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Seed Development, Oil Accumulation and Fatty Acid Composition of Drought Stressed Rapeseed Plants Affected by Salicylic Acid and Putrescine

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Abstract

Two field experiments were carried out in 2017 and 2018 to evaluate the impacts of salicylic acid (1 mM SA) and putrescine (1 mM Put) on leaf osmolytes, seed reserve and oil accumulation and fatty acid composition of rapeseed (*Brassica napus* L.) under different watering levels (irrigations after 70 and 150 mm evaporation as normal irrigation and severe drought stress, and 70 → 90 → 110 → 130 → 150 and 70 → 100 → 130→150 as gradual and moderately gradual water deficits, respectively). The experiments were laid out as split plot on the bases of randomized complete block design in three replications. Water stress increased the contents of glycine betaine, proline, soluble sugars, and proteins. Application of SA and Put further enhanced the contents of glycine betaine and soluble sugars, while reduced proline content of leaves. Seed filling duration, seeds per plant, plant biomass and seed yield were decreased with increasing irrigation intervals. Exogenous SA and Put enhanced all of these parameters under different irrigation intervals. Oil accumulation in seeds was diminished as water stress severed. The gradual water deficit considerably reduced the impacts of drought stress on yield related traits and oil content per seed, due to stress acclimation of plants. Oil content of seeds was augmented by SA and Put treatments through prolonging seed filling duration, particularly under limited irrigations. Percentages of palmitic acid and stearic acid (saturated fatty acids) were not affected by water limitation. However, unsaturated fatty acids of linoleic and linolenic acids were reduced, and oleic acid was enhanced due to water shortage. Unsaturation index was improved by SA and Put treatments under severe water stress as a result of decreasing oleic acid and increasing linoleic and linolenic acids contents. The SA spray was the best treatment for improving rapeseed seed and oil production under normal and stressful conditions.

Keywords Fatty acids · Putrescine · Rapeseed · Salicylic acid · Seed filling · Water deficit

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Einfluss von Salicylsäure und Putrescin auf die Samenentwicklung, die Ölakkumulation und die Fettsäurezusammensetzung von trockenheitsgestressten Rapspflanzen

Zusammenfassung

In den Jahren 2017 und 2018 wurden zwei Feldversuche durchgeführt, um die Auswirkungen von Salicylsäure (1 mM SA) und Putrescin (1 mM Put) auf die Blattosmolyte, die Samenreserve und die Ölakkumulation sowie die Fettsäurezusammensetzung von Raps (*Brassica napus* L.) unter verschiedenen Bewässerungsniveaus (Bewässerungen nach 70 und 150 mm Verdunstung als normale Bewässerung und schwerer Trockenstress sowie 70 → 90 → 110 → 130 → 150 und 70 → 100 → 130 → 150 als allmähliches bzw. mäßig allmähliches Wasserdefizit). Die Versuche wurden als Split Plot auf der Grundlage eines randomisierten vollständigen Blockversuchs mit drei Wiederholungen angelegt. Wasserstress erhöhte den Gehalt an Glycinbetain, Prolin, löslichen Zuckern und Proteinen. Die Anwendung von SA und Put erhöhte den Gehalt an Glycinbetain und löslichen Zuckern weiter, während der Prolingehalt der Blätter sank. Die Dauer der Kornfüllung, die Samen pro Pflanze, die Pflanzenbiomasse und der Samenertrag nahmen mit zunehmenden Bewässerungsintervallen ab. Exogenes SA und Put verbesserten alle diese Parameter bei unterschiedlichen Bewässerungsintervallen. Die Ölakkumulation in den Samen nahm mit abnehmendem Wasserstress ab. Das allmähliche Wasserdefizit verringerte die Auswirkungen von Trockenstress auf ertragsbezogene Merkmale und den Ölgehalt der Samen erheblich, was auf die Stressakklimatisierung der Pflanzen zurückzuführen ist. Der Ölgehalt der Samen wurde durch SA- und Put-Behandlungen erhöht, indem die Dauer der Kornfüllung verlängert wurde, insbesondere bei eingeschränkter Bewässerung. Der prozentuale Anteil von Palmitinsäure und Stearinsäure (gesättigte Fettsäuren) wurde durch die Wasserbegrenzung nicht beeinflusst. Die ungesättigten Fettsäuren Linol- und Linolensäure wurden jedoch reduziert und die Ölsäure wurde durch den Wassermangel erhöht. Der Index der ungesättigten Fettsäuren wurde durch SA- und Put-Behandlungen unter starkem Wasserstress verbessert, da der Gehalt an Ölsäure sank und der an Linol- und Linolensäure stieg. Die SA-Anwendung war die beste Behandlung zur Verbesserung der Raps- und der Ölproduktion unter normalen und stressigen Bedingungen.

