Industrial Crops and Products 30 (2009) 372–379

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Water deficit effects on caraway (*Carum carvi* L.) growth, essential oil and fatty acid composition

Bochra Laribi[a,*], Iness Bettaieb[b], Karima Kouki[a], Ali Sahli[a], Abdelaziz Mougou[a], Brahim Marzouk[b]

[a] *National Agronomic Institute of Tunisia, 43, Av. Charles Nicolle-1082, Tunis, Tunisia*
[b] *Aromatic and Medicinal Plants Unit, CBBC, BP 901, 2050 Hammam-Lif, Tunisia*

ARTICLE INFO

Article history:
Received 1 June 2009
Received in revised form 8 July 2009
Accepted 10 July 2009

Keywords:
Water deficit
Carum carvi L.
Growth
Seed yield
Fatty acids
Essential oil
Petroselinic acid
Carvone
Limonene

ABSTRACT

The Mediterranean region suffers of drought which affects plant behaviour regarding biochemical responses. Accordingly, the effects of water deficit on growth, essential oil and fatty acid composition of caraway (*Carum carvi* L.) seeds were investigated. Plants were treated with different levels of water deficit: control, moderate water deficit and severe water deficit. Plant growth (height, fresh and dry matter weight) was significantly reduced by severe water deficit. This last caused also important reductions of the seed yield and yield components. Drought decreased significantly seed total fatty acid contents and particularly the petroselinic ones whose proportions decreased significantly by 12.17 and 18.47%, in comparison with the control, under moderate water deficit and severe water deficit, respectively. Besides, moderate water deficit increased the essential oil yield (expressed as g/100 g on the basis of dry matter weight). The main essential oil constituents were carvone and limonene which showed an increasing of their contents under water deficit levels. Thus, water deficit induced a significant reduction in growth parameters and fatty acid content, and an increase in the essential oil compounds. These bioactive compounds have been required in many industrial products.

1. Introduction

Among the different environmental constraints, drought is the most abiotic factor limiting crop productivity. Wang et al. (2003) have estimated the average of yield decrease on a worldwide scale due to water deficit at more than 50%. Also, this last affects fatty acid composition of many crops (Hamrouni et al., 2001; Boschin et al., 2008; Bettaieb et al., 2009) and cause changes in essential oils yield and composition of aromatic plants (Fatima et al., 2002; Khalid, 2006; Petropoulos et al., 2008; Bettaieb et al., 2009). An example of an aromatic plant is caraway (*Carum carvi* L.), a member of Apiaceae family. The water requirements of this species ranged between 11,500 and 12,500 m^3 ha^{-1} (Abdelgawad, 2006). Caraway seeds are used as spice in food due to its pleasant flavour. Caraway essential oil has antioxidant (Yu et al., 2005; Shan et al., 2007; Wojdylo et al., 2007), antibacterial (Alzoreky and Nakahara, 2003; Iacobellis et al., 2005; Shan et al., 2007), fungicidal (Soliman and Badeaa, 2002), insecticidal (Lopez et al., 2008), acaricidal (El-Zemity et al., 2006), larvicidal (Pitasawat et al., 2007) and molluscicidal (Kumar and Singh, 2006) activities. In organic farming, the carvone, which is the main component of caraway essential oil, is used as a natural inhibitor of sprouting, mainly in stored potatoes and onions (Toxopeus and Bouwmeester, 1993; Hartmans et al., 1995; Oosterhaven et al., 1995). In addition, caraway essential oil is important in pharmaceutical applications and also in human medicine due to its diuretic (Lahlou et al., 2007), antihyperglycaemic (Eddouks et al., 2004; Ene et al., 2007; Tahraoui et al., 2007), anti-hyperchlolesterol (Lemhadri et al., 2006) and anticancerous (Naderi-Kalali et al., 2005; Kamaleeswari et al., 2006) properties. On the other hand, caraway seed oil is rich in an unusual fatty acid, the petroselinic one (C18:1*n*−12) which is of potential industrial significance (Murphy et al., 1994; Murphy, 1996).

The present study aims to determine water deficit effects on fatty acid and essential oil composition of caraway seeds when subjected to different water regimes. Indeed, water deficit can change plant behaviour regarding the biosynthesis of bioactive compounds and as no data have been collected regarding caraway lipids and essential oils changes under drought. So, in this paper we investigated for the first time some biochemical responses of this plant when submitted to water deficit.

2. Materials and methods

2.1. Plant material and growth conditions

Caraway seeds were collected from cultivated plants in the region of Haouaria (North-eastern of Tunisia) on July 2006. The

* Corresponding author. Tel.: +216 98 57 40 59.
E-mail address: bochra_laribi@yahoo.fr (B. Laribi).

0926-6690/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2009.07.005

field experiment was carried out in the experimental station of the National Agronomic Institute of Tunisia (3 km northwest Tunis) at latitude 36°55′N and longitude 10°11′W. This site was characterized by a semi-arid climate with a mean annual precipitation of 500 mm (mainly during the winter) and an average temperature of 18 °C. The soil has a clayey-loamy texture with pH 7.9 and consisted of 1.4% organic matter, 0.11% nitrogen, 0.034% phosphorus and 0.006% potassium. The soil pH was measured using a pH meter (pH meter pH 538, Windous Labotechnik WTW). The total nitrogen content was determined by the Kjeldahl method (Bremener and Mulvaney, 1982). Total phosphorus was measured colorimetrically (Murphy and Riley, 1962) after digestion with $HClO_4$ (Adler and Wilcox, 1985). Potassium was determined after extraction by H_2SO_4 (Hunter and Pratt, 1957). The determination of the organic matter was carried out indirectly through the determination of total carbon content (Ben-Dor and Banin, 1989; Davies, 1974).

