arid and semi arid (Rafiee, 2011). A decrease in osmotic potential in response to water stress is a well-known mechanism by which many plants can cope with drought conditions, Most organisms increase the cellular concentration of osmotically active compounds, termed "compatible solutes", under desiccation by drought or lowering of osmotic potential Proline and soluble sugar are important solutes for adaptation to low water potential (Saglam *et al.*, 2010). proline is one amongst the most important cytosolutes and accumulates in plants during the adaptation to various types of environmental stress, such as drought, salinity, high temperature, nutrient deficiency, and exposure to heavy metals and high acidity (Nazarli *et al.*, 2011). Free proline and sugar contents significantly increased in *Vigna radiata* nodules under drought, but nodules had more proline than leaves (Ashraf and Iram, 2005). Osmotic adjustment has been reported also in chickpea under water deficit conditions (Najaphy *et al.*, 2010).

Proteins are compounds of fundamental importance for all functions in the cell, It is well known that alteration of gene expression is always involved in preparing plants for an existence under stress. Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions, Under conditions of water deficit (dehydration) numerous processes are modified or impaired, Water stress affects the protein levels of plants but the results of different authors are contradictory. Some authors show decreased protein levels under water stress. (Bakalova *et al.*, 2008).

Chitosan, the deacetylated derivative of chitin, is one of the abundant, renewable, nontoxic and

biodegradable carbohydrate polymers, and available largely in the exoskeletons of shellfish and insects, chitosan has been widely applied as a functional biopolymer in food and pharmaceuticals (Hanafi, 2012; Cho *et al.*, 2010; Dong *et al.*, 2004; Dai *et al.*, 2009). Chitosan chemical structure, β -1, 4-linked polymer of D-glucosamine, chitosan does not get broken down or digested by human gastrointestinal enzymes. It is the most abundant natural polymer after cellulose (Lamiaa and Barakat, 2011). Chitosan can be made into gelatin, orb, fiber and membrane shapes for various uses, Moreover, because chitosan can be easily obtained and conforms to tissue engineering application requirements; can be implanted into human body and causes no harm; it is a notably suitable material for use in tissue engineering (Hsieh *et al.*, 2007). Chitosan seems to act as an stress tolerance inductor when directly applied to plant tissue, unchaining a hypersensible reaction and lignification, inducing lipid peroxidation, production of defense against pathogens (Ortiz *et al.*, 2007).

MATERIALS AND METHODS

Field studies were conducted during the spring of 2010, Damghan Branch , Iran. The experiment was performed in a split-factorial based on randomized complete block design with three replication. Water treatment, including three levels: control, cut of Irrigation in beginning flowering and seedling stage, cultivars used (Commerical, Ahvaz local, Mashhad local), chitosan spraying in 1 level: (control, Spraying in 5 g/l concentration). Were allocated as main and sub-plots, respectively.

Free proline

Proline was determined following Bates *et al.*, 1973. Fresh plant material (1-0.5 g) was homogenized in 10 ml of 3% sulfosalicylic acid and

the homogenate filtered. The filtrate (2ml) was treated with 2ml acid ninhydrin and 2ml of glacial acetic acid, then with 4ml of toluene, Absorbance of the colored solutions was read at 520 nm.

Total soluble sugars

The amino acids were determined in 50 mg of leaf dry matter powder incubated in 5 mL of sterile distilled water at 100 °C for 30 min. After being

homogenized, it was centrifuged at 2000 ×g for 5 min at 20 °C and the supernatant was removed. The quantification of the total soluble amino acids was executed at 570 nm according to Peoples *et al.*, 1989, and L-asparagine + L-glutamine (Sigma Chemicals) was used as a standard.

Total soluble proteins

The determination of the total soluble proteins was performed in 100 mg of leaf powder incubated in 5 mL of extraction buffer (Tris-HCl at 25 mM and pH 7.6). The mixture was kept in agitation for 2 h, after wards centrifuged at 2000 g for 10 min. at 20 °C and subsequently the supernatant was removed. The quantification of the total soluble proteins was carried out at 595 nm according to Bradford (1976) with albumin bovine (Sigma Chemicals) used as standard.

