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Investigation on nutrients uptake of henna ecotypes under deficit irrigation and nitrogen

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Abstract

For investigation deficit irrigation and nitrogen application effect on nutrients uptake of henna ecotypes, this research was conducted in the form of split-split-plot on the basis of randomized complete block design with four replications in the Research Field of Islamic Azad University, Jiroft, Iran in 2013-2014. The main, sub and sub-sub plot were deficit irrigation (100, 75 and 50% of crop water requirement), ecotypes (Bami, Boushehri and Roudbari) and nitrogen amount (50, 100 and 150 kg/ha). Results revealed that deficit irrigation had a significant effect on calcium, sodium and potassium/sodium ($\alpha \leq 0.01$), and on potassium ($\alpha \leq 0.05$) absorption percent. Nitrogen application effect was significant for potassium and calcium/potassium absorption percent ($\alpha \leq 0.05$). There was no significant difference between evaluated ecotypes. According to mean comparison results, decreasing irrigation level from 100 to 50% of water requirement has led to increase in calcium, potassium and sodium absorption percent; On the other hand, it led to decrease in potassium ratio to sodium. Increasing nitrogen amount from 50 up to 150 kg/ha caused decrease in potassium and finally increased calcium/potassium ratio. It seems that increasing potassium, calcium and sodium in drought stress condition is one of the strategies for drought tolerance. For more details and applied results, it is recommended to evaluate relation between growth and nutrients uptake under water deficit and nitrogen application

***Keywords*:** Henna, ecotype; Water stress; Nitrogen; Nutrients absorption

1. Introduction

Lawsonia inermis is one of the medicinal and industrial plants which have been considered as one on the natural dyes. It has wide uses in textile, carpet weaving, paper making, tanning and cosmetic industries such as hair dye production and soap row (Chaudhary *et al*., 2010). In addition, this plant has pharmacological properties such as anti-fungal and anti-bacterial effect (Azadbakht, 2000). According to genial medicinal properties of henna, it is essential that enough research be done about plant reaction to environmental factors. Water stress is one of the environmental factors which limit plants growth in arid and semi-arid regions (Amerian *et al*., 2001; Johari-Pireivatlou *et al*., 2010; Enneb *et al*., 2015). According to water important role among the other environmental factors (Aliasgharzadeh *et al*., 2006), its lack affect many physiological processes such as photosynthesis, remotion of assimilate to seeds and nutrients transmission (Davis *et al*., 2007); In addition, it led to reduction in nutrients absorption from root and transmission to aerial organs. This could be due to reduction in transpiration rate and membrane permeability (Alam, 1999). On the other hand, lack of soil moisture led to reduction in nutrients distribution rate in soil to root absorption. According to researchers report about drought effect on sodium, potassium and phosphorus absorption, less access to nutrients is the reason of nutrients absorption decrease by plant root in soil. Uptake and nutrients transmission mechanisms in crops such as mass flow, diffusion and osmosis are as a function of the moisture amount in soil; in lack of humidity case, intense and amount of nutrients uptake would be changed (Alizadeh *et al*., 2009).

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Chemical fertilizer management can effectively influence plant productivity under drought and salinity stresses. Therefore, nutrients adding in the chemical fertilizer form can affect plant tolerance under stress condition and its amount (increase/decrease resistance or no effect) depend on the available water (Hussein *et al*., 2013). Viets *et al*. (1954) reported that nitrogen affected other nutrients uptake such as potassium, magnesium, calcium and phosphorous, and in some cases led to more absorption of some elements. Other research on amount of nutrient absorption revealed that water stress and nitrogen deficiency are two important factors in the production limiting (Sodayizadeh and Mansouri, 2015). Nutrients absorption amount depend on climatic condition, water supply, the plant absorption capacity of nitrogen, amount of available nitrogen, time and rate of nitrogen consumption.

According to medicinal importance of henna, evaluating the plant reaction under deficit irrigation has particular importance in nutrients accumulation and their role in improving drought tolerance. According to the conducted studies so far, no study has been done on drought and nitrogen application effect on nutrients *uptake* of henna; therefore, this project was conducted with the goal of evaluating nutrients uptake of henna ecotypes, and according to calcium, potassium and sodium role as stress perceived signal (Matsumoto *et al*. 2002), effective factor in photosynthesis, growth and water absorption (Abdel-Moez, 1996) and regulation osmotic balance (Wang *et al*. 2004), respectively, these nutrients were selected for evaluation.

