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Study irrigation deficit and N fertilizer effect on reproductive components of henna ecotypes in Jiroft, Iran

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ABSTRACT: Water deficit stress and N fertilizer effect on reproductive growth of henna ecotypes were studied in the split-split-plot form on the basis of a randomized complete block design with four replications in 2013-2014 growing season. The main, sub and sub-sub-plot were devoted to deficit irrigation (100, 75 and 50% of water requirement), N fertilizer (50, 100 and 150 kg/ha) and ecotypes (Bami, Boushehri and Roudbari), respectively. The highest seed number and1000-seed yield belonged to 50% of water requirement and water stress increasing (up to 50% of water requirement) resulted in a severe loss of seed yield at all fertilizer levels especially under 150 kg/ha. According to the results, full irrigation with 100 kg/ha of N had the greatest effect on improving reproductive growth of henna; In addition, it can be said that under water deficit conditions, higher N rates should be avoided because they did not absorb.

Keywords: henna ecotypes, water deficit stress, nitrogen, seed yield, *Lawsonia inermis*

INTRODUCTION

Today, human beings rely on a wide range of at least 6000 species for different consumptions. Agricultural studies are naturally focused on main crops, and other plants are rarely studied (Pourdad, 2006). The over-emphasis of agriculture sector officials on a few strategic crops and ignoring other crops has resulted in the loss of agriculture biodiversity. Today, the importance of medicinal herbs is obvious, and they have substituted chemicals in some countries. According to the WHO report in 2003, about 80% of the population of developed countries could not produce their required medications (Orwa *et al*., 2009); Meanwhile, henna (Lawsonia inermis) is an industrial and medicinal herb which is considered as a natural dye and is widely used in different industries including textile, cosmetic, paper, and tannery. Moreover, henna has sterilizing property for skin and hair cleaning and cooling (Chaudhary *et al*., 2010). Anti-fungal and antibacterial effects are henna's pharmaceutical properties (Azadbakht, 1999).

Population growth and climate changes have posed a tough challenge for plant researchers and breeders in the 21st century to produce useful plants in water-limited environments, whereas 75% of global water consumption is dedicated to agriculture (Molden, 2007). On the other hand, about 26% of arable lands in the world are located in arid regions (Atlin and Frey, 1990). In addition, the fluctuation of precipitation distribution due to global warming may increase the risks of plants exposure to drought. Almost all plant species have a degree of drought stress tolerance, but the tolerance varies with cultivar and variety (Larcher, 2003). Plants are exposed to different types of stresses in natural environmen and their growth is negatively affected by them. Temperature, light, available water, and nutrients are examples of abiotic factors effectively influencing the growth of higher plants. Among these factors, drought is the main factor that limits crop production (Reddy *et al*., 2004). Drought significantly influence the plants survival and yield through changing their morphology and physiology (Claeys and Inzé, 2013). Furthermore, studies show that water deficit can negatively affect plant organs growth and its morphology (Jamal *et al*., 2014). In a study on the effect of three irrigation levels on the morphology of henna, Enneb *et al*. (2015) found that leaf area, leaf number and stem length were decreased by increasing irrigation intervals. In total, yield potential of henna is closely related with water availability.

Although other factors like nutrients can influence its yield potential, these factors themselves can be limited by water availability. According to the studies, water deficit affects N metabolism in plants too (Tarighaleslami *et al*., 2012). It is necessary to supply the plants with adequate N under stress conditions. On the other hand, suitable N amount can improve drought tolerance of the plants under stress conditions by improving their water relations and photosynthesis (Zhang *et al*., 2007). Studies indicated that N consumption in soil can affect nitrate concentration in plant latex that resulting in higher pH and abscisic acid. This increase reduces stomatal conductance under drought stress (Haffmann and Kosegarten, 1995; Sharp and Davies, 2009). The interaction between N and water availability has a close relationship with plant type, N form and sowing method (Guo *et al*., 2007). Khosk *et al*. (2004) reported that on time and proper application of chemical fertilizers was one of the factors that improved henna yield. They revealed that 120 kg $ha^{-1}a$ of N and 60 kg $ha^{-1}a$ of P resulted in 225 cm higher plant height, 27.84 more number of auxiliary branches, 175.9 g higher fresh leaf weight, 52.7 g higher leaf dry weight per plant and the highest dye intensity (1.29 m/u). In a study on the effect of density and N on henna, Askari *et al*. (2012) reported that the highest number of leaves per plant (90 leaves/plant) was obtained from the density of 7 plants/m2 treated with 100 kg ha^{-1} of N and that it was about 85 leaves/plant at the density of 11 plants/m^2 treated with 50 kg ha^{-1} of N. The highest leaf dry weight (about 20 g) was obtained from the density of 8 plants/m^2 treated with 100 kg ha^{-1} of N. In a study on *Silybum marianum*, Agharahimi (2002) obtained the highest number of fruits, seeds per fruit, auxiliary branches and yield per ha from N rate of 150 kg $ha^{-1}a$.