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and means were compared using Duncan's range test at P = 0.05. All calculations were performed in Statistical analysis Version SAS 9.1 (2007) (Mistake, 2009) software for Windows program and Excel software was used for drawing diagrams.

RESULTS

Soluble protein

The Result from the ANOVA statistical analysis indicated that Water deficit caused effect significant (P<0.05) in Protein content and the other between treatments in protein content no had a significant (*Table 1*). The mean comparison shows that maximum amount Protein related to (D1: complete Irrigation, 26.79%) and the minimum amount Protein obtain from (D2: Water deficit in beginning of Flowering stage, 21.04%) (*Table 2*). Water deficit caused decrease in protein content (*Figure 1*).

Total Soluble Sugars

The Result Shown that Water deficit caused increase a significant (P<0.05) in total soluble sugars, also a had cultivars between different a significant (P<0.01) and a had Factorial effect different Between(Water deficit *Cultivar) and (Water deficit *Cultivar*Solution) a significant (P<0.05).(*Table1*).

Table 2 shown that no had different a significant between water deficit levels but rate of Control treatment highest amount. The most amount of sugars obtain of water deficit Levels (D2:Water deficit in beginning of flowering stage, D3: Water deficit in beginning of seedling stage, 0.042%) and minimum amount related to treatment control(D1: complete Irrigation, 0.014%) (*Figure 1*). Also the mean comparsion shown that a had cultivars between a significant, the most amount related to cultivar (V2: Ahvaz local, 0.047%) and the lost amount related to cultivar (V1: commercial local, 0.017%). (*Table 2*). *Figure 2* shown the most amount carbohydrate related to (D3: Water deficit in beginning of seedling stage, V2: Ahvaz local cultivar, 0.063%) and the lost amount obtain from

(D1: complete Irrigation, V1: commercial cultivar, 0.001%).

Proline content

The results from the ANOVA statistical analysis indicated water deficit caused effect significant ($P < 0.05$) and no had significant other treatments.

(Table 1). The mean comparison indicated between treatment different Irrigation had a significant. maximum proline content related to (D3: water deficit in beginning seedling stage) and minimum proline content related to (D1: complete Irrigation). (Table 2), (Figure 1).

