



Influence of Seed Priming on Seed Yield, Oil Content and Fatty Acid Composition of Safflower (*Carthamus tinctorius* L.) Grown Under Water Deficit

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Abstract

Safflower oil is a rich source of omega-3 fatty acids that is important for health. Drought stress can severely decrease the productivity and oil quality of safflower. Thus, in order to study the effect of hydropriming and melatonin-seed-priming on fatty acid composition and yield of Safflower under water deficit conditions a field experiment was carried out as split-factorial in a randomized complete block design with four replicates during 2017 and 2018 growing seasons. The oil concentration of the safflower seeds was determined by soxhlet extraction method. Fatty acids of safflower's oilseed were transformed to their methyl esters (FAME), and a gas chromatograph equipped with a flame ionization detector (FID) was used for determination of fatty acids. The results indicated that melatonin-seed priming increased grain yield, HI and oil yield of safflower under drought condition. Drought stress led to a significant decrease in oil yield, and recently harvested seeds had higher oil yield across both years. The highest amount of oil yield was obtained by hydropriming on seeds which had been stored for 8 years, and recently harvested seeds with 576.50 and 645.57 kg.ha⁻¹, respectively under no-stress condition. Melatonin-seed-priming improved the oil quality of safflower under drought with an increase of unsaturated fatty acids of safflower especially omega 6 and omega 3. Melatonin-seed priming increased the amount of Σ PUFA, Σ UFA/ Σ SFA, P/S and DBI across both years in comparison with unprimed seeds. Seed priming improved the quality of oil and productivity in both recently harvested and stored seeds. It can be concluded that melatonin-seed priming improved the productivity, oil content and composition especially in stored seeds and under drought stress.

Keywords Biostimulator · Iodine value · Melatonin · Oilseed · Seed deterioration

Abbreviations

| | |
|------------|--|
| BBCH scale | (Biologische Bundesantalt, Bundesortenamt and Chemische Industrie) |
| DBI | Double bond index |
| Mel | Melatonin |
| SFA | Saturated fatty acid |
| UFA | Unsaturated fatty acids |

| | |
|----------------------------|--|
| Σ UFA/ Σ SFA | Ratio of total unsaturated to saturated fatty acids |
| PUFA | Poly unsaturated fatty acids |
| P/S | Ratio of poly unsaturated fatty acids to saturated fatty acids |

Introduction

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop in arid and semi-arid regions of the world, because of its high tolerance and adaptation capacity to any environment with limited rainfall (Weiss 2000; Zanetti et al. 2013). Safflower has been investigated by degrees due to its medicinal value and health care properties and is used in herbal medicine in east Asia for the promotion of bone formation and in the treatment of osteoporosis and rheumatism (Bessada et al. 2015). Oil crops are essential ingredients for food supply due to the growing world population. With

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the human population expected to reach 9 billion by 2050, it has been estimated that 44% of the required additional calories will come from oil crops (Teh et al. 2017). Today, the safflower is cultivated as an oilseed crop (de Oliveira et al. 2018). Safflower oil contains saturated fatty acids such as palmitic (C16:0) and stearic (C18:0) and the unsaturated fatty acids such as oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) (Nazari et al. 2017). The oleic acid has excellent stability and a bland taste for frying oil (Smith 1993), while linoleic acid decreases the cholesterol level in the blood (Wilson et al. 2006). Standard safflower oil contains about 6–8% palmitic acid, 2–3% stearic acid, 16–20% oleic acid, and 71–75% linoleic acid (Velasco and Fernandez-Martinez 2001). The lipid profile of a crop determines its worth for industrial and nutritional applications (Schulte et al. 2013). Although safflower has great potential to be grown under severe environmental conditions with a high-value edible oil, it is an outlasted and underutilized crop around the world (Emongor 2010).

Demand for high seed quality, rapid and uniform seedling emergence, has become a priority in meeting the current requests for high standards in the agricultural market (Paparella et al. 2015). Seeds have the highest quality (viability and vigor) at physiological maturity, and seed storage can result in a continuous loss of vigor and eventually death of seeds (Wang et al. 2018). It is important to prevent or reduce the loss of vigor and viability during storage of seeds (Walters et al. 2005).

Drought is the most critical warning to world food security and crop productivity that is particularly important in the context of climate change and an increasing world population especially at arid and semiarid regions in the world (Wojtyla et al. 2016). One of the strategies for imparting higher drought tolerance on plants is seed priming (Farooq et al. 2009). Seed priming involves control hydration of seeds that allows imbibition of water and seeds can pass the first phase of water uptake but they will not reach the third phase of water uptake (Wojtyla et al. 2016). Some researchers reported that seed priming improves the deleterious effects of seed ageing (Butler et al. 2009; Goel et al. 2003). One of the major factors affecting the efficiency of the priming duration of the priming process and seed priming must be done before radicle protrusion (Wojtyla et al. 2016).

Application of chemical compounds as priming agents has been found to improve plant tolerance to environmental stresses in various crops such as soybean (Wei et al. 2015), rice (Zheng et al. 2016) and wheat (Shan et al. 2011). A natural metabolite which is being evaluated by numerous researchers as a biostimulator growth-promoting molecule is the indoleamine molecule melatonin (Mel), which is also involved in multiple physiological processes in plants (Arnao and Hernández-Ruiz 2014). Melatonin (N-acetyl-5-methoxytryptamine) is a low-molecular-weight biomolecule applied

as a bio-stimulant (Arnao and Hernández-Ruiz 2014). The priming potential of melatonin as an exogenously applied agent is the result of its dual mode of action as both a direct antioxidant molecule and also a trigger of antioxidant responses in plants (Zhang et al. 2015). Application of melatonin improves the tolerance of crops to drought stress by maintaining the green color of leaves and lateral root formation (Wei et al. 2015).

In the current study we aimed to investigate the effects of seed priming on oil productivity and composition of safflower with different initial seed quality under different water irrigation. Our hypotheses were (1) melatonin-seed-priming enhances the seed quality of safflower especially in seeds with a lower initial quality, and (2) melatonin-seed-priming decreases the detrimental effect of drought and helped safflower to maintain a normal productivity and quality of safflower's oil under water deficit stress. The aims of this study were (1) to compare stored and recently harvested seeds on the response to seed priming, (2) to assess the effects of melatonin-seed priming and hydropriming on enhancing the quality of stored seeds, and (3) to evaluate the effects of seed priming on seed yield and fatty acid composition of safflower's oil under drought conditions.

Materials and Methods

Plant Material and Chemical Reagents

Seeds of safflower (*Carthamus tinctorius* L.; Goldasht cv.), were prepared from Seed and Plant Improvement Institute, Karaj, Iran. Two seed lots were used for this experiment; one lot was stored for 8 years under natural conditions (20–28 °C; 55–60% relative humidity), and the other was recently harvested seeds. Also the melatonin (N-acetyl-5-methoxytryptamine) was purchased from Sigma-Aldrich company and 0.1 (Mel1) and 0.5 (Mel2) mM concentrations were used for this experiment.

Determination of Seed Priming Duration and Seed Priming Performance

Before the experiment, the initial moisture content was determined in stored and recently harvested seeds of safflower in accordance with the research by Larsen et al. 2004. The moisture content of stored and recently harvested seeds were 7.03 and 6.71% (based on dry weight). For determination of the seed priming duration an experiment was conducted based on the research by Larsen et al. (2004) for various seed priming treatments. For this purpose, 50 seeds were weighted for each treatment and were moistened (in water, Mel1, or Mel2) on filter papers and in petri dishes. Petri dishes were incubated at 25 °C during the imbibition.