Given the fact that henna has appropriate price stability and economical prosperity in Jiroft as compared to other crops, it is regarded as a low-risk crop. Therefore, it is important to recommend a suitable N rate and ecotype of henna with the capability of producing higher yield under climatic and economical conditions, precipitation deficiency and the fall of groundwater in the studied region.

MATERIALS AND METHODS

The effect of different rates of N and deficit irrigation was studied on growth traits, quantitative and qualitative yield 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. 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^{-1} from urea source) and the sub-sub-plot was devoted to ecotype at three levels (Bami, Boushehri and Roudbari). 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 estimated as presented in Table 1.

Table 1: Results of soil analysis of 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

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. The amount of water to apply was calculated on the basis of daily evaporation from evaporation pan and the different levels of deficit irrigation treatments were calculated by the following formula:

$$ET_0 = K_p \times E_{pan}$$

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

$$ET_c = ET_0 \times K_c$$

where, ET_C was the water used by plant, and K_c was 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. Also, N fertilizer was applied at three phases (the first phase about 2 weeks after the transfer of the seedlings, the second phase about one month later and the third phase before flowering). The studied ecotypes were procured from agriculture research centers of Southern Roudbar, Boushehr and Bam.

The recorded traits were flowering branches number, mean inflorescence number, fruits number per inflorescence, fruits number per plant, seeds number per fruit, seeds number per plant, 1000-seed weight and seed yield. These traits were measured on five plants which were randomly sampled from the plots. To measure 1000-seed weight, the seeds were counted by seed-counting machine and weighed by a 0.001-precision scale. Seed yield was estimated on an area of 10 m^2 during which the plants were flailed to have the seeds separated. Then, they were weighed. The analysis of variance and means comparison were done by SAS software package (Ver. 7) and MSTAT-C software package, and the graphs were drawn by MS-Excel software package.

RESULTS AND DISCUSSION

A. Flowering branches number

Analysis of variance revealed (Table 2) that the number of flowering branches was affected by various levels of deficit irrigation ($\alpha \leq 0.01$), N amount ($\alpha \leq 0.01$) and ecotype ($\alpha \leq 0.05$). In addition, the interaction of deficit irrigation × N, deficit irrigation × ecotype, and deficit irrigation × N × ecotype was significant for this trait ($\alpha \leq 0.01$).

Means comparison for the interaction between these three factors showed (Table 3) that under 100% of water requirement and 100 kg ha^{-1}a of N application resulted in the highest flowering branches number in Bami and Roudbari ecotypes. Furthermore, more intense water stress, i.e. irrigation to supply 75% of water requirement and 100 kg ha^{-1} of N application resulted in the highest flowering branches number in Bami and Boushehri ecotypes. Although irrigation to supply 75% of water requirement resulted in lower branches in Roudbari ecotype than irrigation to supply 100% of water requirement, this decrease in branch number was not statistically significant. The increase or decrease in N rate at irrigation levels to supply 100 and 75% of water requirement reduced flowering branches number and showed significant differences with that 100 kg ha^{-1} of N. However, as irrigation was decreased to supply only 50% of water requirement, N rates had no significant effect on flowering branches number of the studied ecotypes (Table 3).

Nonetheless, as water deficit was increased, the efficiency of N application reduced without any significant effects on the improvement of the studied traits. Water stress reduced photosynthates production, auxiliary stems number and consequently auxiliary branches number. These results are consistent with Oveisi Omrani (2013).

