



Effect of drought stress on indigo and seed yield of *Indigofera tinctoria* ecotypes

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ABSTRACT

The objective of the the current study was to elucidate the effect of drought stress on indigo yield and to derive certain morphological traits of indigo ecotypes. This experiment was laid out in a split-plot arranged at a randomized complete block design, with three replications in the Jiroft Agricultural and Natural Resources Research and Education Center, Kerman, Iran, during 2013-14 growing seasons. The main plot and the subplot included drought stress (100, 75, and 50% of crop water requirement) and indigo ecotypes (Jiroft, Roudbar, Rigan, and Ghale Ganj), respectively. The obtained results showed that morphological and indigo content attributes revealed significant differences between the control and the drought-stress plants. Increasing water stress (up to 50% of crop water requirement) resulted in a severe loss of leaf dry mass, plant height, and seed yield of 25, 25 and 58%, respectively, in comparison to the control condition; the highest indigo yield and content obtained from the mid-stress level. Moreover, interaction effects of drought and ecotypes on leaf dry mass, seed, and indigo yield were significant; thus, the LDM and seed yield was the greatest in the Jiroft ecotype in the control, the indigo yield was the greatest under mid-level stress, and it was the lowest in the Ghale Ganj ecotype under severe water-stress level. Therefore, the results of this research suggest that the Jiroft ecotype will perform well with respect to drought tolerance and will be useful for cultivation in the semi-arid regions of Jiroft.

Key words: Drought stress, Indigo content, Indigo yield, Leaf dry weight

Water deficit is considered to be a major abiotic factor that affects many aspects of plant physiology which limits growth and crop production (Maghsoudi *et al.* 2019). In spite of the fact that almost all plant species have a degree of drought-stress tolerance, tolerance varies with cultivar and variety. However, understanding the morphological and physiological basis is necessary to select and breed plants for improving crop water stress tolerance (Maghsoudi *et al.* 2019).

Indigofera is grown in many countries of the world as an ornamental plant, for the production of indigo dye, and as an herbal drug (Luiz-Ferreira *et al.* 2011). In South East, Iran, it is cultivated as an industrial crop in rotation after wheat and cucurbitacea plants. Indigo dye is the oldest natural dye and is found as glucoside indican in the leaf plant *Indigofera tinctoria*; it has been known in India for about 4000 years (Samanta and Agarwal 2009). *Indigofera tinctoria* is used to treat many human diseases (Asuntha *et al.* 2010, Verma *et al.* 2010). Indigo does not naturally occur in plants but is made during the extraction process from the hydrolysis and the oxidation of indican glucosides; it is, therefore, a

secondary metabolite (Taati *et al.* 2014). Ecological changes are the most important factors that effectively influence the quality and the quantity of the metabolite products of the medicinal plant. Literature suggests that increased water stress severity resulted in a significantly reduced growth, dry leaf yield, and indigo content and yield (Mohammed 2006, Sarhadi *et al.* 2014). In addition, drought stress generally leads to an increase in the secondary metabolite of medicinal plants (Roazmjoo *et al.* 2008).

Therefore, the aim of this study was to evaluate the effect of drought-stress on leaf dry mass, seed yield, and indigo yield and content in order to reach the suitable ecotype of indigo, with the capability of producing higher yields under arid and semi-aride conditions.

MATERIALS AND METHODS

The effect of different levels of deficit irrigation was studied on the qualitative and the quantitative aspects of the yield of indigo ecotypes in a split-plot experiment based on a randomized complete block design, with three replications in a research farm located in the Jiroft Agricultural and Natural Resources Research and Education Center (28.3242 °N; 57.5115 °E and 2056 m altitude), Kerman, Iran, during the 2013-14 growing seasons. The main plot was employed to indicate deficit irrigation at three levels (irrigation to

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supply at 100, 75, and 50% of crop water requirement) and the subplot was employed to demonstrate the four ecotypes of indigo (Jiroft, Roudbar, Rigan and Ghale Ganj).

The operation of the plough and the preparation of the farm included a deep plough, two vertical disks, leveling, furrowing, mound, and plot making. The seeds were cultivated in the field on May 22 over a period of two years. The plots were prepared with six sowing rows such that their length and intervals were 4 m and 50 cm, respectively. Seed-sowing was performed in rows with 3–5 seeds in one hole, which were spaced 30 cm apart. The main plots were spaced 2 m apart and the subplots were spaced 1 m apart; 2 m of spacing was left between the replications.

The amount of water applied was calculated on the basis of the daily evaporation from the evaporation pan and different levels of deficit irrigation treatments were calculated as;

$$ET_o = K_p \times E_{\text{pan}}$$

where, ET_o was evapotranspiration of the base crop, K_p was the evaporation from the pan, and E_{pan} was the coefficient of the pan (which was assumed as 0.7).