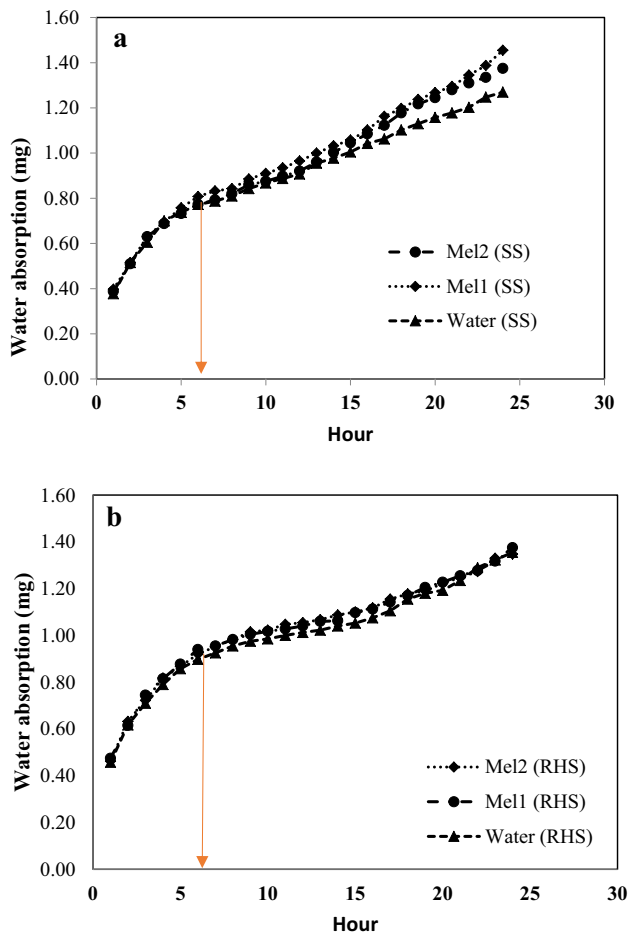


Fig. 1 Determination of seed priming duration for (a) stored seed (SS), and (b) recently harvested seed (RHS)

Seeds were regularly removed from the petri dishes to determine the seed weight after being surface-dried with paper towels. Water uptake was recorded for 24 h with 1 h intervals. According to the results of this initial experiment, the seed priming duration was determined 6 h after submerging seeds in distilled water for hydropriming and two melatonin concentrations (Fig. 1). The ratio of seed weight to solution volume was 1:5 (g/ml) (Basra et al. 2004). Seeds were dried to their original moisture content (11.3%) after 24 h at room temperature (approximately 22 °C, 45% relative humidity).

Experimental Design and Field Conditions

In order to study the effect of seed priming on fatty acid composition and yield of Safflower under water deficit conditions a field experiment was carried out at a research farm on Aburaihan Campus, University of Tehran (35° 28' N, 51° 36' E and 1020 masl), Iran, during the 2017 and 2018 growing seasons. This location is an arid region (according to the de Martonne climate classification) characterized by

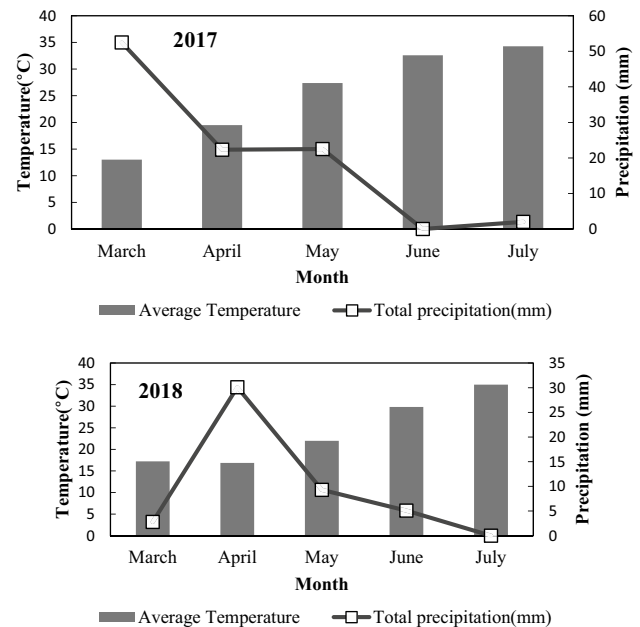


Fig. 2 Variation of temperature and rainfall in Aburaihan Campus meteorology station during 2016 and 2017 growing seasons

warm and dry summers. Long-term (30 years) mean annual rainfall and temperature are 141 mm and 15.6 °C, respectively. Minimum and maximum average temperatures and rainfall amounts during the two growing seasons are summarized in Fig. 2.

The average temperature was cooler in 2018 than in 2017 (1.17 °C cooler). The average temperature in June 2017, which corresponds to safflower flowering time, was 2.77 °C warmer than in June 2018. The average rainfall amount in 2018 was 52.36% lower compared with that in 2017. The rainfall amount in both years was trivial in June. Therefore, after irrigation to reach 85% moisture depletion of field capacity, there was no effective rainfall during flowering, and the experimental stress was not affected by rainfall in both crop seasons. As a result, noticeably stronger environmental stresses, such as lack of rain during flowering and pollination, were higher in 2018 compared with 2017.

The physical and chemical characteristics of the soil and water were determined three weeks before planting. The soil sample was collected at a depth of 0–30 cm. The sample was air dried, crushed and tested for electrical conductivity (EC), pH, total nitrogen (N) using Kjeldahl method (Bremner 1960), available phosphorus (P) by the Olsen procedure (Olsen et al. 1954), available Potassium (K) using flame photometer (Mehlich 1953), boron (B) by azomethine h colorimetric method (Parker and Gardner 1981) and organic carbon through sulfuric acid using the Walkley–Black method (Walkley and Black 1934). For analyzing iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) atomic absorption spectroscopy was used (Tandon 2005). The texture of

Table 1 Physico-chemical properties of the soil and characteristics of water used for irrigation

| Soil properties | Values | Water characteristics | Values |
|------------------------------------|--------|---|--------|
| EC (dSm ⁻¹) | 7.57 | EC (dSm ⁻¹) | 5.39 |
| pH | 8.21 | pH | 7.71 |
| Organic carbon (%) | 1.4 | Ca ²⁺ (mequiv. l ⁻¹) | 17 |
| Total N (%) | 0.14 | Mg ²⁺ (mequiv. l ⁻¹) | 19 |
| Available P (mg kg ⁻¹) | 79 | Na ⁺ (mequiv. l ⁻¹) | 29.4 |
| Available K (mg kg ⁻¹) | 732 | Cl ⁻ (mequiv. l ⁻¹) | 22.5 |
| Fe (mg kg ⁻¹) | 4.23 | SO ₄ (mequiv. l ⁻¹) | 37.28 |
| Zn (mg kg ⁻¹) | 2.3 | SAR | 6.92 |
| Mn (mg kg ⁻¹) | 8.94 | HCO ₃ (mequiv. l ⁻¹) | 5.62 |
| Cu (mg kg ⁻¹) | 0.83 | TDS (ppm) | 2440 |
| B (mg kg ⁻¹) | 1.34 | SSP (%) | 44.95 |

the research field was clay loam. Details of soil properties are shown in Table 1. Also, the water sample was analyzed for 11 parameters, namely electrical conductivity (EC), pH, sodium adsorption ratio (SAR), total dissolved solids (TDS), soluble sodium percentage (SSP), calcium (Ca), magnesium (Mg), sodium (Na), chloride (Cl), sulphate (SO₄), bicarbonate (HCO₃) (Tandon 2005) (Table 1).

The experimental design was split-factorial in a randomized complete block design with four replicates. The factors were; two drought levels (normal irrigation or irrigation to reach 50% soil moisture depletion of field capacity (non-stress)) and irrigation from the beginning of flowering to the end of pollination stage reaching 85% of soil moisture depletion of field capacity (drought stress) were randomized to the main plots. Subplots were 8 treatments in number and consisted of a factorial combination of seed quality (stored seed and recently harvested seeds) and seed priming (unprimed (control), hydropriming(hydro), melatonin 0.1 mM (Mel1) and melatonin 0.5 mM (Mel2).