B. Inflorescence number

Inflorescence number per plant was significantly affected under deficit irrigation condition ($\alpha \leq 0.01$), N amount ($\alpha \leq 0.05$) and the interactions of deficit irrigation × N, N × ecotype, and deficit irrigation × N × ecotype at the 1% level (Table 2). According to the means comparison (Table 3), although the highest number of inflorescence was related to Bami ecotype under irrigation to supply 100% of water requirement and 100 kg ha-1 of N, no significant differences were observed in inflorescence number between Bami and other ecotypes under irrigation to supply 75% of water requirement and three N fertilization rates. Leaves start shedding under moisture stress during vegetative period and then, they acquire the moisture required for their survival from the stem and even petiole. As a result, fruit formation and growth, seed-setting, and seeds number per fruit and per plant decreased considerably (Aliari and Shekari, 1996).

Table 2: Analysis variance of the studied traits.

SOV	df	Means of squares							
		Flowering branches/plant	Inflorescences/plant	Fruits/inflorescence	Fruits/plant	Seeds/fruit	Seeds/plant	1000-seed weight	Seed yield
Replication	3	67.09	25.06^{ns}	39.25^{ns}	27655.59^{ns}	18.58^{ns}	7431051.49^{ns}	0.002^{ns}	3204.12^{ns}
Deficit irrigation (A)	2	3233.02**	1671.06**	928.01**	2526963.45**	1129.06**	1847188134.17**	0.05**	3161709.93**
Error A	6	175.59	10.68	7.38	15391.70	10.31	5943374.44	0.003	16339.08
Nitrogen (B)	2	950.19**	39.12*	81.84**	289478.92**	237.67**	166789001.73**	0.001	377617.65**
A × B	4	431.22**	110.55**	63.49**	159845.84**	138.74**	208863534.46**	0.005**	300826.85**
Error B	18	35.70	9.01	8.26	9685.48	16.92	11234394.14	0.001	9125.93
Ecotype C	2	181.02*	27.37	8.25	9028.67	74.34**	51391901.01*	0.001	17717.53
A × C	4	185.26**	19.63	20.45**	44589.34**	21.78*	3936084.20	0.001	1722.57
B × C	4	42.55	98.23**	32.70**	11289.81	18.39*	16561544.13	0.002	23916.99*
A × B × C	8	124.37**	66.82**	13.91**	19233.46**	17.50*	15656038.16	0.001	18895.81*
Error C	54	38.54	8.63	3.33	6287.83	8.21	8202527.16	0.001	7227.81
CV (%)		14.56	9.16	8.97	11.80	12.47	19.28	4.07	14.09

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

Table 3: Means comparison of the interaction between deficit irrigation, N fertilizer and ecotype.

			Flowering branches/plant	Inflorescences/plant	Fruits/inflorescence	Fruits/plant	Seeds/plant	Seed yield (kg ha^{-1})
100% of water requirement	50 kg ha^{-1} of N	Bami	43.25 cde	21.25 k	26.00 bc	548.25 gh	24.00 d..g	456.89 k
		Boushehri	38.50 c..g	35.75 b..f	21.25 d..g	754.25 f	18.25 i..m	456.07 k
		Roudbari	45.25 bcd	32.00 d..h	22.25 c..f	712.25 f	24.00 d..g	589.36 j
	100 kg ha^{-1} of N	Bami	66.00 a	42.50 a	24.50 cd	1095.25 a	31.00 bc	1072.97 a
		Boushehri	47.50 bcd	32.50c..h	32.75 a	1064.00 ab	25.25 d..f	952.34 abcd
		Roudbari	57.00 ab	35.50 b..f	28.25 b	999.00 abc	31.75 b	1042.18
	150 kg ha^{-1} of N	Bami	46.50 bcd	28.75 ghi	23.75 cde	681.75 fg	36.75 a	930.97 ab
		Boushehri	46.75 bcd	33.50 c..g	21.50 def	719.50 f	34.50 ab	983.41 abc
		Roudbari	48.00 bc	30.25 e..h	23.50 c..f	708.50 f	30.75 bc	833.95 defg
75% of water requirement	50 kg ha^{-1} of N	Bami	42.00 c..f	38.50 abc	20.00 e..h	767.75 ef	27.00 cd	775.68 fghi
		Boushehri	41.00 c..f	42.25 a	17.50 g..j	739.75 f	23.25 d..h	692.48 hig
		Roudbari	36.50 c..h	42.25 a	19.50 f..i	830.00 def	20.00 g..l	652.69 ij
	100 kg ha^{-1} of N	Bami	66.75 a	36.25a..e	22.25 c..f	806.75 def	20.75 e..j	701.30 ghi
		Boushehri	60.75 a	38.50 abc	21.25 d..g	837.25 c..f	20.50 f..k	649.18 ij
		Roudbari	47.50 bcd	37.50 a..d	24.25 cd	970.25 a..d	21.50 e..i	739.47 fghi
	150 kg ha^{-1} of N	Bami	44.00 b..e	37.75 a..d	22.25 c..f	751.25 f	26.75 cd	716.36 ghi
		Boushehri	48.25 bc	41.00 ab	22.50 c..f	924.75 b..e	25.50 de	858.10 cdef
		Roudbari	40.25 c..f	37.25 a..d	23.00 c..f	935.25 a..d	23.50 d..g	804.87 efgh
50% of water requirement	50 kg ha^{-1} of N	Bami	36.00 c..h	26.50 h..k	19.75 e..i	474.75 hi	15.75 klm	304.73 lmn
		Boushehri	35.00 c..h	26.25 h..k	15.50 jk	383.75 ij	16.50 j..m	249.53 mn
		Roudbari	29.00 fgh	23.50 ijk	14.00 jkl	331.00 ij	15.00 m	205.54 mn
	100 kg ha^{-1} of N	Bami	24.75 h	29.50 f..i	16.00 ijk	473.00 hi	21.00 e..j	419.93 kl
		Boushehri	30.50 e..h	26.50 h..k	16.50 h..k	437.25 jij	18.25 i..m	340.58 km
		Roudbari	34.75 c..h	22.50 jk	13.25 kl	296.50 j	16.75 i..m	202.79 mn
	150 kg ha^{-1} of N	Bami	35.50 c..h	21.50 k	13.50 kl	287.25 j	18.75 h..m	219.55 mn
		Boushehri	33.75 d..h	21.25 k	13.75 jkl	291.50 j	18.00 i..m	258.04 mn
		Roudbari	26.25 gh	28.50 g..j	11.50 l	325.00 ij	15.50 lm	177.62 n