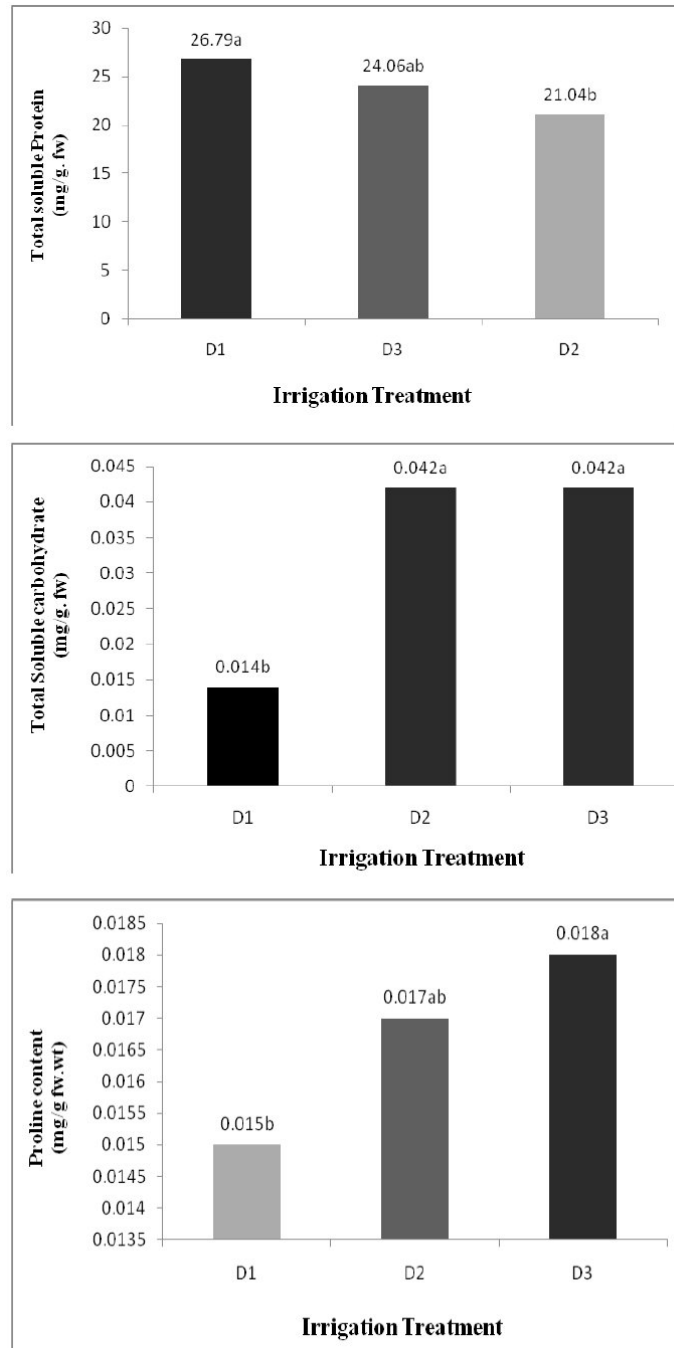


Figure 1: Effect water stress on soluble Protein, Total Soluble carbohydrate, Proline content. Letters on bars indicate results of Duncan’s multiple range test different letters on the histograms indicate that the means differ significantly ($P < 0.05$).

D1: complete Irrigation, D2: water deficit in beginning seedling stage, D3: water deficit in beginning flowering stage

Table 1: Analysis of variance (Mean Square) water Deficit, cultivars and spraying chitosan on studied traits

Source of Variation	DF	Protein (mgg ⁻¹ fw)	Total soluble sugars(mgg ⁻¹ fw)	Pro content(mgg ⁻¹ fw.wt)
Replication	2	13.6 ^{ns}	0.0001 ^{ns}	0.000007 ^{ns}
Water deficit	2	149.0*	0.004*	0.00005*
Main Error	4	13.0	0.0002	0.000007
Cultivar	2	7.7 ^{ns}	0.004**	0.0000008 ^{ns}
Spraying	1	2.02 ^{ns}	0.0001 ^{ns}	0.000001 ^{ns}
Cultivar*water deficit	4	18.6 ^{ns}	0.0005*	0.000004 ^{ns}
water deficit * Spraying	2	1.5 ^{ns}	0.00008 ^{ns}	0.000001 ^{ns}
Cultivar* Spraying	2	16.0 ^{ns}	0.00006 ^{ns}	0.000002 ^{ns}
Cultivar* water deficit * Spraying	4	1.4 ^{ns}	0.0003*	0.000002 ^{ns}
Error	30	11.2	0.00013	0.0000065
Coefficient of variation%		13.9	24.8	15.0

ns, *, and **, non-significant, significant at P < 0.05 and P < 0.01, respectively. Df. Degree of freedom

Table 2: Effect of various treatments Irrigation, cultivar, Spraying on studied traits

Treatments	Protein (mgg ⁻¹ fw)	Soluble Carbohydrate(mgg ⁻¹ fw)	Pro (mgg ⁻¹ fw.wt)
Irrigation			
D ₁	26.79a	0.014b	0.015b
D ₂	21.04b	0.042a	0.017ab
D ₃	24.06ab	0.042a	0.018a
Cultivars			
V ₁	23.4a	0.017c	0.017a
V ₂	23.7a	0.047a	0.016a
V ₃	24.7a	0.034b	0.017a
Solution			
M ₁	23.7a	0.031a	0.016a
M ₂	24.1a	0.034a	0.017a
Factorial Effect			
D ₁ V ₁	25.9abc	0.001g	0.020a
D ₁ V ₂	28.53a	0.028de	0.011a
D ₁ V ₃	25.95abc	0.014ef	0.016a
D ₂ V ₁	20.81d	0.035cd	0.02a
D ₂ V ₂	20.54d	0.050b	0.016a
D ₂ V ₃	21.78 cd	0.040bc	0.02a
D ₃ V ₁	23.74bcd	0.014f	0.018a
D ₃ V ₂	22.04bcd	0.063a	0.021a
D ₃ V ₃	26.40ab	0.047bc	0.018a

Data represent the mean values of three replicates. Within a column, mean values followed by different letters are statistically different based on Duncan's range test at P = 0.05.