Each plot was 2 × 4 m² and included 4 rows. The length of the rows was four meters, and a free row was set between plots as a margin. The intra-row spacing was 5 cm; therefore, the final plant density was approximately 400,000 plant ha⁻¹. Safflower seeds were disinfected with fungicide prior to planting. Weeds and insects were effectively controlled all through the field experiment.

Water Deficit Treatments

For precision understanding of phenological stages of safflower the BBCH¹ scale was used for this purpose (Flemmer et al. 2015). The BBCH scale provides an accurate and

simplified approach to identify plant phenological growth stages based on easily observable external morphological characteristics that use a decimal code for growth stages of different plant species (Lancashire et al. 1991). In accordance with the BBCH scale water deficit stress was applied when 50% of the florets opened in concordance with BBCH = 65. All experimental plots were simultaneously and equally irrigated up to the BBCH = 65. After that, control irrigation plots were irrigated up to the harvest time, while drought-stressed plots were irrigated only after determination of soil moisture to achieve 85% of moisture depletion of field capacity. Soil water was determined based on soil moisture release curve, which indicates the relationship between soil water potential and soil moisture content (Saxton et al. 1986; Soltani et al. 2017). According to this method, the soil moisture at field capacity and permanent wilting point was 0.3168 and 0.1584 g.cm⁻³, respectively. The gravimetric method was used for measuring soil moisture (Carter and Gregorich 2008; Jones 2006; Kirkham 2005), based on the method presented by Nazari et al. (2017).

Productivity (Grain Yield and Harvest Index)

According to the BBCH scale, the best stage for harvest is principal growth stage 9 that was determined by BBCH = 99 (Flemmer et al. 2015). For harvesting, the samples consisted of a 2 m² area in the center row of each plot after leaving two rows empty on the border areas to avoid border effects. All samples were dried at 75 °C to constant weight and then weighed to calculate yield and harvest index.

Determination of Oil Concentration, Fatty Acid Profile, and DBI

The oil concentration of the safflower seeds was determined by Soxhlet extraction method and petroleum benzene was used as a solvent (Movahhedy-Dehnavy et al. 2009). Fatty acids were transformed to their methyl esters (FAME), following the method of Metcalfe et al. (1966), and a gas chromatograph (Unicam 4600, Cambridge, England) equipped with a flame ionization detector (FID) was used for this purpose. The programming of the column temperature was the same as reported by Movahhedy-Dehnavy et al. (2009).

A double bond index (DBI), as an indicator of the unsaturation FA fraction, using the relative percentage of each fatty acids (Xu et al. 2011), the ratio of oleic to linoleic acids (*O/L* ratio) (Singkham et al. 2011), and Iodine value (IV) (Mercer et al. 1990) were calculated by using the following formulas:

$$(1) \text{ DBI} = 0 \times ([16:0] + [18:0]) + 1 \times ([16:1] + [18:1] + [20:1]) + 2 \times ([16:2] + [18:2]) + 3 \times ([18:3] + [20:3])$$

¹ Biologische Bundesanstalt, Bundessortenamt and Chemische Industrie.

$$(2) \text{ IV} = (\% \text{oleic acid} \times 0.8601) + (\% \text{linoleic acid} \times 1.7321) + (\% \text{eicosenoic acid} \times 0.7854)$$

Statistical Analyses

For analysing data the SAS Ver. 9.1 (SAS Institute Inc 2004) software and GLM procedure on this software were used for analysis of variance (ANOVA). Mean comparison (LSMEANS) was performed by slicing interaction method with the PDIFF option. For calculating Pearson correlation coefficients in SAS the PROC CORR option used for this purpose. The Microsoft excel was used for drawing the charts.

Results and Discussion

Productivity (Grain Yield, Biological Yield, Harvest Index and, Oil Yield)

The seed yield of safflower significantly decreased in the first year but not in the second year (Table 2). It seems that the lower temperature in June 2018 compared with 2017 and 5.1 mm precipitation, in concordance with flowering and anthesis stages of safflower, lead to non-significant effects of drought stress on seed yield in the second year. There was no difference between grain yield of stored and recently harvested seeds in both years. The results showed that the grain yield had a little change under different situations and treatments but melatonin-seed priming improved grain yield. Under non-stress conditions in stored seeds, the highest amount of grain yield observed in Mel2 (2974.87 kg. ha⁻¹) and Mel1 (2957.87 kg. ha⁻¹). Melatonin-seed-priming at the lowest concentration (Mel1) had a great influence on grain yield in stored and recently harvested seeds, thus Mel1 increased grain yield up to %19.53 in comparison with unprimed seeds in the first year.

The total biomass was not affected by drought stress in both years (data not shown). There was no significant difference in the total biomass between stored and recently harvested seeds over the two years. Seed priming decreased total biomass in stored and recently harvested seeds under non-stress and drought stress conditions. For example, in stored seeds, Mel2 decreased total biomass by 33.35% in both years in comparison with unprimed seeds under non-stress conditions. Drought stress decreased harvest index (HI) in both years (Table 2). The results showed that the Mel1 increased HI by %14.05 under non-stress conditions in stored seeds in both years. The highest value of HI belonged to Mel2 with 41.24% in recently harvested seeds. It seems that melatonin-seed priming decreased total biomass for increasing assimilates transfer toward seeds and increased the HI.

Drought stress led to a significant decrease in oil yield and recently harvested seeds had higher oil yield in both years. The highest amount of oil yield belonged to hydropriming in stored and recently harvested seeds with 576.50 and 645.57 kg. ha⁻¹, respectively, under non-stress conditions (Table 2). Melatonin-seed priming increased oil yield in stored seeds under drought stress. In the present study, the grain yield was significantly correlated with oil yield ($r=0.90$, $P<0.01$) and total biomass ($r=0.90$, $P<0.01$) (data not shown). The flowering stage of safflower is the most sensitive stage to drought stress (Farooq et al. 2009; Movahhedy-Dehnavy et al. 2009) and any water shortage at this stage led to yield losses (Nazari et al. 2017). The main reason for a decreased yield is interruption the flux of assimilates to the reproductive organelles (Farooq et al. 2009). There have been several reports on the decrease in seed yield and biological yield of safflower under water deficit in the past (Lovelli et al. 2007; Movahhedy-Dehnavy et al. 2009; Sampaio et al. 2016). The pre-anthesis assimilates reserves in safflower are very important for achieving higher yields (Koutroubas et al. 2004). Dry climate conditions have a negative influence on the rate of photosynthesis and sink size of safflower grains which can limit safflower production (Koutroubas et al. 2004). However, Singh et al. (2016) suggested that the climate during maturity stage must be dry and warm for achieving higher seed yields in safflower. A reduction in oil yield previously reported for canola (Safavi Fard et al. 2018) and *Sesamum indicum* L. (Kadkhodaie et al. 2014) under drought stress. It seems that melatonin-seed priming and hydropriming have considerable effects on increasing oil and grain yield of safflower under both conditions especially in drought conditions. Wojtyla et al. (2016) concluded that seed priming can enhance tolerance to drought by regulating gene expression and protein abundance of multiple stress-responsive pathways. In a meta-analysis study, Soltani and Soltani (2015) reported that seed priming has a considerable influence of grain yield and they suggested hydropriming as a cost effective method for increasing grain yield. Also Zhang et al. (2015) reported that a pre-treatment of seeds with melatonin increased the growth of plants and resulted in maintaining a developed root system and improvement in the photosynthetic capacity under drought stress. Liang et al. (2019) indicated that a pre-treatment of seeds with melatonin increased the stomatal conductance and produced a significant increase in photosynthetic capacity because the stomata remained open.