2. Materials and Methods

The effect of different rates of N and deficit irrigation was studied on growth traits and nutrient absorption of henna ecotypes in a split-split-plot experiment based on a randomized complete block design with four replications in Research Farm of Islamic Azad University of Jiroft, Iran in 2013-2014 (Fig. 1).

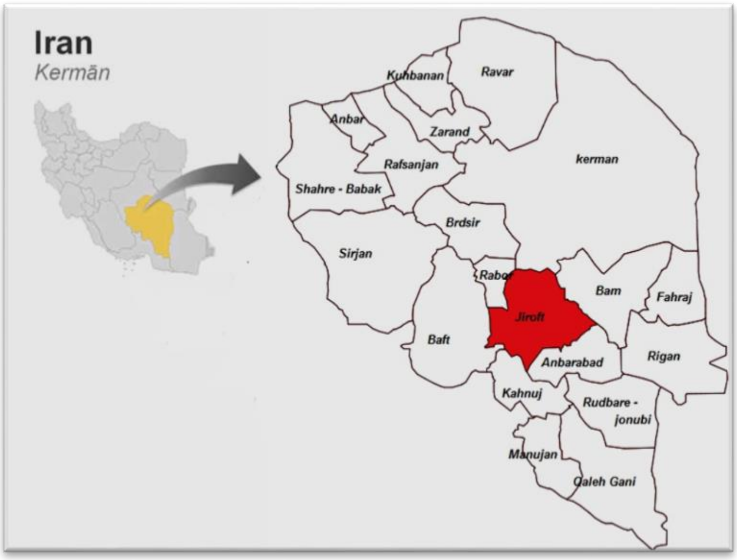


Fig. 1. Location of the studied area

2.1. Studied variables

The main plot was devoted to deficit irrigation at three levels (irrigation to supply 100%, 75% and 50% of water requirement), the sub-plot was devoted to N fertilization at three rates (50, 100 and 150 kg/ha from urea source) and the sub-sub-plot was devoted to ecotype at three levels (Bami, Boushehri and Roudbari) (Fig. 2). Before the experiment, two combined samples were taken from the soil profile (from four points as zigzag) at the depths of 0-30 and 30-60 cm to find out its physical and chemical status. The soil samples were analyzed as presented in Table 1.

2.2. Study seeds cultivation and field preparation

Since henna seeds have long dormancy period, gibberellic acid (1000 ppm) was applied to break their dormancy. The seeds were cultivated in germplasm in March. When the seedlings were reached to 15 cm, they were transferred to the main farm. The plots were prepared with 6 sowing rows that their length and interval were 6 m and 50 cm, respectively. The main plots were spaced 3 meters apart, the sub-plots were spaced 1 meter apart and the sub-sub-plots were spaced 75 cm apart. 2 m spacing was left between replications. The deficit irrigation treatments were applied by evaporation pan.