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

For this trait, the application of N until certain levels of water deficit could decrease and neutralize free oxygen radicals and so, prevent cellular destruction and oxidative stress. But as water deficit was increased to 50% of water requirement, N application lost its improving effect on reproductive growth.

C. Fruits number

Fruits number per inflorescence and per plant was affected by deficit irrigation and N fertilizer. Fruits number per inflorescence was impacted by all interactions ($\alpha \leq 0.01$), but the interaction of N × ecotype was not significant for fruits number per plant (Table 2). The highest fruits number per inflorescence was belonged to Boushehri ecotype irrigated to supply 100% of water requirement and fertilized with 100 kg ha^{-1} of N; whereas, the highest number of fruits per plant of Bami and Boushehri ecotypes was obtained under full irrigation and N at the rate of 100 kg ha^{-1}, and it was obtained under irrigation to supply 100% of water requirement and N rate of 100 kg ha^{-1} in Roudbari ecotype (Table 3). Adequate N availability increases the fertile flowers number in plants; therefore, caused increase in higher number of fruits per inflorescence and consequently, higher number of fruits per plant and seeds per plant. In a study on *Silybum marianum*, Agharahimi (2002) obtained the highest number of fruits per hectar by 150 kg ha^{-1} of N application.

D. Seeds number

Seeds number per inflorescence and per plant was significantly affected by different levels of deficit irrigation, N and ecotype. All interactions were significantly for it but only the interaction between deficit irrigation and N was significant for the number of seeds per plant (Table 2). The highest number of seeds per fruit was related to Bami and Boushehri ecotypes which obtained in full irrigation and 150 kg ha^{-1} of N application (Table 3). The highest seeds number per plant (Fig. 1) was related to Bami ecotype (1616 seeds per plant) and no statistically significant difference was observed in the number of seeds between Boushehri and Roudbari ecotypes. In addition, it was found that the highest seeds number per plant as affected by irrigation and N regimes was obtained from the irrigation to supply 100% of water requirement fertilized with 100 and 150 kg ha^{-1} of N.

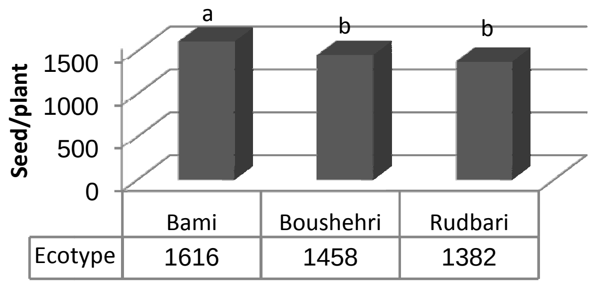

Fig. 1. Effect of ecotype on the number of seeds in henna plants.