Oil Content and Fatty Acids Profile

Oil Content

The oil content of safflower significantly decreases with drought stress (Table 2). The oil content was higher in

Table 2 Effect of water deficit stress “WS” (W1-non-stress: normal irrigation or irrigation to reach 50% soil moisture depletion of field capacity, W2: irrigation from the beginning of flowering to the end of pollination stage reaching 85% of soil moisture depletion of field capacity), seed quality “SQ” (SS stored seed, RHS recently harvested seed) and seed priming “SP” (Control: unprimed seed, Hydro hydropriming on distilled water, Mel1 melatonin-seed-priming at 0.1 mM concentration, Mel2 melatonin-seed-priming at 0.5 mM concentration) on grain yield, harvest index, oil yield and oil content of safflower in 2017 and 2018 growing seasons

| WS | SQ | SP | Grain yield (kg.ha ⁻¹) | | Harvest index (%) | | Oil yield (kg.ha ⁻¹) | | Oil content (%) | | |
|-----|-----|---------|------------------------------------|--------------------|--------------------|------------------|----------------------------------|------------------|------------------|------------------|-----------------|
| | | | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | |
| W1 | SS | Control | 2704.17 ± 115.75a | 2712.97 ± 19.45a | 36.945 ± 1.277a | 31.596 ± 4.485b | 533.49 ± 26.90ab | 574.75 ± 5.79a | 19.710 ± 0.185b | 21.184 ± 0.101a | |
| | | Hydro | 2795.43 ± 182.51a | 2415.87 ± 337.35a | 36.968 ± 0.828a | 36.160 ± 2.774ab | 576.50 ± 42.07a | 521.04 ± 76.40a | 20.595 ± 0.175a | 21.505 ± 0.179a | |
| | | Mel1 | 2637.36 ± 168.90a | 2957.87 ± 160.29a | 37.893 ± 0.511a | 39.649 ± 1.066a | 554.89 ± 26.48ab | 515.23 ± 24.44a | 21.102 ± 0.347a | 17.437 ± 0.148b | |
| | | Mel2 | 2570.75 ± 135.42a | 2974.87 ± 184.41a | 34.546 ± 0.448a | 35.364 ± 0.704ab | 505.02 ± 28.77b | 529.32 ± 36.17a | 19.636 ± 0.215b | 17.770 ± 0.126b | |
| | RHS | Control | 3006.06 ± 62.57a | 2628.75 ± 205.22a | 33.645 ± 1.513b | 28.367 ± 1.420b | 619.41 ± 16.80a | 505.92 ± 42.49a | 20.598 ± 0.188a | 19.219 ± 0.112c | |
| | | Hydro | 2724.79 ± 155.65a | 2890.17 ± 480.20a | 36.424 ± 0.179ab | 26.085 ± 3.956b | 550.55 ± 35.02b | 645.57 ± 109.72a | 20.204 ± 0.509a | 22.302 ± 0.134a | |
| | | Mel1 | 2859.88 ± 149.94a | 2733.35 ± 401.63a | 34.451 ± 0.463b | 37.301 ± 0.984a | 584.22 ± 25.59ab | 584.19 ± 85.01a | 20.45 ± 0.213a | 21.393 ± 0.164b | |
| | | Mel2 | 2738.99 ± 46.96a | 2157.00 ± 283.44a | 41.248 ± 4.766a | 40.879 ± 1.499a | 530.67 ± 8.97b | 486.15 ± 66.68a | 19.377 ± 0.175b | 22.498 ± 0.173a | |
| | W2 | SS | Control | 2573.60 ± 108.97a | 3044.62 ± 116.56a | 32.344 ± 1.495ab | 32.838 ± 1.593a | 496.12 ± 22.07a | 566.63 ± 23.18ab | 19.274 ± 0.147b | 18.607 ± 0.139b |
| | | | Hydro | 2284.02 ± 104.00a | 2642.76 ± 136.42a | 30.757 ± 0.740b | 29.208 ± 2.026a | 462.02 ± 21.87a | 611.79 ± 33.67a | 20.228 ± 0.251a | 23.137 ± 0.096a |
| | | | Mel1 | 2607.92 ± 21.67a | 2449.760 ± 250.19a | 36.686 ± 1.112a | 30.927 ± 2.248a | 511.32 ± 1.27a | 428.69 ± 47.89b | 19.611 ± 0.190ab | 17.454 ± 0.180c |
| | | | Mel2 | 2589.81 ± 60.79a | 2682.43 ± 292.32a | 33.162 ± 1.915ab | 33.240 ± 3.035a | 521.04 ± 15.99a | 468.47 ± 47.95ab | 20.108 ± 0.153a | 17.512 ± 0.185c |
| RHS | | Control | 2339.75 ± 146.45b | 3201.01 ± 106.49a | 33.499 ± 2.522a | 34.903 ± 0.849a | 488.55 ± 20.71ab | 642.78 ± 18.76a | 20.961 ± 0.437a | 20.089 ± 0.088c | |
| | | Hydro | 2217.96 ± 4.98b | 2679.06 ± 233.820a | 36.060 ± 1.929a | 32.291 ± 0.825a | 474.18 ± 2.63ab | 488.43 ± 47.46a | 21.379 ± 0.125a | 18.186 ± 0.172d | |
| | | Mel1 | 2796.89 ± 26.75a | 2603.66 ± 485.54a | 36.964 ± 3.397a | 30.151 ± 3.894a | 536.14 ± 2.22a | 534.22 ± 95.98a | 19.172 ± 0.113b | 20.585 ± 0.125b | |
| | | Mel2 | 2240.60 ± 140.36b | 2850.20 ± 216.58a | 35.143 ± 0.928a | 31.305 ± 1.286a | 437.39 ± 23.09b | 633.50 ± 49.28a | 19.557 ± 0.193b | 22.219 ± 0.140a | |

For a given year LSMEANS (along with ± standard error) within each column of each section followed by the same letter are not significantly different (P < 0.05)

W1 No-stress, W2 water stress, SS Stored seed, RHS Recently harvested seed

recently harvested seeds as opposed to stored seeds in both years. Under non-stress conditions, the hydropriming played a constant role in sustaining high amounts of oil content in stored and recently harvested seeds in both years. Under drought stress conditions, the highest amount of oil content was obtained in hydropriming (23.13%) in stored seeds while it had the highest level in Mel2 (22.22%) for recently harvested seeds (Table 2). In the present study, the average amount of oil content was 20% that was slightly lower than that reported by Knowles and Ashri (1995); oil content of safflower ranged from 21.2 to 25.8% in spring sowing. Since the soil was saline in our experiment it may cause a decrease in oil content in grains (Table 1). It has been reported that salinity stress reduced oil content of safflower grains (Yeilaghi et al. 2012), the main reason for this decrease maybe related to the toxic effect of sodium ions that led to disorders in enzymatic activity and metabolic process of cells (Ghassemi-Golezani and Farhangi-Abri 2018). Improved oil content by seed priming was previously reported in safflower (Bastia et al. 1999) and in *Linum usitatissimum* L. (Rehman et al. 2014).