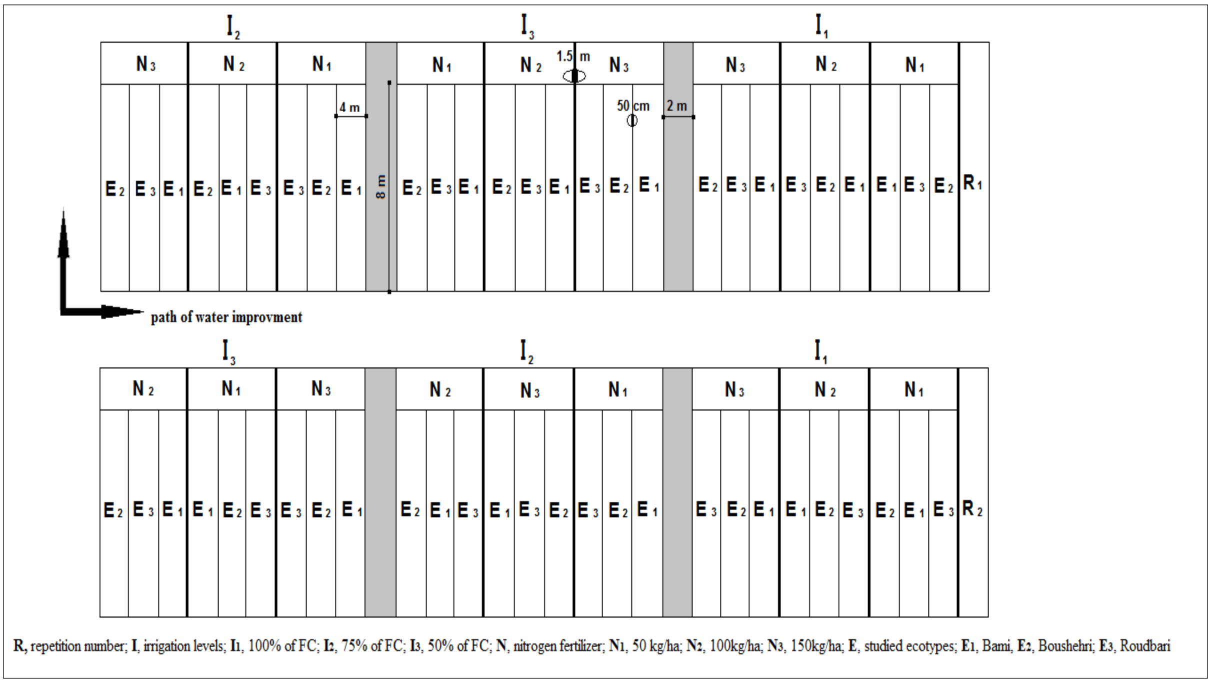


Fig. 2. Schematic figure of the plots

Table 1. Results of soil analysis of the studied farm

Soil texture	pH	EC (dS m^{-1})	Absorbable K (ppm)	Absorbable P (ppm)	Total N (%)	Organic C (%)	Depth
Loam-sandy	7.6	1.64	240	4.2	0.023	0.115	0-30
Loam-sandy	7.6	2.01	230	6.5	0.03	0.02	30-60

2.3. applied irrigation methods

It should be noted that local farmers irrigate henna in traditional way and in every 7 or 10 days but in this project, the amount of used water was calculated on the basis of daily evaporation from evaporation pan and the different levels of deficit irrigation treatments were calculated by Eq.

Eq. (A.1): $ET_0 = K_p \times E_{pan}$ (1)

where, ET_0 is evapotranspiration of base crop, K_p is the evaporation from pan, and E_{pan} is coefficient of pan (assumed as to be 0.7). Then, the amount of water to apply was calculated by Eq.

Eq. (A.2): $ET_c = ET_0 \times K_c$ (2)

where, ET_C is the water used by plant, and K_c is crop factors which was assumed to be 1.1 given the fact that henna is a shrub. Then, the amount of irrigation water was determined for each treatment and was applied by a water volume counter according to the area of the plot.

2.4. Nitrogen application

N fertilizer was applied at three phases (1: two weeks after seedlings transmission, 2: one month later and 3: before flowering). Local farmers use nitrogen in side-dressing form and after each harvesting (150 to 200 kg/ha each time). The studied ecotypes were procured from agriculture research centers of southern Roudbar, Boushehr and Bam.

2.5. Nutrients measurement

The leaves elements were measured in peak of vegetative stage. For sodium and potassium measurement, a part of plant samples were converted to ashes in an electric furnace with temperature of 550 °c and then digested with 02.0 normalized of hydrochloric acid. The extract was used for measuring sodium and potassium concentration by Flame Photometer, and calcium by titration method (Emami, 1996).

2.6. Statistical analysis

After ensuring data normalization, variance analysis and mean comparison were done by SAS (Ver. 7) and MSTAT-C software, respectively.

3. Results and Discussion

3.1. Calcium percent

Variance analysis indicated that deficit irrigation was significantly affected calcium amount in leaves ($\alpha \leq 0.01$). There was no significant difference between nitrogen amounts, ecotypes and treatments interaction on it (Table 2).