Then, the amount of water to be applied was calculated as;

$$ET_c = ET_o \times K_c$$

where, ET_c was the water used by the plant and K_c were the crop factors that were assumed to be 1/1, given the fact that indigo 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.

The quantitative data (plant height, pod/plant-seed/pod, 1,000-seed weight, and seed yield) were recorded from random selection at five plants from each plot and from each replication. In order to derive the qualitative data (leaf dry weight, indigo content), two harvesting sessions were performed on an area of at least 2 m² in the middle row from each plot after 65 and 135 days of sowing the plant. To measure the 1000-seed weight, the seeds were counted by a seed-counting machine and weighed by a 0.001 precision scale. The seed yield was estimated for an area of 10 m², during which the plants were flailed to have the seeds separated. Then, they were weighed. The indigo content of the leaves was extracted according to the protocol described by Stoker *et al.* (1998) and Sales *et al.* (2006), with some modification. From each plot, the leaf samples were cut into small pieces and a bulk sample was prepared (1 g weighed). The weighed sample was immersed in 10 ml of distilled water, boiled for 10 min, cooled rapidly in ice water, and then added to 200 ml of Ca(OH)₂ solution (pH value = 11). The samples were then aerated for 30 seconds and allowed to stand at room temperature for one hour, before acidification with 50 µl of 50% HCL (pH value = 2). After allowing the sample to stand for 30 min, according to the standard curves, the sample extracts were centrifuged at 5000 RPM for 5 min, and the absorbance was measured at 611 nm. A calibration curve was prepared by using various

amounts of standard indigo that were obtained by dissolving 8 mg of standardized indigo in 20 ml of H₂SO₄; this was diluted to 500 ml with distilled water and then prepared as different concentrations of these solutions. The analysis of variance and the means comparison were performed by the SAS software package (Version 7) and the MSTAT-C software package. The graphs were drawn by MS Excel.

RESULTS AND DISCUSSION

Leaf dry weight: As pictured in the variance analysis results, the drought-stress treatment is significant in relation to leaf dry weight at the 1% level. In addition, considering the results of the comparison between the average of the main effects of drought stress. The stress resulted in a respective 7 and 25% reduction in leaf dry weight at 75 and 50 percent of the water requirement in comparison to the control level. The interaction effect of drought stress and the ecotype on the dry weight leaf was significant. The leaf dry weight was the greatest in the Jiroft ecotype (5284.83 Kg) in the control level and the lowest in the server stress level Ghale Ganj ecotype (3289.68) (Fig 1). Sarhadi *et al.* (2014) reported that drought stress (50% F.C) resulted in a reduction in the dry weight leaf, the total fresh biomass, and the total dry biomass of 20, 44, and 38%, respectively, in comparison to the control condition since the greater plant fresh- and dry-weight under water-limited conditions are desirable characteristics. A common adverse effect of water stress on crop plants involves the reduction of fresh and dry biomass production (Maghsoudi *et al.* 2019). Plant productivity under drought-stress is strongly related to the process of dry matter partitioning and temporal biomass distribution (Sarhadi *et al.* 2014).

Content and yield of indigo: The indigo content of leaf ecotypes were affected by various levels of drought stress ($\alpha \leq 0.01$). The indigo content was respectively increased by 29 and 10% at 75% and 50% of the water requirement in comparison to the control condition. Furthermore, the means comparison showed that indigo content significantly

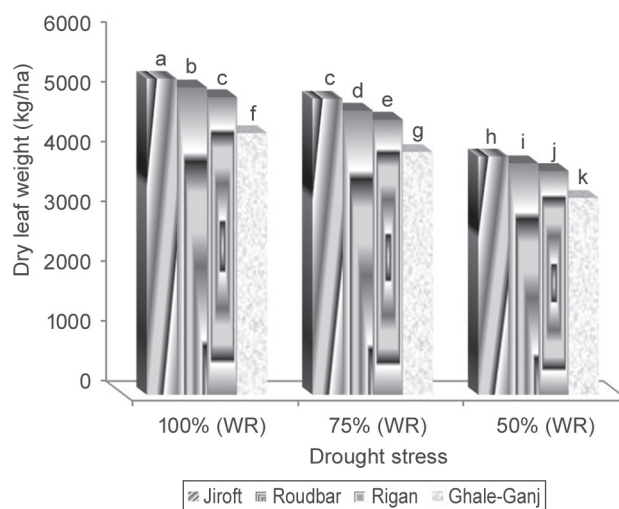


Fig 1 Interaction between drought stress and ecotype on dry leaf weight.

varied between the ecotypes studied. The means were greater in both the Jiroft and the Roudbar ecotypes (0.67) in comparison to the Ghale Ganj (0.66) and the Rigan ecotype (0.65). There was no significant difference between the Ghale Ganj and the Rigan ecotype (Table 1). In addition, in previous studies related to *Indigofera*, the response to the water irrigation interval (7, 10, and 15 days) was reported by Mohammed (2006), which showed that the greatest indigo content was obtained from 10 days of irrigation interval, and was increased to 8 and 28%, respectively, in comparison to other treatments (7 and 15 days). Since, we were observed that water deficit increased of secondary metabolites in many plant, such as chamomile (Razmjoo *et al.* 2008) resulted in that indigo contents as secondary metabolites, Not only affects genetic characteristics, but also climatic and growth conditions.