Saturated Fatty Acids(SFA)

The range of palmitic acid was between 6.01 to 7% and SFA was not affected by drought stress in either year (Table 3). In stored seeds under non-stress conditions, Mel2 and hydropriming increased palmitic acids by 6.4 and 3.52%, respectively compared with unprimed seeds. The range of stearic acid in the present study was between 0.41 to 3.370% in both 2017 and 2018. In the present study the stearic acid was decreased by drought stress in both years. Melatonin-seed-priming (Mel1) increased stearic acid in stored and recently harvested seeds under non-stress conditions (Table 3). The average amount of \sum SFA was 9.239% in both years. In the present study, the \sum SFA was decreased by drought. Mel2 increased \sum SFA up to 7.66% in stored seeds under non-stress conditions while Mel2 increased \sum SFA by % 1.25 in recently harvested seeds. It seems that decreased \sum SFA in our experiment originated from drought resistance capacity in safflower. The range of palmitic in the present study is in accordance with Gecgel et al. (2007) and Sabzalian et al. (2008). Researchers are not in agreement about the effects of drought stress on palmitic acid; some researchers reported a decrease (Ashrafi and Razmjoo 2010), some reported an increase (Nouraei et al. 2016), and some reported no change at all (Kim et al. 2006). In accordance with our results, Neđeral et al. (2014) reported that palmitic acid was not influenced by change in environment conditions such as temperature or rainfall. Similar to our finding, Ashrafi and Razmjoo (2010) reported that stearic acid decreased with drought stress. Although some researchers reported that stearic was subject to slight changes (Nazari

et al. 2017) or increased (Dwivedi et al. 1993) with drought stress. The total amount of saturated fatty acid decreased throughout both years as previously reported by Ashrafi and Razmjoo (2010). They indicated that saturated fatty acids of safflower decreased with drought stress and suggested that imposing drought may enhance the quality of safflower oil seed due to reducing the requirement for a hydrogenation process which may otherwise produce undesirable trans-fats in food that decreased oil quality. Environmental conditions have a great influence on grain quality traits, oil concentration and the saturated fatty acids composition (Jalilian et al. 2012).

Mono Unsaturated Fatty Acids [Palmitoleic (C16:1) (Cis, Omega-7), Oleic Acid (C 18:1) (Cis, Omega-9) and Eicosenoic Acid (C20:1) (Cis, Omega-9)]

The average amount of palmitoleic acid in the present study was 0.063% in both years (Table 3). In the present study the drought stress decreased palmitoleic acids in both years. Melatonin-seed-priming increased the palmitoleic acid in both recently harvested and stored seeds and drought treatments. The range of oleic acid was between 13.11 to 18.75% among treatments (Table 3). Drought stress had no significant effect on the oleic acid in 2017 but it increased due to drought stress in 2018. The oleic acid was higher in stored seeds than recently harvested seeds in both years. Under non-stress conditions, stored seeds showed no response to seed priming. However, hydropriming and Mel2 increased oleic acid content by 20.33 and 5.28% in recently harvested seeds under non-stress conditions. The amount of eicosenoic acid was between 0.03 to 0.33% in both two years (Table 3). Melatonin-seed priming increased the amount of eicosenoic acid in stored and recently harvested seeds up to 19.88 and 151.40% (on average over both years), respectively under non-stress conditions. Under drought stress conditions in stored seeds, Mel2 and hydropriming had 0.221 and 0.114% eicosenoic acid (Table 3).

In the present study, the \sum MUFA was increased by drought stress only in 2018 and there was no change in 2017. The results showed that \sum MUFA was higher in stored than recently harvested seeds during both years. Seed priming decreased \sum MUFA in stored seeds under non-stress conditions. The Mel2 (16.35%) and hydropriming (16.59%) had the highest \sum MUFA in recently harvested seeds with the same condition. Nazari et al. (2017) in a comprehensive study on oil characteristics of five *Carthamus* species under drought stress reported that oleic acid increased in the event of drought in all five species. The increased oleic acid in the present study were similar to the findings of Nouraei et al. (2016) on globe artichokes. Although some researchers reported that oleic decreased in the event of drought stress such as in safflower (Ashrafi and Razmjoo 2010) and

Table 3 Effect of water deficit stress “WS” (W1-non-stress: normal irrigation or irrigation to reach 50% soil moisture depletion of field capacity, W2: irrigation from the beginning of flowering to the end of pollination stage reaching 85% of soil moisture depletion of field capacity), seed quality “SQ” (SS stored seed, RHS recently harvested seed) and seed priming “SP” (Control: unprimed seed, Hydro: hydropriming on distilled water, Mel1 melatonin-seed-priming at 0.1 mM concentration, Mel2 melatonin-seed-priming at 0.5 mM concentration) on fatty acids profile and related characteristics