Table 2. Analysis of variance of the studied traits of *Lawsonia inermis*

SOV	df	Means of squares					
		calcium	potassium	sodium	Calcium/Potassium	Calcium/Sodium	Potassium/Sodium
Replication	3	0.01ns	0.03ns	0.004ns	0.02ns	0.22ns	6.20*
Deficit irrigation (A)	2	0.58**	0.53*	0.05**	0.09*	0.92ns	19.36**
Error A	6	0.01	0.05	0.003	0.01	0.29	0.90
Nitrogen (B)	2	0.007ns	0.11*	0.001ns	0.03*	0.001ns	5.11ns
A × B	4	0.00ns	0.01ns	0.001ns	0.002ns	0.02ns	0.41ns
Error B	18	0.004	0.02	0.002	0.007	0.53	3.86
Ecotype C	2	0.003ns	0.05ns	0.003ns	0.003ns	0.70ns	2.47ns
A × C	4	0.01ns	0.006ns	0.016**	0.007ns	0.84ns	13.22**
B × C	4	0.004ns	0.02ns	0.002ns	0.002ns	0.69ns	4.69ns
A × B × C	8	0.009ns	0.02ns	0.001ns	0.01ns	0.52ns	0.64ns
Error C	54	0.007	0.04	0.003	0.009	0.62	2.76
CV (%)	-	11.77	15.65	20.74	17.71	26.23	28.67

ns, * and ** show non-significance and significance at the 5 and 1% levels, respectively.

According to means comparison, calcium concentration was soared by increasing irrigation intervals. 50% of water requirement led to significant soar in comparison with others. It was increased from 0.59 (in 100% water requirement) and 0.67% (in 75% water requirement) to 0.84% (Table 3).

Table 3. Means comparisons of irrigation and nitrogen levels effect on nutrient absorption of *Lawsonia inermis*

	Calcium (%)	Potassium (%)	Sodium (%)	Calcium/ potassium	Calcium/ sodium	Potassium/ sodium
Irrigation levels (water requirement %)						
100	0.59b	1.25b	0.20b	0.48b	3.12a	6.64a
75	0.67b	1.28ab	0.25ab	0.53a	2.82a	5.33b
50	0.84a	1.47a	0.27a	0.58a	3.08a	5.41b
Nitrogen amount (kg/ha)						
50	0.69a	1.40a	0.24a	0.50b	3.00a	6.19a
100	0.69a	1.32b	0.24a	0.53ab	3.01a	5.76a
150	0.72a	1.29b	0.25a	0.56a	3.08a	5.44a

Means with similar letter(s) in each column did not show significant differences in Duncan's Multiple Range Test.

Sardans and Uelas (2008), Afsharmanes *et al*. (2009) and Hussein and Safaa (2013) reported that drought stress caused increase in calcium percent of aerial organs. According to Matsumoto *et al*. (2002), sever osmotic stress led to increasing in cytoplasmic calcium and had stress perceived signal role. By abscisic acid synthesis and transmission to leaves caused by stress, calcium channels in stomata guard cells would be activated and stomata would be closed (Abdul-Majid *et al*., 2007). Whereas, no significant difference of leaves calcium between irrigation and non-irrigation condition was reported in some other research (Palomo *et al*., 1999). Differences between reported results could be related to plants need of calcium, levels and types of used fertilizer, seasonal and climatic condition and type of studied plant.

3.2. Potassium percent

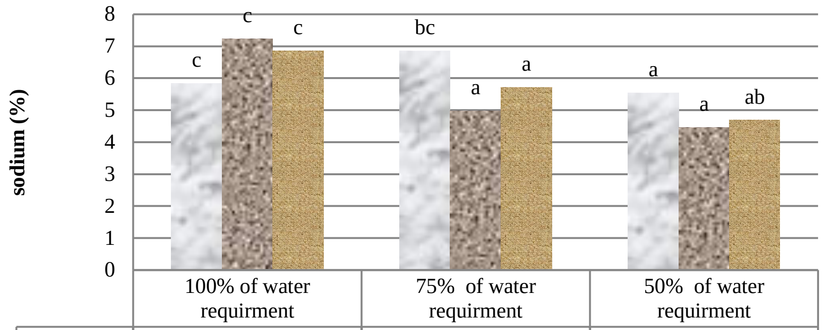
According to variance analysis (Table 2), Potassium percent was significantly affected by deficit irrigation and nitrogen amount ($\alpha \leq 0.05$).

Increasing irrigation intervals and water stress through it led to addition potassium; it was significantly increased from 1.25% (in 100% water requirement) to 1.47 (in 50% water requirement). On the other hand, increasing nitrogen level from 50 to 100 kg/h led to decrease in leaves potassium accumulation. There was no significant difference between 100 and 150 kg/ha of potassium consumption on it (Table 3). Our results were matched with Lionel-Jordan and Sylvain (2004) findings on potassium increasing under drought stress. Under drought condition, crop increase potassium concentration in roots and aerial organs to heighten resistance to energy consumption and this have positive effect on photosynthesis, growth and water absorption (Abdel-Moez, 1996). Ashraf *et al*. (2002) reported that sodium and potassium absorption were increased under deficit irrigation due to regulation of osmotic pressure and potassium role in the control of stomata opening and closing. Generally, increasing drought stress caused decrease in some elements absorption (like nitrogen) from roots and transmission to aerial organs but it increased potassium absorption (Omid and Habib, 2010).