2.2. Experimental design and water deficit treatment

A completely randomized experimental design was conducted as a factorial bloc with three replications. Plot area was of 2.8 m^2. Caraway seeds were sown manually in the field on March 01, 2007 with row spacing of 0.4 m and by respecting a density of 125 plants m^{-2}. Fertilization consisted of 250, 200 and 100 kg ha^{-1} of P_2O_5, K_2O and N, respectively, incorporated uniformly to the soil before sowing, and supplemented by 100 kg ha^{-1} of N brought twice during the crop cycle. Drip irrigation was provided with a flow of 3.8 l h^{-1}. Pre-irrigation was done immediately after sowing for uniform emergence and establishment of seedlings before starting the water deficit treatment.

Then, plants were subjected to three different water levels: 100% [control (C)], 50% [moderate water deficit (MWD)] and 25% [severe water deficit (SWD)] of crop evapotranspiration (ETc) by means of the software MABIA, which is an irrigation scheduling computer program (Sahli and Jabloun, 2005, 2006; Jabloun and Sahli, 2004, 2007, 2008). The calculation procedures used by this model are based on dual crop coefficient approach according to the United Nations Food and Agriculture Organization (FAO) Penman–Montheith equation (Allen et al., 1998). In addition, weeds were controlled by hand when needed. Harvest was on June 29, 2007. Seeds harvested were air-dried and stored at 4 °C until use for further analysis.

2.3. Growth, seed yield and its components parameters

For each treatment, measurements of plant height, fresh and dry matter weights were evaluated by destructive harvests of seven randomly selected plants from the centre rows of each plot. Plants were harvested at the soil surface and immediately weighed (fresh matter weight). After that, plants were wrapped in a clean paper bags, labelled and then oven-dried at 65 °C for 48 h to constant weight and reweighted (dry matter weight). Their dry matter contents were computed using the following equation:

$$DM\,(\%) = \frac{DMW}{FMW} \times 100$$

where DM: dry matter, FMW: fresh matter weight, and DMW: dry matter weight.

For each treatment seed yield and yield components such as the main branches number per plant, the umbels number per plant and umbellets (umbellules) per umbel, seed yield and 1000 seed weight were determined at the end of the experiment.

2.4. Total lipids extraction

Total lipids from seeds were extracted by the modified method of Bligh and Dyer (1959). Thus, seeds were fixed by boiling water to inactivate the tissue phospholipases (Benson and Strickland, 1960) and then ground manually using a mortar and pestle. A chloroform/methanol/hexane mixture (4:3:2, v/v/v) (Marzouk and Cherif, 1981) was used for total lipid extraction. Finally, the fixation water was added to collect the lipids extracted during fixation. After resting for 24 h at +4 °C, two phases were obtained and the chloroformic phase (lower phase) containing the seeds lipids was dried under a stream of nitrogen; then, the residue was dissolved in a known volume of toluene–ethanol (4:1, v/v) at −20 °C for further analyses. Total lipid extraction was made in triplicate.

2.5. Fatty acid methylation and analysis

Total fatty acids were converted into their methyl esters using 3% sodium methylate in methanol according to the method described by Cecchi et al. (1985). Heptadecanoïc acid methyl ester (C17:0) was used as an internal standard inorder to quantify fatty acids. The superior phase that contains fatty acid methyl esters (FAMEs) was aspired and the solvent volume reduced in a stream of nitrogen, prior to analysis.

Methyl esters obtained were analyzed by gas chromatography using a Hewlett-Packard 6890 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector (FID), an electronic pressure control (EPC) injector. The esters were separated on a RT-2560 capillary column (100 m length, 0.25 mm i.d., 0.20 mm film thickness). The oven temperature was kept at 170 °C for 2 min, followed by a 3 °C min^{-1} ramp to 240 °C and finally held there for an additional 15 min period. Nitrogen was used as carrier gas at a flow rate of 1.2 ml min^{-1}. The injector and detector temperatures were maintained at 225 °C.

2.6. Essential oil isolation

Whole air-dried seeds (50 g) were subjected to hydrodistillation for 90 min (time fixed after a kinetic survey during 30, 60, 90 and 120 min. The optimal yield was obtained at 90 min). The hydrodistillation was performed by a simple laboratory Quik-fit apparatus which consisted of a 1000 ml steam generator flask, a distillation flask, a condenser and a receiving vessel. The obtained distillate was extracted using diethyl-ether as solvent (v/v) and dried over anhydrous sodium sulphate. The organic layer was then concentrated at 35 °C using a Vigreux column and the essential oil stored at −20 °C prior to analysis.

2.7. Essential oil gas chromatography analysis

Essential oil analyses were performed using a Hewlett-Packard 6890 gas chromatograph equipped with a flame-ionization detector (FID) and an electronic pressure control (EPC) injector. A polar HP Innowax (PEG) column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The carrier gas was nitrogen (N_2, U) with a flow rate of 1.6 ml min^{-1}. The split ratio was 60:1. The analyses were performed using the following temperature program: oven temperature isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C min^{-1} and isotherm at 225 °C during 10 min. Injector and detector temperatures were held, respectively, at 250 and 300 °C.

2.8. Gas chromatography–mass spectrometry

The GC–MS analyses were performed on a gas chromatograph HP 6890 (II) interfaced with a HP 5973 mass spectrometer (Agilent