Water deficit increasing up to irrigation to supply 75% of water requirement fertilized with 50 kg ha^{-1} of N increased seed number from 11740 to 18900 seeds per plant, but 100 and 150 kg ha^{-1} of N application this irrigation level resulted in lower number of seeds than at full irrigation, so that seeds number was decreased by 26.56 and 20.76% in these two levels, respectively (Fig. 2). Nakayama *et al*. (2007) reported that N accumulation rate in leaves was decreased under water deficit conditions caused the loss of photosynthesis. In fact, drought disrupts N uptake, and drought-sensitive genotypes accumulate less N and are in more damage. Therefore, none of the N levels improved seed number under the treatment of irrigation to supply 50% of water requirement.

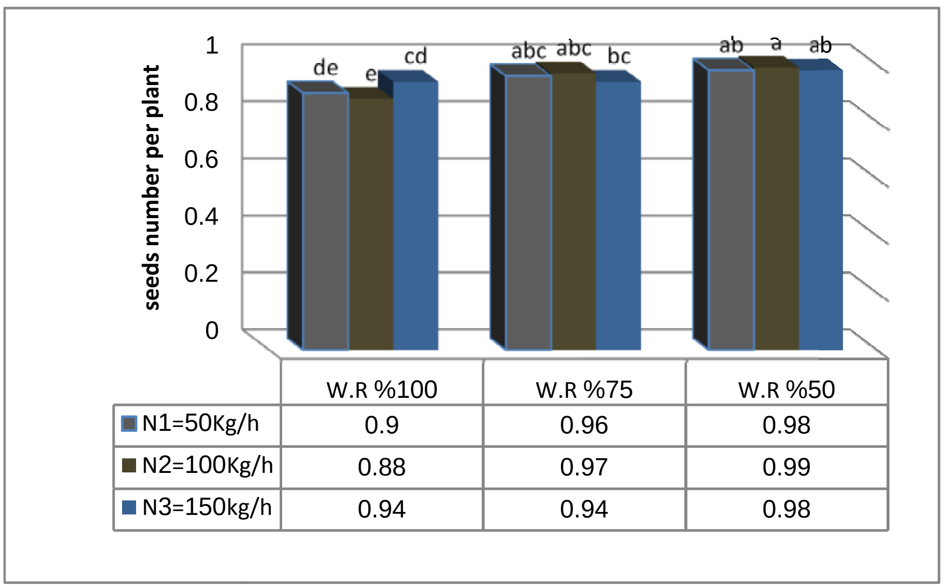

Fig. 2. The interaction between deficit irrigation and N for the number of seeds per henna plant.

E. Thousand-seed weight

Thousand-seed weight was affected by deficit irrigation and the interaction between deficit irrigation and N fertilization at the 1% level (Table 2). According to means comparison, although the highest 1000-seed weight of 0.99 g was related to 50% of water requirement fertilized with 100 kg ha^{-1} of N, no significant difference was observed between them and the treatment of irrigation to supply 50% of water requirement fertilized with 50 or 150 kg ha^{-1} of N; the treatment of irrigation to supply 75% of water requirement fertilized with 50 or 100 kg ha^{-1} of N were in the same condition too (Fig. 3).

Full irrigation resulted in the loss of 1000-seed weight which can be associated with higher number of seeds per plant and fruit under full irrigation because as seed number per plant and fruit is increased, photosynthates are distributed among more sinks resulting in the loss of 1000-seed weight. (Kumar and Singh, 1994) stated that water deficit stress resulted in higher 1000-seed weight and lower number of seeds per plant.

