Industrial Crops & Products 109 (2017) 346–357

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Towards improving the agronomic performance, chlorophyll fluorescence parameters and pigments in fenugreek using zeolite and vermicompost under deficit water stress

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ARTICLE INFO

Keywords:
Trigonella foenum-graecum
Photosynthetic pigments
Drought
Chemical nitrogen
Secondary metabolite

ABSTRACT

A comprehensive study has not been carried out so far to determine the outcome and impact of chlorophyll (Chl) fluorescence parameters and photosynthetic pigments in relation to qualitative and quantitative properties in fenugreek, especially in regions with limited water availability. Fenugreek (*Trigonella foenum-graecum*) is one of the most important medicinal plants in the world. To determine the effects of water deficit stress, nitrogen fertilization and zeolite on Chl fluorescence, pigments, trigonelline concentration and seed yield of fenugreek, a split factorial experiment was laid out in a randomized complete block design with three replications in a semi-arid region of Iran in 2014 and 2015. Five irrigation regimes (unstress control, irrigation at 60% available soil water (ASW); mild stress, irrigation at 40% ASW during the vegetative and reproductive stages; severe stress, irrigation at 20% ASW during the vegetative and reproductive stages) were randomized to the main plots. Subplots consisted of a factorial combination of three nitrogen treatments (untreated plots, vermicompost @ 2.7 t ha^{-1} and urea @ 11 kg ha^{-1}) and two zeolite treatments (@ 0 and 9 t ha^{-1}). The results demonstrated that water deficit stress decreased maximum Chl fluorescence (F_m), variable Chl fluorescence (F_v), the photochemical efficiency of PSII (F_v/F_m), Chl (a, b and $a + b$), leaf area index (LAI), biological yield and seed yield but minimum fluorescence (F_O), carotenoids, Anthocyanin and trigonelline concentration increased under water deficit stress. There were negative and significant correlations between F_o and LAI, biological and seed yield and on the other side, positive and significant correlations between total Chl. and the above mentioned traits. Water deficit stress led to the photo inhibition of photosynthesis. In most treatments, both vermicompost and zeolite application increased LAI, biological yield, seed yield and trigonelline concentration in fenugreek. Greater concentration of trigonelline in fenugreek under mild water deficit stress conditions may not necessarily supply give economic benefits, as higher concentration is often compensated by lower seed yield.

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual and herbaceous crop, which belongs to the legume family (Fabaceae). It is widely cultivated as one of the most important medicinal and industrial plants, a high quality forage crop, or a nitrogen (N) fixing cover crop and green manure in agricultural farm in the world. This crop is native to an area extending from Europe (Ukraine and Greece) to Asia (Iran to northern India and China) and north and east Africa (Ethiopia and Egypt) (Dadrasan et al., 2015). As this plant is cultivated across diverse areas, environmental conditions are unpredictable, and therefore abiotic stresses may occur. To improve a crop's productivity under stress conditions, a good management of inputs in time, amount and form, understanding important physiological processes and defense mechanisms to avoid stress are required (Ibáñez et al., 2010). Various environmental stresses lead to the direct and indirect effects on the performance of the photosynthetic mechanism (Hazrati et al., 2016; Hazrati et al., 2017). Drought stress due to lack of available water in the soil, especially in arid and semi-arid regions (as that of Iran), is one of the most important constraints for photosynthesis and thus for plant growth and plant

Abbreviations: F_m, maximal fluorescence level from dark adapted leaves; F_O, minimal fluorescence level from dark-adapted leaves; F_v/F_m, maximal photochemical efficiency of the active center of PSII in the dark; Chl, Chlorophyll; Anth, anthocyanin

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http://dx.doi.org/10.1016/j.indcrop.2017.08.049
Received 14 February 2017; Received in revised form 14 August 2017; Accepted 27 August 2017
0926-6690/

production. Furthermore, in these regions, the unfavorable distribution of rainfall during the growing season and the year-to-year fluctuations represent a major constraint on plant growth (Afshar et al., 2014). The ability of plants to maintain the photosynthetic mechanism under water stress conditions is very important. In this regard, stomata closure is one of the fast reactions which prevents the waste of water. Environmental stresses such as deficit water stress lead to inactivation of photosystem1 (PSI) and photosystem 2 (PSII) which may also lead to the electron transport respiratory chain. Zhang et al. (2011) reported that photosynthetic efficiency reduced by increasing the fluorescence of Chl in drought stress conditions. Thus, the measurement of Chl fluorescence can be used to determine the status of plant physiology and the damage to photosynthetic mechanism. The effects of environmental stresses such as drought and salinity, have been investigated using the measurements of the light quantum efficiency of PSII (Hazrati et al., 2016). Also, Ranjbar Fordoei and Dehghani Bidgholi, (2016) reported that the function of photosynthetic mechanism can be investigated by the measurement (F_v/F_m) which is indicative of the quantum function of PSII reaction center and shows a close correlation with net photosynthesis and quantum yield of intact leaves of the plant. According to Zhang et al. (2011), although PSII is largely resistant to drought stress, drought can prevent the electron transfer in PSII and therefore reduces the efficiency of PSII and increases the amount of Chl fluorescence. In severe stress, carotenoids and Anth concentration (due to its antioxidant properties) increase to prevent further destruction of Chlorophyll in light oxidation. Increase in carotenoids and Anth concentration and decrease in Chl concentration (*a, b* and total) under unfavorable environmental conditions have been reported in other studies (Abdalla and El-Khoshiban, 2007; Hazrati et al., 2016).