| WS | SQ | SP | Palmitic acid (SFA) (%) | | Stearic (SFA) (%) | | Palmitoleic (UFA) (%) | | Oleic acid (UFA) % | | Eicosenoic acid (UFA) (%) | |
|----|-----|---------|-------------------------|------------------|--------------------------|-----------------|-------------------------------|-------------------|--------------------|------------------|---|------------------|
| | | | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| W1 | SS | Control | 6.788 ± 0.090a | 6.830 ± 0.148a | 2.346 ± 0.123a | 2.717 ± 0.112a | 0.138 ± 0.0034b | 0.063 ± 0.0024b | 18.419 ± 0.153a | 14.535 ± 0.190a | 0.194 ± 0.0030b | 0.090 ± 0.032b |
| | | Hydro | 7.022 ± 0.073a | 6.701 ± 0.128a | 2.411 ± 0.119a | 2.526 ± 0.163a | 0.105 ± 0.0049c | 0.084 ± 0.0022a | 15.335 ± 0.177c | 14.109 ± 0.083a | 0.224 ± 0.0106a | 0.078 ± 0.0026c |
| | | Mel1 | 6.918 ± 0.082a | 6.732 ± 0.115a | 2.344 ± 0.025a | 2.532 ± 0.198a | 0.042 ± 0.0022d | 0.087 ± 0.0024a | 15.132 ± 0.113c | 14.172 ± 0.087a | 0.129 ± 0.0038c | 0.104 ± 0.0043a |
| | | Mel2 | 7.223 ± 0.130a | 6.657 ± 0.216a | 2.615 ± 0.083a | 2.611 ± 0.086a | 0.147 ± 0.0038a | 0.061 ± 0.0022b | 16.45 ± 0.166b | 13.517 ± 0.161b | 0.241 ± 0.0309a | 0.087 ± 0.0037bc |
| W2 | RHS | Cotrol | 6.989 ± 0.3181a | 7.079 ± 0.103a | 2.319 ± 0.108a | 2.520 ± 0.185a | 0.052 ± 0.0018a | 0.00 ± 0c | 15.336 ± 0.151b | 13.707 ± 0.174c | 0.127 ± 0.0039b | 0.038 ± 0.0028c |
| | | Hydro | 6.761 ± 0.1996a | 6.688 ± 0.117b | 2.380 ± 0.147a | 2.462 ± 0.182a | 0.059 ± 0.0016a | 0.051 ± 0.0016 b | 14.406 ± 0.141c | 16.494 ± 0.165a | 0.115 ± 0.0068b | 0.046 ± 0.0024c |
| | | Mel1 | 6.839 ± 0.1277a | 6.883 ± 0.122ab | 2.416 ± 0.127a | 2.749 ± 0.136a | 0.044 ± 0.0020b | 0.048 ± 0.0026 b | 15.605 ± 0.226b | 13.632 ± 0.161c | 0.075 ± 0.0046c | 0.092 ± 0.0041b |
| | | Mel2 | 6.774 ± 0.1214a | 7.118 ± 0.090a | 2.380 ± 0.099a | 2.605 ± 0.165a | 0.041 ± 0.0014b | 0.107 ± 0.0046a | 16.149 ± 0.097a | 14.855 ± 0.179b | 0.164 ± 0.0043a | 0.142 ± 0.0024a |
| | SS | Control | 6.766 ± 0.1763a | 6.864 ± 0.110ab | 2.430 ± 0.125a | 2.397 ± 0.149a | 0.063 ± 0.0030c | 0.038 ± 0.0016b | 15.361 ± 0.150ab | 15.785 ± 0.161b | 0.0732 ± 0.0039c | 0.095 ± 0.0030b |
| | | Hydro | 6.805 ± 0.072a | 6.802 ± 0.147ab | 2.311 ± 0.120a | 2.401 ± 0.181a | 0.032 ± 0.0018d | 0.00 ± 0c | 15.609 ± 0.185ab | 15.620 ± 0.160b | 0.106 ± 0.0047b | 0.114 ± 0.0028a |
| | | Mel1 | 6.698 ± 0.239a | 6.787 ± 0.138b | 2.620 ± 0.269a | 2.437 ± 0.153a | 0.078 ± 0.0026b | 0.067 ± 0.0028a | 15.317 ± 0.191b | 15.947 ± 0.077ab | 0.065 ± 0.0070c | 0.085 ± 0.0037c |
| | | Mel2 | 7.091 ± 0.122a | 7.151 ± 0.113a | 2.471 ± 0.103a | 2.287 ± 0.157a | 0.088 ± 0.0026a | 0.00 ± 0c | 16.584 ± 0.184a | 16.394 ± 0.174a | 0.221 ± 0.0083a | 0.058 ± 0.0034d |
| | RHS | Control | 6.527 ± 0.189a | 6.916 ± 0.084a | 2.332 ± 0.121a | 2.427 ± 0.163a | 0.058 ± 0.0022c | 0.051 ± 0.0022ab | 15.429 ± 0.203bc | 15.521 ± 0.182a | 0.095 ± 0.0047b | 0.092 ± 0.0049b |
| | | Hydro | 6.828 ± 0.200a | 6.849 ± 0.107a | 2.449 ± 0.141a | 2.333 ± 0.153a | 0.076 ± 0.0021b | 0.047 ± 0.0034b | 15.279 ± 0.153c | 15.189 ± 0.160ab | 0.122 ± 0.0034a | 0.085 ± 0.0037b |
| | | Mel1 | 6.719 ± 0.192a | 6.807 ± 0.090a | 2.460 ± 0.106a | 2.484 ± 0.185a | 0.121 ± 0.0022a | 0.055 ± 0.0016a | 15.835 ± 0.134b | 15.065 ± 0.066b | 0.114 ± 0.0033ab | 0.063 ± 0.0030c |
| | | Mel2 | 6.5310 ± 0.187a | 6.8855 ± 0.102a | 0.434 ± 0.010b | 2.4450 ± 0.167a | 0.0425 ± 0.0017d | 0.0540 ± 0.0018ab | 17.0892 ± 0.115a | 13.5130 ± 0.163c | 0.1242 ± 0.0051a | 0.1730 ± 0.0038a |
| WS | SQ | SP | Linoleic acid (UFA) % | | Linolenic acid (UFA) (%) | | Eicosatrienoic acid (UFA) (%) | | Oleic/linoleic(OL) | | Ratio of poly unsaturated fatty acids to \sum SFA (P/S) | |
| | | | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 | 2017 | 2018 |
| W1 | SS | Control | 71.422 ± 0.181c | 74.949 ± 0.060c | 0.325 ± 0.0164a | 0.339 ± 0.0175a | 0.220 ± 0.0096b | 0.2490 ± 0.0163a | 0.258 ± 0.0014a | 0.194 ± 0.0025a | 7.878 ± 0.019ab | 7.930 ± 0.222a |
| | | Hydro | 74.388 ± 0.173a | 75.791 ± 0.137b | 0.288 ± 0.0094a | 0.319 ± 0.0107a | 0.219 ± 0.0116b | 0.2035 ± 0.0055b | 0.206 ± 0.0018b | 0.186 ± 0.0014b | 7.945 ± 0.143ab | 8.272 ± 0.076a |
| | | Mel1 | 74.752 ± 0.159a | 75.781 ± 0.123b | 0.314 ± 0.0094a | 0.340 ± 0.0171a | 0.233 ± 0.0012a | 0.0730 ± 0.0454c | 0.202 ± 0.0020b | 0.187 ± 0.0014b | 8.130 ± 0.037a | 8.253 ± 0.296a |
| | | Mel2 | 72.466 ± 0.183b | 76.397 ± 0.165a | 0.315 ± 0.0106a | 0.326 ± 0.0189a | 0.211 ± 0.0059b | 0.2332 ± 0.0051ab | 0.227 ± 0.0028b | 0.177 ± 0.0024c | 7.426 ± 0.164b | 8.331 ± 0.292a |
| | RHS | Control | 74.735 ± 0.261b | 76.180 ± 0.090a | 0.314 ± 0.0110a | 0.214 ± 0.0071b | 0.240 ± 0.0116ab | 0.1635 ± 0.0053b | 0.205 ± 0.0026b | 0.180 ± 0.0024c | 8.127 ± 0.320a | 7.994 ± 0.226a |
| | | Hydro | 75.331 ± 0.206a | 73.792 ± 0.127c | 0.289 ± 0.0096a | 0.291 ± 0.1000a | 0.230 ± 0.0106b | 0.1730 ± 0.0043b | 0.191 ± 0.0014c | 0.223 ± 0.0026a | 8.299 ± 0.085a | 8.117 ± 0.084a |
| | | Mel1 | 74.360 ± 0.158b | 76.135 ± 0.115a | 0.310 ± 0.0107a | 0.308 ± 0.0101a | 0.241 ± 0.0147ab | 0.1982 ± 0.0096ab | 0.2100 ± 0.0034b | 0.179 ± 0.0024c | 8.093 ± 0.024a | 7.973 ± 0.223a |
| | | Mel2 | 73.716 ± 0.188c | 74.529 ± 0.165b | 0.308 ± 0.0079a | 0.313 ± 0.0109a | 0.242 ± 0.0115a | 0.2240 ± 0.0100a | 0.219 ± 0.0018a | 0.199 ± 0.0027b | 8.126 ± 0.1814a | 7.721 ± 0.079a |
| W2 | SS | Control | 74.459 ± 0.198a | 74.211 ± 0.158ab | 0.326 ± 0.0132a | 0.276 ± 0.0099a | 0.233 ± 0.0127ab | 0.2365 ± 0.0173a | 0.206 ± 0.0014b | 0.212 ± 0.0024b | 8.195 ± 0.092a | 8.068 ± 0.031a |
| | | Hydro | 74.394 ± 0.188a | 74.506 ± 0.158a | 0.275 ± 0.0144b | 0.286 ± 0.0135a | 0.214 ± 0.0088c | 0.2475 ± 0.0208a | 0.210 ± 0.0030b | 0.209 ± 0.0016b | 8.223 ± 0.151a | 8.1533 ± 0.017a |
| | | Mel1 | 74.661 ± 0.192a | 73.855 ± 0.157bc | 0.322 ± 0.0134a | 0.282 ± 0.0104a | 0.243 ± 0.0156a | 0.2395 ± 0.0192a | 0.205 ± 0.0029b | 0.215 ± 0.0006b | 8.094 ± 0.237a | 8.065 ± 0.078a |
| | | Mel2 | 73.067 ± 0.110b | 73.748 ± 0.147c | 0.333 ± 0.0156a | 0.271 ± 0.0229a | 0.227 ± 0.0157b | 0.2230 ± 0.0156a | 0.227 ± 0.0028a | 0.222 ± 0.0028a | 7.711 ± 0.164a | 7.886 ± 0.239a |
| | RHS | Control | 74.598 ± 0.213b | 74.415 ± 0.170c | 0.306 ± 0.0075ab | 0.288 ± 0.0146b | 0.242 ± 0.0117b | 0.2350 ± 0.0199a | 0.207 ± 0.0032bc | 0.208 ± 0.0028a | 8.516 ± 0.328b | 8.035 ± 0.197a |
| | | Hydro | 74.621 ± 0.226ab | 74.847 ± 0.161b | 0.305 ± 0.0087b | 0.351 ± 0.0169a | 0.173 ± 0.0046c | 0.2195 ± 0.0134a | 0.205 ± 0.0026c | 0.202 ± 0.0024ab | 8.125 ± 0.277b | 8.214 ± 0.055a |
| | | Mel1 | 74.151 ± 0.134b | 74.902 ± 0.136b | 0.338 ± 0.0170ab | 0.327 ± 0.0163a | 0.174 ± 0.0089c | 0.2332 ± 0.0130a | 0.213 ± 0.0020b | 0.201 ± 0.0012b | 8.136 ± 0.092b | 8.124 ± 0.095a |
| | | Mel2 | 75.180 ± 0.169a | 76.365 ± 0.192a | 0.349 ± 0.0194a | 0.290 ± 0.0084b | 0.253 ± 0.0193a | 0.1980 ± 0.0092a | 0.227 ± 0.0010a | 0.177 ± 0.0016c | 10.904 ± 0.290a | 8.237 ± 0.050a |