DISCUSSION

Proline content

A common response to water deficit in plants is the accumulation of osmo protectants such as proline (Moradshahi *et al.*, 2004). Proline accumulation is responsible for the hydration of biopolymers surviving as readily utilizable energy source and serving as a nitrogen source compound during periods of inhibited growth (Kala and Godara, 2011).

A marked increase in proline content in the leaves of could be an indicator of its high drought tolerance (Ashraf and Iram, 2005). Proline accumulation is believed to play adaptive roles in plant stress tolerance (Mafakheri *et al.*, 2011). Also, Din *et al.*, 2011 found that metabolic factors such as free proline contents in leaves increased significantly under sever drought stress. Thus, it appears that increase in proline contents during drought stress induction is an adaptive mechanism

in castor oil.

Total Soluble Sugar

Soluble sugars is an important constituent and source of energy for all living organisms, plants manufacture this organic substance during photosynthesis and breakdown during respiration (Seyyednejad and Koochak, 2011). Under water stress condition the breakdown of polysaccharides caused an accumulation of soluble sugars which help maintenance of turgor (Nazarly *et al.*, 2011). Under drought conditions, the accumulation of soluble sugars seemed to be associated with drought tolerance in many plant species, soluble sugars also contributed to improving drought tolerance of peas, sugar beets and black poplars (Liu *et al.*, 2011). The accumulation soluble sugars the cell under stress by balancing the osmotic strength of the cytosol with that of the vacuole and the external environment (Abdalla, 2011). In order to tolerate drought stress, plants will accumulate high

concentration of low molecular-mass organic solutes such as soluble sugars or other amino acids to regulate the osmotic potential of cells aiming at improving absorption of water under drought stress (Abbaspour *et al.*, 2011).

Total Soluble Protein

It seems that decrease in soluble protein during drought stress was due to a severe decrease in photosynthesis. Photosynthesis decreased in Drought stress and Material for protein synthesis weren't provided, therefore, protein synthesis dramatically reduced or even stopped (Mohammadkhani and Heidari, 2007). The progressive reduction of total soluble proteins during water deficiency in the plants was induced by proteolysis, with the liberated amino acids used during the plant osmotic adjustment, This fact indicates a slow recuperation of this parameter probably because the proteins depend on other nitrogen compounds for synthesis (Costa and LoBato, 2009). our result are in agreement with those of (Iqbal and Bano, 2009; Bayramov *et al.*, 2010).

CONCLUSION

Progressive water deficit stress increased concentration of proline and soluble sugars in castor oil leaves. The accumulation of the osmolytes can help the castor bean plant to maintain the cell turgor and the structural integrity of membranes. suggested that lower accumulation of osmolyte function in protecting macromolecules either by protecting the tertiary structure of protein or by scavenging ROS (reactive oxygen species) produced in response to drought, the other soluble sugars content and proline improve stress tolerance by protecting and stabilizing membranes and enzymes during stress conditions. In generally, Osmotic

adjustment is a mechanism to maintain water relations and sustains photosynthesis by maintaining leaf water content at reduced water potentials. Damghan regions ingredient arid and semi arid in Iran, also castor bean herb is drought tolerant, the experimental our, cultivars between no had a significant different of proline and protein content. But, cultivars between had a significant different of soluble sugars, the result show that cultivar Ahvaz local the most amount of soluble sugars. therefore suggested that Ahvaz Local cultivar in drought stress condition rate of other cultivar Toleranter, we can be with attention Damghan Climate condition, there Cultivate Ahvaz local cultivar.

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