3.3. Sodium percent

Different irrigation levels and interaction between irrigation and ecotypes had significant effect on sodium percent (Table 2).

Results of Table 3 revealed that intensification drought level caused increase in leaves sodium and the highest percent was observed in 50% water requirement (Table 3). Sodium percent changes in studied ecotypes under deficit irrigation showed that it went higher in all ecotypes by increasing irrigation intervals from 100% to 75% water requirement. Boushehri and Roudbari ecotype had more sodium than the other one (Fig. 3) but no significant difference was observed between them by reaching to 50% of water requirement.



	100% of water requirment	75% of water requirment	50% of water requirment
Bami	5.84	6.86	5.54
Boushehri	7.23	4.98	4.46
Roudbari	6.86	5.71	4.69

Fig. 3. Means comparison of the interaction between irrigation and ecotype on sodium percent of *Lawsonia inermis*

This results were consistent with Sener and Dorothy (2012) findings. Akhundi *et al*. (2007) reported that sodium and potassium of *Medicago sativa* aerial organs increased under drought condition. Sodium is soluble cations in the soil of arid and semi-arid regions. Most of the plants are sensitive to sodium high concentration because it disrupts ions stability inside the cell and lead to membrane bad function and weakening of metabolic reactions (Wang *et al*. 2004); On the other hand in many of the xerophytes, sodium plays major role in regulation of osmotic balance with entering into vacuoles. In this way, many of xerophytes or drought resistant plants tolerant sodium temporary increase in apoplast by raising water amount of mesophyll cells. So, salts be more diluted and the crops increase their capacity for salt absorption from apoplast solution (Heydari, 2001). Bohnert *et al*. (1999) believe that sodium increase in drought condition and the plant try to get it out or send it to vacuole for blockage toxicity. Abbasi and Ebadi (2012) reported that nitrogen consumption had no significant effect on calcium and sodium absorption percent which were matched with our results.

3.4. Calcium/potassium

Results of variance analysis indicated that the calcium ratio to potassium significantly changed under deficit irrigation and nitrogen levels ($\alpha \leq 0.05$).

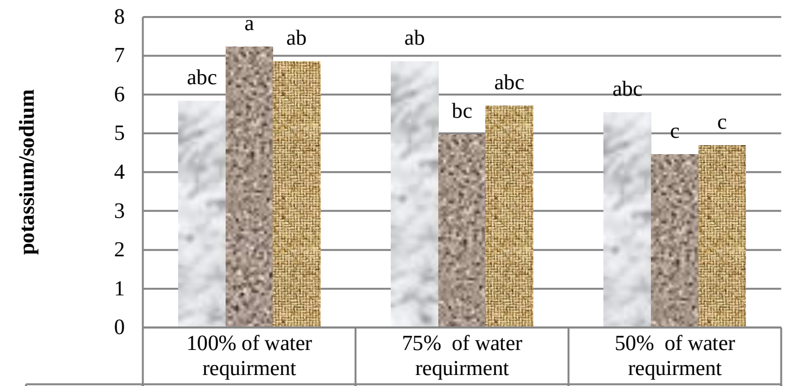
By increasing water stress level, the calcium ratio to potassium was enhanced due to two ions increasing under this condition. Moreover, this ratio was increased by adding nitrogen consumption which led to potassium percent reduction. According to potassium location in the denominator of ratio, it was increased. Researches revealed that nutrients amount decrease in aerial organs but calcium ratio to potassium increased under drought condition (Sener and Dorothy, 2012).

3.5. Potassium/sodium

According to variance analysis (Table 2), none of the studied treatments had significant effect on the calcium ratio to sodium. Deficit irrigation and interaction between irrigation and ecotype significantly affected potassium/sodium ($\alpha \leq 0.01$).