For a given year LSMEANS (along with ± standard error) within each column of each section followed by the same letter are not significantly different ($P < 0.05$)

W1 No-stress, W2 water stress, SS Stored Seed, RHS Recently Harvested Seed

canola (Aslam et al. 2009). Carvalho et al. (2006) claimed that the climate and growing conditions have great influence on the fatty acids composition in grains. Also Nazari et al. (2017) concluded that the main reason for increased oleic under drought stress is due to earlier maturity of plants that will result in a shorter period of grain filling and as a consequence a shorter time cycle for conversion of oleic to linoleic acid. An increase in oleic/linoleic acid ratio was already reported under water stress conditions occurring during grain filling in different sunflower genotypes (Laribi et al. 2009).

Poly Unsaturated Fatty Acids [Linoleic Acid (c18:2) (Cis, Omega-6), α -Linolenic Acid (c18:3) (Cis, Omega-3) and Eicosatrienoic Acid (c20:3) (Cis, Omega-3)]

The amount of linoleic acid was 70.97–76.84% that increased with drought stress in 2017 and reversibly decreased in 2018 (Table 3). The linoleic acid content was higher in recently harvested seeds than stored seeds over both years. Melatonin-seed-priming (Mel1) and hydropriming increased linoleic acid in stored seeds under non-stress conditions in both years. The highest amount of linoleic acid belonged to Mel2 (76.397%) but melatonin-seed-priming did not increase linoleic acid in recently harvested seeds (Table 3). The range of α -linolenic acid was between 0.19 to 0.39% in our experiment that was increased by drought stress in 2017 and was not affected in 2018. The highest amount of α -linolenic acid belonged to Mel1 (0.340%) in stored seeds under non-stress conditions (Table 3). Under drought stress conditions, it was observed that in stored and recently harvested seeds, the melatonin-seed-priming and hydropriming increased α -linolenic acid content in comparison with unprimed seeds. The eicosatrienoic acid amount was 0.019–0.298% that decreased with drought stress in 2017 and increased in 2018 (Table 3). In the present study, melatonin-seed-priming influenced eicosatrienoic acid content in stored and recently harvested seeds in both two years under non-stress conditions but recently harvested seeds were more affected by melatonin. Under drought stress conditions the Mel2 and hydropriming with 0.243 and 0.247% respectively gained a higher amount of eicosatrienoic acid.

Drought stress increased the \sum PUFA in 2017 and caused a decrease in 2018. The results showed that melatonin-seed-priming increased \sum PUFA in stored seeds; up to 3.25% compared with unprimed seeds under non-stress conditions. In recently harvested seeds, the higher amount of \sum PUFA with 76.64 and 75.85% belonged to Mel1 and hydropriming respectively under non-stress conditions. Under drought stress conditions in stored seeds, Mel1 and hydropriming gained a higher amount of \sum PUFA. Mel2 increased the amount of \sum PUFA of up to 1.70% (on average in both years) in comparison with unprimed seeds. In the present study,

the average amount of \sum UFA was 90.64%. The amount of \sum UFA increased under drought over both years. Nedřal et al. (2014) indicated that by decreasing the amount of rainfall, the value of oleic acid and saturated fatty acids decreased, while linoleic acid and linolenic acid increased in pumpkins. On the contrary, some researchers reported that drought decreased linoleic acid in safflower (Ashrafi and Razmjoo 2010; Nazari et al. 2017). It has also been reported that drought stress had a slight impact on the fatty acid composition of soybean seeds Dornbos and Mullen 1991). Increased \sum UFA in the present study was previously supported by Zhang et al. (2005) stating that increased unsaturated fatty acids content is a defense mechanism. The ratio of \sum UFA/ \sum SFA increased with drought over both years. The highest \sum UFA/ \sum SFA ratio belonged to treated seeds with Mel2 (9.83 and 9.72 in stored and recently harvested seeds, respectively) under non-stress conditions.

Zhang et al. (2017) reported that an increase in unsaturation levels of fatty acids under drought stress is a possible defence mechanism against the detrimental effect of drought on the turf grass plant. The linoleic and α -linolenic acid or essential fatty acids are responsible for fluidity of membranes so affecting the enzymes and receptors of membrane-bound (Das 1991, 2006). The ratio of essential fatty acid/non-essential showed no change in drought in 2017 but decreased in the event of drought in 2018. The highest value of this ratio was obtained in treated seeds with Mel1 (4.89) and hydropriming (4.78) in stored seeds under drought stress conditions. The highest value of essential fatty acid/non-essential ratio (5.67) belonged to Mel2 and was followed by hydropriming (4.90).

The average Ratio of Poly Unsaturated Fatty acids to \sum SFA (P/S) was 8.16. The P/S index increased by drought stress over both years (Table 3). The results indicated that hydropriming was successful in stored and recently harvested seeds under non-stress conditions, and seed priming treatment increased P/S index in both years. The P/S index increased up to 1.79 and 4.17% (on average in both years) in stored and recently harvested seeds, respectively. The highest value of P/S index was obtained in recently harvested seeds and Mel2 (10.90) under drought stress conditions (Table 3). Although Kang et al. (2005) reported an increase in P/S index led to low level of lipid deposition in human body. In contrast with our results, Nazari et al. (2017) reported that P/S index was not significantly affected by various environments. They also suggested that safflower has high stability under drought condition (Nazari et al. 2017). It is also reported that the P/S index and fatty acid composition may be affected by abiotic stresses (Sabzalian et al. 2008).

The oleic to linoleic ratio increased with drought in 2018 but not in 2017 (Table 3). In the present study the seed priming decreased O/L in stored seeds under non-stress conditions but recently harvested seeds had a positive response

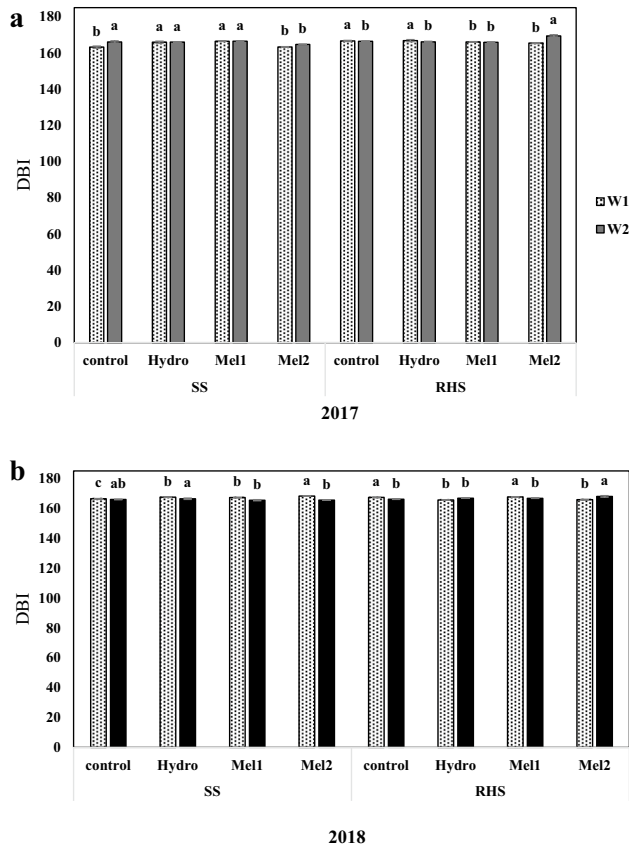


Fig. 3 Effect of water deficit stress “WS” (W1-non-stress: normal irrigation or irrigation to reach 50% soil moisture depletion of field capacity, W2: irrigation from the beginning of flowering to the end of pollination stage reaching 85% of soil moisture depletion of field capacity), seed quality “SQ” (SS stored seed, RHS recently harvested seed) and seed priming “SP” (Control: unprimed seed, Hydro: hydropriming on distilled water, Mel1: melatonin-seed-priming at 0.1 mM concentration, Mel2: melatonin-seed-priming at 0.5 mM concentration on double bond index (DBI), fatty acids in 2017 (a) and 2018 (b) growing seasons. LS MEANS within each column of each section followed by the same letter are not significantly different ($p \leq 0.05$)