Only a few studies have shown how photosynthesis and Chl fluorescence (*ChF)* relate when nitrogen is the limiting factor. Cendrero-Mateo (2013) reported that low nitrogen led to lower chlorophyll concentration and thus lower, photosynthetic rate, fluorescence yield, and light-use efficiency in wheat and when nitrogen was sufficient and photosynthesis was highest, *ChF* decreased because these two processes compete for available energy.

Macro- and micro-nutrients are essential for plant life, and their deficiency can strongly compromise plant life cycles and yields. Among macro-nutrients and inputs, nitrogen is a major nutrient that limits plant growth and development worldwide and the demand for it is likely to grow in the future (Mokhtassi-Bidgoli et al., 2013). On the other hand, environmental stresses and nutrient deficiency affect photosynthetic pigments and can inhibit photosynthesis (Ashraf and Harris, 2013). The photosynthetic rate and Chl concentration vary in plants due to different moisture conditions and nutritional status. Many studies have shown that reduced availability of nitrogen reduces the quantum function of electron transfer in PSII and its maximum efficiency. In addition, nitrogen deficiency results in the destruction of PSII, because it is a necessary component of the Chlorophyll molecule (Conming and Zang, 2000).

The use of agronomic techniques including organic fertilizers and zeolites (microporous aluminisilicate minerals used as commercial adsorbents and catalysts) and/or reinforcement of biological conditions of the soil may be effective in reducing the effects of drought stress. Researchers have reported the positive effects of nitrogen chemical fertilizers on the yield of fenugreek (Tunctürk et al., 2011; Abbasdokht et al., 2016). Also, intensive agriculture with the widespread use of chemical fertilizers, especially nitrogen can cause an increase in costs and environmental pollution. A key component of sustainable agriculture is use of organic fertilizers such as vermicompost. Keshavarz Afshar et al. (2014) indicated that vermicompost and poultry manure can improve the yield of milk thistle (*Silybium marianum* L.) and minimize the negative impact of drought stress.

Also, zeolites, due to their high porosity, are able to improve plant growth by increasing the long-term availability of water and nutrients (Hazrati et al., 2017). Several researchers have reported that fenugreek produces high seed yield and trigonelline with suitable irrigation and adequate nutrition (Abbasdokht et al., 2016; Dadrasan et al., 2015).

Considering the importance of medicinal plant production such as fenugreek on the one hand, and the spread of drought stress on the other, it is important to improve the nutritional status of fenugreek in such a way that the effects of drought stress are modified. A comprehensive study has not been carried out so far to determine the outcome and impact of Chl fluorescence parameters and photosynthetic pigments in relation to qualitative and quantitative properties in fenugreek under above-mentioned conditions. Hence, the current study was aimed to evaluate the effects of water deficit stress at different growth stages along with nitrogen and zeolite management on Chl fluorescence parameters and pigments to find out their relationships to qualitative and quantitative yield during plant growth stages.

2. Material and methods

2.1. Field site description

A two-year experiment was laid out at Research Field in the Faculty of Agriculture, Tarbiat Modares University, Tehran, (35°44′N′ 51°09′E, and 1265 m above sea level), Iran in 2014 and 2015. This area is a semi-arid area (according to the Köppen climate classification) characterized by warm and dry summers, a long-term (30 years) mean annual rainfall of 232.6 mm and temperature of 17.6 °C, respectively. The daily weather data recorded during the trial period in each year are given in Fig. 1.

Two months prior to planting, soil samples were taken from 0 to 30 cm depth in order to determine the physical and chemical properties. The soil texture was sandy loam. The physical and chemical properties of different layers of the experimental soil are presented in Table 1.

2.2. Cultural practices and experimental design

After field preparations, this area was divided into 90 experimental plots each of which were 6 m long with five rows, 0.3 m apart. Between all the main plots, a 3 m alley was kept to eliminate the influence of lateral water movement. Plant to plant distance in each row was 12.5 cm. The seed of fenugreek landrace from central plateau of Iran was planted @ 20 kg ha^{-1} on 9 May 2014 and 10 May 2015.

A split-plot factorial experiment was laid out in a randomized complete block design with three replications. Five irrigation regimes including unstress at vegetative and reproductive stages, i.e. irrigation at 60% ASW during the vegetative and reproduction stage (I_1); irrigation at 40% ASW during the vegetative stage (I_2) and the reproductive stage (I_3); irrigation at 20% ASW during the vegetative stage (I_4) and the reproductive stage (I_5) were randomized in main plots. Subplots consisted of a factorial combination of three nitrogen fertilization treatments (F_1: untreated plots, F_2: vermicompost @ 2.7 t ha^{-1} and F_3: urea @ 11 kg ha^{-1}) and two zeolite treatments (@ 0 and 9 t ha^{-1}). Average distance between emergence and flowering was 38 and 36 days in 2014 and 2015, respectively. The amount of vermicompost was calculated based on N contents in manure and soil and mineralization percent per year (50%). Vermicompost and zeolite (clinoptilolite type) were broadcasted and mixed with soil prior to planting in the respective plots. The vermicompost characteristics are presented in Table 1. Zeolite analysis indicated a cation exchange capacity (CEC) of 200 meq 100^{-1} g and the other chemical properties of zeolite include: silicon dioxide (SiO_2) 65%; aluminum oxide (Al_2O_3) 12%; sodium oxide (Na_2O) 1%; potassium oxide (K_2O) 3% and calcium oxide (CaO) 2.5%.

To ensure successful establishment, all plots received full irrigation until seedling attained 4–6-leaf growth stage (20 days after planting (DAP)), then irrigation treatments were initiated. Soil moisture content in each plot was monitored daily using a time domain Reflectometry