The effects of various levels of drought-stress on the indigo yield were significant ($a \leq 0.01$). The indigo yield was greater in the mid-stress level (35 kg), and increased by 20 and 24%, respectively, in comparison to the control and the server stress levels. The interaction effect of drought (stress $\times$ ecotype) on the indigo yield was significant. The indigo yield was the greatest in the Jiroft ecotype (37.92 Kg/ha) and the lowest in the Ghale- Ganj ecotype (21.29 Kg/ha) under mid- and severe-stress levels, respectively (Fig 2). Angelini *et al.* (2004) reported that the indigo content and the yield in the three lines of polygonum was strongly affected by the climatic condition; the variation in indigo content was 12–25 g/ha fresh weight and the potential indigo yield was up to 326 kg/ha. The greatest yield was obtained from rainy conditions. Moreover, Laitonjam and Wangkheirakpam (2011) suggested that the activity of the enzyme beta-glucosidase was the highest in fresh plant leaves and decreased with exposure to heat and dryness. In fact, a reduction in soil moisture may reduce the availability of nutrients for the plant and, consequently, reduce plant growth, height, and yield (Razmjoo *et al.* 2008). Drought-

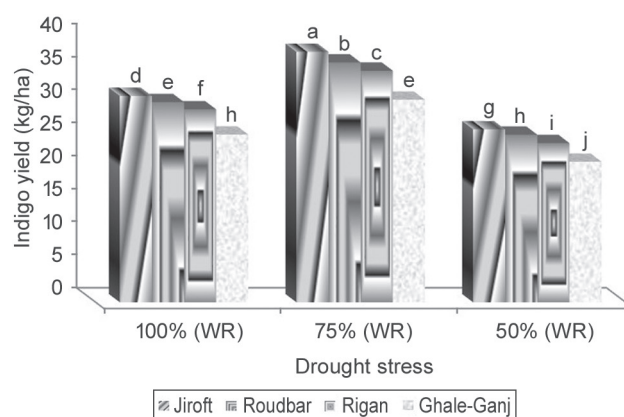


Fig 2 Interaction between drought stress and ecotype on indigo yield.

stress reduces plant growth; thus, the carbon fixed during photosynthesis could be used to form secondary metabolites (Maghsoudi *et al.* 2018).

Plant height: This trait in the total ecotypes significantly differed based on the drought-stress levels ($a \leq 0.01$). The results showed that the greatest height of the plant obtained from normal conditions (100% WR) and plant height was decreased to 7% and 25% at mid- and severe-stress levels, respectively. In addition, a significant difference in plant height was also found between the four ecotypes. The plant height was the greatest in the Jiroft ecotype (94.01 cm) and the lowest in the Ghale Ganj ecotype (82.92 cm). Angelini *et al.* (2007) reported that the plant dry biomass of *Indigofera* were strongly affected by water-deficit and plant height decreased by 18% in comparison to the normal condition. Enneb *et al.* (2015) showed that water-stress significantly reduced LA and the stem length of henna by 65% and 44%, respectively, in comparison to control. The reduction in height may always be associated with a decline in cell enlargement under water-stress. Water-stress greatly

Table 1 The effects of drought stress and ecotypes on the traits studied in Indigo 2013–2014

	Leaf dry weight (kg/ha)	Indigo content (g/kg)	Indigo yield (kg/ha)	Plant height (cm)	No. of pod-plants	No. of seed- pods	1000-seed weight (g)	Seed yield (kg/ha)
Year								
2013	3144.11a	0.62a	26.05b	84.43a	244.4a	4.72a	3.48a	301.10a
2014	4690.31a	0.7a	32.81a	94.10a	261.71a	4.81a	4.04a	372.25a
Stress								
100% (WR)	4942.08a	0.58c	29.05b	99.91a	282.1a	5.31a	4.2a	448.79a
75% (WR)	4588.54b	0.75a	35.000a	93.10b	265.36b	4.98b	3.96b	377.01b
50% (WR)	3721.02c	0.64b	24.24c	74.77c	211.7c	4.01c	3.11c	184.23c
Ecotype								
Jiroft	4738.58a	0.67a	31.79a	94.01a	271.51a	4.81a	3.85a	372.66a
Roudbar	4585.6b	0.67a	30.67b	91.5b	258.81b	4.89a	3.83a	352.45b
Rigan	4337.89c	0.66b	29.47c	88.62c	246.63c	4.7a	3.78b	328.58c
Ghale Ganj	3906.77d	0.65b	25.79d	82.92d	235.27d	4.68a	3.57c	293.02d