Schlüsselwörter Fettsäuren · Putrescin · Raps · Salicylsäure · Kornfüllung · Wasserdefizit

Introduction

Rapeseed is an important crop due to the high oil contents in the seeds. Different rapeseed cultivars contain 37% to 45% seed oil (Balalic et al. 2017). Moreover, seed yield and oil percentage are essential in the profitability of this crop (Robertson and Holland 2004). Seed filling of the crops from the fertility stage to the physiological maturity of rapeseed can be divided into three stages: 1) the dry weight of the seed increases slowly at the early stages of seed filling, 2) the linear stage of seed filling up to achieving 90% of the seed dry weight that is also called the effective seed filling duration, 3) the dry matter content of the seed increases slightly. At the end of this stage, the relationship between the mother plant and the seed is cut off (Menendez et al. 2019). Seed filling duration and rate and also seed yield could be influenced by environmental factors such as drought (Ghassemi-Golezani et al. 2015) and salinity (Ghassemi-Golezani et al. 2018a; Lotfi et al. 2018).

An important metabolic way of reducing the harmful impacts of drought stress on plants is the synthesis and accumulation of osmolytes such as glycine betaine (GB), soluble sugar, soluble protein and proline in leaf tissues. This can improve osmotic adjustment of plants to prevent cell dehydration, which is necessary for cell growth (Ashraf et al. 2011). These osmolytes can enhance plant tolerance to environmental stresses through osmotic regulation, energy transfer, carbon storage and energy, elimination of hydroxyl radicals, regulation of cellular oxidative potential, reduction of pH and maintenance of cell turgor required for the stability of cell structure, proteins and membranes (Rontain et al. 2002).

Although water deficit during different developmental stages reduces the yield of rapeseed, the effects of stress during flowering and pod development are more deleterious (Bilibio et al. 2011; Rashidi et al. 2017). Seed yield of rapeseed depends on the number of seeds per pod and 1000-seed weight. The effect of drought stress on seed yield reduction was mainly affected by lower number of seeds per plant (Faraji et al. 2009).

Oil begins to accumulate in developing seeds 1–2 weeks after flowering, depending on environmental conditions (Bhardwaj and Hamama 2003). Seed yield, oil content and oil composition (individual fatty acids) may be changed by water deficit (Aghdam et al. 2019). Seed yield and oil content of safflower under drought stress could be diminished due to decreased availability of carbohydrates for oil synthesis (Mohammadi et al. 2018b). The seven major fatty acids of *Brassica napus* seeds are palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosanoic (C22:0), and erucic (C22:1) acids (Beyzi et al. 2019). Rapeseed oil is valuable due to high ratio of unsaturated to saturated fatty acids and also because of containing valuable omega-3 alfa-linolenic acid (Rashidi et al. 2017). It was reported that percentages of linoleic (C18:2) and linolenic (C18:3) acids reduced, while the oleic acid (C18:1) enhanced as a consequence of rising water limitation (Joshan et al. 2019).

Foliar spray of plant growth regulators such as salicylic acid (SA) may improve drought tolerance of plants (Farhangi-Abriz and Ghassemi-Golezani 2018; Ghassemi et al. 2019). The SA stimulates nutrient uptake and translocation (Wada et al. 2010), photosynthesis and growth of plants (Janda et al. 2014). This hormone also mediates the response of plants to adverse environmental conditions such as water stress. It was reported that SA can enhance antioxidant enzymes activities, thereby maintains membrane integrity and improves plant performance (Ghassemi-Golezani et al. 2019). Ghassemi-Golezani et al. (2018b) showed that exogenous salicylic acid increases seed and essential oil yield of ajowan under drought stress. Foliar treatment of SA also enhances oil quality of soybean seeds by increasing unsaturation index (Ghassemi-Golezani and Farhangi-Abriz 2018).

Putrescine (Put) as an important polyamine can regulate plant growth and acts as a secondary messenger in signaling pathways (Kusano et al. 2008). Polyamines influence the expression of genes and induce stress related proteins in plants (Farooq et al. 2009b). The level of such polyamines are increased in plant tissues in response to salt and water stresses (Farooq et al. 2009b; Kubiś et al. 2014). The increased levels of Put under drought are consistent with the induction of genes for Put synthesis, indicating the involvement of Put in signal transduction. This growth regulator has properties for neutralizing and stabilizing of cell membranes (Farooq et al. 2009a). Polyamines also play major roles in stress tolerance by scavenging free radicals, involving in activation of expression of genes encoding antioxidant enzymes, and acting directly as stress-protecting compounds (Takahashi and Kakehi 2010). The Put can increase osmolytes and photosynthetic pigments, especially under drought stress conditions (Mostafaei et al. 2018).