to seed priming. The highest O/L ratio was obtained with Mel2 in stored seeds in both years (Table 3). Our results were similar to previous works on globe artichokes (Nouraei et al. 2016) and soybeans (Bellaloui et al. 2013) in which O/L ratio increased under drought stress. The O/L ratio indicates stability, maintenance ability and quality of the oil (Andersen and Gorbet 2002). This index increased when the plant was faced with water stress (Nouraei et al. 2016). More stability of oleic acid in comparison to linoleic acid (a higher value of O/L ratio) gives more durability and longer shelf-life to the oil and its derived products (Chaiyadee et al. 2013).

The average amount of double bond index (DBI) was 166.27. In the present study, the drought stress increased DBI index in 2017 while it decreased it in 2018 (Fig. 3).

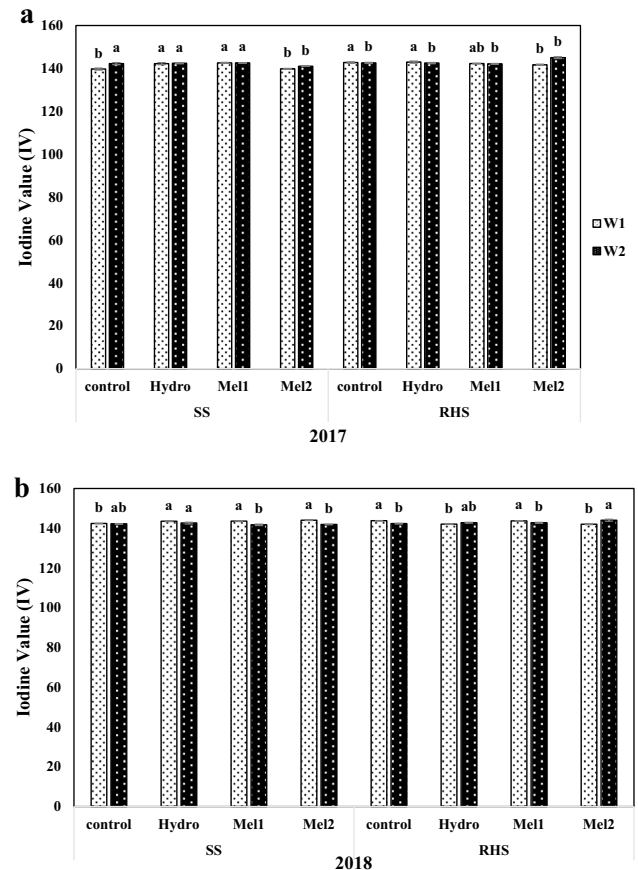


Fig. 4 Effect of water deficit stress “WS” (W1-non-stress: normal irrigation or irrigation to reach 50% soil moisture depletion of field capacity, W2: irrigation from the beginning of flowering to the end of pollination stage reaching 85% of soil moisture depletion of field capacity), seed quality “SQ” (SS stored seed, RHS recently harvested seed) and seed priming “SP” (Control: unprimed seed, Hydro: hydropriming on distilled water, Mel1: melatonin-seed-priming at 0.1 mM concentration, Mel2: melatonin-seed-priming at 0.5 mM concentration on iodine value (IV), fatty acids in 2017 (a) and 2018 (b) growing seasons. LS MEANS within each column of each section followed by the same letter are not significantly different ($p \leq 0.05$)

Under non-stress conditions in stored seeds, melatonin-seed-priming increased DBI up to 1.51% in comparison with unprimed seeds in both years. In recently harvested seeds, the response to seed priming was weak however hydropriming and Mel1 with 166.80 and 167.56 had higher DBI than control. Under drought stress conditions in stored seeds, the difference between seed priming and unprimed seeds was very low however in this situation, Mel and hydropriming had higher DBI in comparison with other treatments (Fig. 3). There are controversial reports on DBI under drought stress. Some of researchers reported that DBI decreased under drought (Sánchez-Martín et al. 2018; Xu et al. 2011) and some, reported that DBI increased under drought (Zhang et al. 2017).

The iodine value (IV) in our experiment was 138.57–146.07 in both years (Fig. 4). The IV decreased with drought in 2017 but increased in 2018. The IV shows the degree of unsaturation of the oil. Melatonin-seed-priming and hydropriming under non-stress conditions in stored seeds with increased IV up to 1.6 and %1.27 (on average in both years) in comparison with unprimed seeds over both two years. Under drought stress conditions in stored seeds, Mel1 and hydropriming had a higher IV but in recently harvested seeds the Mel2 was superior in both years (144.52) (Fig. 4). Knowles and Mutwakil (1963) reported that iodine value in safflower varies from 138 to 145 and the average amount of IV in oil of safflower collected from Iran was 143. This value must be between 75 and 150 for food products and this value for safflower was 139.9. The iodine value is the indicator of stability and health characteristics of oil (Thomas 2000). There are controversial reports on iodine values because some researchers reported that IV decreased with drought (Nouraei et al. 2016) and some reported that IV was unchanged under drought conditions (Ali et al. 2012). The high level of IV indicated the increase in unsaturated fatty acids fraction thus decreasing the oil stability and increased sensitivity to oxidation (Erickson 1990). Bessada et al. (2015) showed that safflower seeds contain serotonin, a molecule that has very common characteristics with melatonin, and the anti-oxidative property of serotonin probably plays a role in the fatty acids composition of safflower oil. We do not know the reason for the improvement in the oil composition by melatonin. However, melatonin can decrease lipid peroxidation when it is placed between the polar heads of polyunsaturated fatty acids (Reiter et al. 2009).

In the present study, iodine value significantly correlated with linoleic ($r=0.91$, $P<0.01$), DBI ($r=0.98$, $P<0.01$) while a reverse relationship was observed between iodine value and palmitoleic ($r=-0.45$, $P<0.01$) and O/L ($r=-0.66$, $P<0.01$) (data not shown). The most effective fatty acid for increasing DBI was linoleic because there was a significant relationship between them ($r=0.88$, $P<0.01$) while a reverse relationship was observed between DBI and oleic ($r=-0.54$, $P<0.01$).

Conclusion

Melatonin-seed-priming has improved productivity traits of safflower under both irrigation regimes in both 2017 and 2018. Melatonin-seed-priming increased seed yield of safflower after two seed quality treatments, and also seed quality plays an important role in the effectiveness of melatonin-seed priming on enhancement of oil yield. Hydropriming and melatonin-seed priming improved the productivity, oil content and composition of safflower, especially in stored seeds and drought stress. The oil quality of safflower is

related to polyunsaturated fatty acid. Melatonin-seed priming increased omega 3 and omega 6 even under drought conditions. Although the melatonin-seed-priming plays a significant role in increasing the properties of different seed quality under drought conditions but hydropriming also had an acceptable role on increasing oil quality and characteristic of safflower in harsh environments equal to melatonin-seed-priming and could be used as a more cost-effective treatment than melatonin-seed-priming in similar situations.

Compliance with Ethical Standards

Conflict of Interest None.

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