In each culomn, means that have at least one common letter didn't show significant difference in 1% level.

suppresses cell expansion and cell growth due to low turgor pressure (Enneb *et al.* 2015). Sankar *et al.* (2008) reported that a reduction in the height of bean plants was associated with the enlargement of the leaf cell and plant senescence in *Abelmoschus esculentus* under water-stress. The results of Angelini *et al.* (2004) confirm our results in this research.

Number of pods per plant: The result from variance analysis showed that the significance of drought-stress treatment and the number of pods in plant is 1%. In addition, a comparison between the average of the main effect of drought-stress showed that drought stress levels resulted in a 6% and a 25% reduction of pods per plant in comparison to the control level. The difference was the significance between the four ecotypes ($\alpha \leq 0.01$). The pods per plant quantity was the greatest in the Jiroft ecotype (271.51) and the lowest in the Ghale Ganj ecotype (235.27). The difference was 13%.

Maghsoudi *et al.* (2019) reported that water-stress significantly reduced yield and component yield in the wheat cultivar since *Indigofera* plants are sensitive to water-stress at the flowering stage (Khorramdel *et al.* 2016). Thus, the water-stress during blossoming may increase the number of sterile flowers and the immature pods.

Number of seeds in pods: Variance analysis of the number of seeds in the pod showed that drought stress significantly affects this trait at a probability level of 1%. Therefore, considering the results of comparison between the average of the main effect of drought stress in comparison to the full-irrigation treatment, irrigation in 75% and 50% of the water requirement resulted in a respective 6% and a 24% reduction of seeds in the pod. In addition, there was no significant difference in the seed pods between the ecotypes studied. Enneb *et al.* (2015) reported that the a deficient water for 50% water requirement in three henna ecotypes resulted in the reduction of the number and the seed yield in total N-nutrition levels. Therefore, environmental factors are as effective as genetic factors on the trait.

1000-seed weight: The results of the variance analysis showed that seed weight was affected significantly by 1% drought stress. According to the mean comparison, although, the highest thousand-seed weight (4.2 g) was related to normal irrigation conditions, but the thousand-seed weight decreased by 6% and 26% in the treatment of the irrigation to supply at 75% and 50% of the water requirement, respectively. The 1000-seed weight was also significantly different across the ecotypes studied. The Jiroft and the Roudbar ecotypes had greater 1000-seed weights (average 3.84 g) in comparison to the Regan (3.78 g) and the Ghale Ganj ecotypes (3.57 g). The results presented in this study were also assisted by the results of Sarhadi *et al.* (2014).

Seed yield: Drought stress treatment is significant in relation to seed yield at the 1% level. In addition, considering the results of the comparison between the averages of the main effects of drought stress, stress levels of 75% and 50% water-requirement resulted in a reduction in seed yield by 16% and 58%, respectively, in comparison to the control

level. Seed yield significantly varied between ecotypes. Seed yield was the greatest in the Jiroft ecotype (372.66 kg/ha) and the lowest in the Ghale Ganj ecotype (293.02 kg/ha), with a 21% difference. Thus, the greatest seed yield was obtained in the Jiroft ecotype at the full irrigation level and the lowest seed yield was obtained in the Ghale Ganj ecotype in the severe stress level. In fact, it can be said that seed yield under drought stress is effectively related to leaf dry matter production. There was no significant difference in seed yield between the Jiroft and the Roudbar ecotypes under severe stress level or between the Rigan and the Ghale Ganj ecotypes. Our finding was also in agreement with the results reported by Sarhadi (2014) and Angelini *et al.* (2004) on *Indigofera tinctoria*.

The conclusion is that although significant genetic variability existed for the effective selection and the genetic improvement of the tissue and whole-plant drought tolerance among control and drought-tolerant plants for morphological characteristics (plant height, leaf dry mass, and number of reproductive organs) and quality traits. Since secondary metabolite production and its osmoprotection effects are useful pointers of whole plant's drought-tolerance and the advantage of economic value are also important (which could be applied as a worthy indices), the cultivation purpose of indigo as a dye plant for indigo or seed production is important. Our results show that the amount of indigo content increased at the moderate-stress level, but at severe deficit irrigation stress, the plants seem to be more sensitive to drought.

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