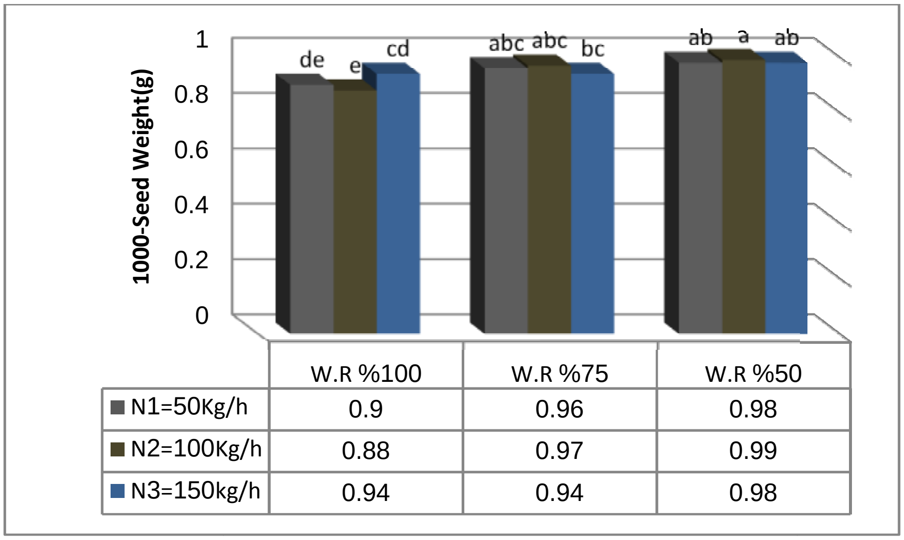

Fig. 3. The interaction between deficit irrigation and N for 1000-seed yield of henna.

Seed yield was influenced by deficit irrigation and N fertilization at the 1% level and the interactions of deficit irrigation × N, N × ecotype, and deficit irrigation × N × ecotype at the 1, 5 and 5% levels, respectively (Table 2). According to the means comparison of all three factors (Table 3), full irrigation fertilized with 100 kg ha^{-1} of N produced the highest seed yield in Bami (1072.97 kg ha^{-1}) and Roudbari (1042.18 kg ha^{-1}). In addition, Boushehri ecotype produced the highest seed yield under full irrigation that fertilized with 100 or 150 kg ha^{-1} of N. The increase in water stress up to 50% of water requirement resulted in severe loss of seed yield at all fertilization levels, especially 150 kg ha^{-1} of N which can be related to the negative impact of water stress on the number of inflorescences, fruits and seeds per plant. In addition, the increase in N rate from 50 to 100 kg ha^{-1} increases photosynthesis rate resulting in higher seed weight and yield (Ebrahimi *et al*., 2011). N application enhances photosynthesis through increasing the growth and duration of plant parts like leaves resulting in higher seed yield (Daneshvar *et al*., 2008). Other research, also, report higher seed yield under higher N rate (Vukovic *et al*., 2008; Hatami *et al*., 2009) although this increase only up to a certain level improves yield and more increase in N rate starts to reduce seed yield.

According to the results, full irrigation fertilized with 100 kg ha^{-1} of N had the strongest effect on the improvement of vegetative growth of henna. It can be concluded that higher N rates should be avoided under water deficit conditions because they are not absorbed.

F. Coefficients of correlation

As can be seen in table of coefficients of correlation (Table 4), seed yield had positive, significant correlation with the studied indices. The highest correlations were observed between the number of flowering branches with the number of fruits per inflorescence and the number of seeds per plant (92.0 and 86.0, respectively). The correlation between the number of fruits per inflorescence and the number of inflorescences per plant was negative. In addition, the number of seeds per fruit was negatively correlated with the number of inflorescences per fruit and also, 1000-seed weight had negative correlation with the number of inflorescences per fruit so that as the number of inflorescences per plant increased, the number of seeds per fruit and 1000-seed weight decreased. All variables showed positive, highly significant correlation with seed yield. In total, given the high number of treatments in the present study, even low coefficients were significant because of the increase in degrees of freedom in significance test of coefficients of correlation which is in agreement with Golparvar and Madani (2008).

Table 4: Coefficients of correlation between the estimated reproductive traits of henna.

	Inflorescences/plant	Fruits/inflorescence	Fruits/plant	Seeds/fruit	Seeds/plant	1000-seed weight	Seed yield
Inflorescences/plant	1						
Fruits /inflorescence	-0.42*	1					
Fruits/plant	0.68**	0.64**	1				
Seeds/plant	-0.55**	0.76**	0.81**	1			
Seeds/plant	0.50**	0.75**	0.55**	0.51**	1		
1000-seed weight	-0.31*	0.64**	0.38*	0.78**	0.31*	1	
Seed yield	0.35*	0.57**	0.58**	0.65**	0.44*	0.46*	1
Flowering branch no.	0.51**	0.91**	0.76**	0.86**	0.82**	0.65**	0.63**

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

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