The ratio decreased by increasing water stress intensity which was matched with Ahkundi *et al*. (2004) findings about *Medicago sativa* changes under water deficit. They reported that potassium, sodium and calcium concentration of aerial organs increased in low water condition but the potassium ratio to sodium decreased. According to Yarnia (2003) report, the potassium ratio to sodium and calcium to sodium of *Medicago sativa* aerial organs were important criterion in diagnosis of salinity resistance and tolerant varieties showed higher potassium ratio to sodium.

The highest ratio amount was observed in Boushehri ecotype but there was no significant difference between it and other ecotypes under 100% of water requirement (Fig. 4).



	100% of water requirment	75% of water requirment	50% of water requirment
Bami	5.84	6.86	5.54
Boushehri	7.23	4.98	4.46
Roudbari	6.86	5.71	4.69

Fig. 4. Means comparison of the interaction between irrigation and ecotype on potassium/sodium of *Lawsonia inermis*

Potassium ratio to sodium was significantly decreased in Boushehri and Roudbari ecotype by increasing irrigation intervals up to 50% of water requirement. Changing irrigation levels had no significant effect on Bami ecotype. Potassium absorption and transmission decrease in potassium-rich environment and increase by potassium adding to sodium-rich soils; finally, lead to plant yield increase. It should be noted that maintaining proper sodium concentration in salinity condition is necessary for plant (Pasban *et al*., 2008). Khan *et al*. (1997) reported that potassium concentration of herbal tissues slake with increasing sodium or sodium ratio to calcium in root environment. Sodium ratio to potassium in leaves have close resistance with salinity tolerance (Shachtman *et al*., 1992).

Generally, results of this project showed that henna resist getting drought by increasing calcium, potassium and sodium ions accumulation. Adding nitrogen levels from 50 up to 150 kg/ha led to potassium percent decrease and increase sodium ratio to potassium, but it had no significant effect on calcium and sodium absorption. For more details and applied results, it is recommended to evaluate the relation between growth and nutrients absorption under water deficit and nitrogen application.

3.6. Correlation coefficient

According to correlation between measured traits of henna (Table 4), there was significant positive correlation between calcium and potassium which was matched with findings of Alkateeb (2006). Calcium showed negative and significant correlation with sodium, sodium ratio to potassium, sodium ratio to calcium and potassium ratio to calcium. The highest significant positive correlation was observed between sodium ratio to calcium and to potassium. Significant negative correlation between sodium and potassium have been reported in *Brassica napus* (Asghari *et al*., 2012) and *Hordeum vulgare* varieties (Pakniyat *et al*., 1997) under salinity stress. Significant positive and negetive correlation of potassium has been reported on sodium ratio to potassium and on sodium, respectively (Rajabi *et al*., 2006) which was consistent with this project results. Charlengbadil *et al*. (2015) reported that sodium had high positive correlation with calcium, potassium ratio to sodium had negative correlation with sodium of *Saccharum Officinarum* leaves which were matched with our results.

Table 4. Correlation between studied traits of *Lawsonia inermis*

	potassium	sodium	Sodium/potassium	Sodium/calcium	Potassium/calcium	
potassium	1					
sodium	-0.25*	1				
Sodium/potassium	-0.34*	0.90**	1			
Sodium/calcium	-0.60**	0.90**	0.87**	1		
Potassium/calcium	0.41**	0.26*	0.51**	-0.41**	1	
calcium	0.39*	-0.47**	-0.77**	-0.54**	-0.66**	1

ns, * and ** show non-significance and significance at the 5 and 1% levels, respectively.

4. Conclusion

In arid and semi-arid regions, occurrence probability of abiotic stresses as drought is high. This factor caused changes in nutrients uptake, and henna increased calcium, potassium and sodium accumulation in its leaves to reduce drought effect and their highest amount belonged to 50 % of water requirement. However, different varieties and ecotypes show various tolerance ranges, and Bami ecotype had the highest sodium percent and potassium to sodium ratio in water deficit condition. Nitrogen application was significant for potassium uptake and increasing its level from 50 to 100 kg/ha reduced potassium accumulation; although, there was significant positive correlation between potassium and calcium but this correlation could not significantly affected calcium percent. It seems that when the plant accumulation of potassium reduced, it increased calcium percent for resistance drought. According to the results of this project, Bami ecotype is introduced as the best ecotype for cultivation in arid and semi-arid regions with similar agro-climate condition. For more details and applied results, it is recommended to evaluate relation between growth and nutrients uptake under water deficit and nitrogen application.

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