Since the efficacy of growth regulators such as SA and Put on grain development and oil quantity and quality of oilseed crops is not clear, this research was laid out to investigate the possible roles of these growth regulators on grain filling, oil accumulation and fatty acid composition of rapeseed grains under severe and gradual water deficits.

Materials and Methods

Experimental Conditions

Two field experiments were conducted in 2017 and 2018 at the Research Farm of the University of Tabriz, Iran (Latitude 38° 05′N, Longitude 46° 17′E, Altitude 1360 m above sea level), to investigate the influence of foliar applications of salicylic acid (SA) and putrescine (Put) on seed development, oil accumulation and fatty acid composition of rapeseed (*Brassica napus* L.) grains under different watering levels. The experiments were laid out as split plot with RCB design in three replications. Irrigation intervals after 70 mm (I_1) and 150 mm (I_4) evaporations from class A pan were considered as normal irrigation and severe water stress, respectively. Waterings after 70 → 90 → 110 → 130 → 150 mm (I_2) and 70 → 100 → 130 → 150 mm (I_3) evaporations were applied as gradual and moderately gradual water deficits to assess drought acclimation of plants. Each irrigation was carried out to achieve 100% field capacity (FC). The rainfall during the growing season was 39.9 mm. The class A pan was not covered, so the raining water was also dropped into the pan and the next irrigations were carried out after reaching the marked evaporation levels within the pan. In this way, the irrigation intervals were not disrupted and only intervals durations a little prolonged. The intervals durations for irrigations were also prolonged at low temperatures. Irrigation treatments were allocated to the main plots and foliar applications of water (control), salicylic acid (SA) (1 mM) and putrescine (Put) (1 mM) were assigned to the sub plots. Each plot consisted of 6 rows with 4 m length and 25 cm distance from each other. In both years, the seeds of a spring rapeseed (cv. Gole yas × H19) were pre-treated with 2 g/kg Benomyl and then were sown on 30 April in about 1 cm depth of the soil (sandy-loam). After sowing, all plots were regularly irrigated (%100 field capacity) up to seedling establishment. The subsequent irrigations were carried out according to the treatments. The weeds were removed from the plots during plant growth and development. Foliar applications of growth regulators were carried out at vegetative and grain formation stages of plants.

Osmolyte Content

The soluble sugar content of leaves at flowering stage was assayed by the phenol sulfuric acid method (Kochert 1978). A fresh leaf sample (0.1 g) was added to 10 ml of 70% ethanol and it was kept in the refrigerator for a week. Then, 1 ml of the solution was mixed with 1 ml of distilled water. Subsequently, 1 ml of phenol 5% and 5 ml of concentrated sulfuric acid were added to this solution and the absorbance was recorded at 485 nm. The soluble sugar content of rapeseed leaves was estimated by a calibration curve of pure glucose and expressed as mg g^{-1} DW.

The soluble protein content was measured according to Bradford (1976). A leaf sample (1 g) was homogenized with 4 mL Na-Phosphate buffer (pH 7.2) and then it was centrifuged for 20 min at 4 °C. The supernatant was pipetted in cuvettes and the absorbance was recorded at 595 nm, using a UV-VIS spectrophotometer (Model Analytikjena Spekol 1500 Germany).

For determination of glycine betaine, a leaf sample (500 mg) was ground and mixed with 5 ml of toluene-water mixture (0.05% toluene) in a plastic tube. The tubes were shaken for 24 h at 25 °C, and then 1 ml of 2 HCl and 0.1 ml of potassium tri-iodide solution was added to 0.5 ml of this sample and again it was shaken in an ice cold water bath for 90 min. The absorbance of organic layer was tested at 365 nm and glycine betaine content was determined as mg g^{-1} dry weight (DW) (Grieve and Grattan 1983).

According to Bates et al. (1973), 500 mg of each leaf sample was homogenized in 5 ml of 3% sulphosalicylic acid. About 2 ml of the extracted sample was mixed with 2 ml of glacial acetic acid and 2 ml of ninhydrin within a tube. All samples were heated at 100 °C for 1 h in a Bain Marie (BM-15 Bain Marie, Magapor SL, Spain) and then were cooled in the laboratory at 25 °C. The mixtures were extracted with toluene, and the absorbances of upper layers were read at 520 nm. The leaf proline content was estimated by the calibration curve of pure proline and expressed as mg g^{-1} fresh weight (FW).

Rate and Duration of Seed Filling

During seed filling and maturity in 2018, five plants were harvested from each plot in 5 days' intervals at six stages. Seeds were detached from the pods and each sample was separately dried in an oven at 80℃ for 48 h and then weighed. Maximum seed weight and seed filling duration were estimated by a two-piece regression model, using SAS 9.4:

$$W = \begin{cases} a + bt & t < tm \\ a + bt & t \geq tm \end{cases}$$

Where W is seed mass, *a* is the intercept, *b* is the slope, *t* is days after flowering and tm is the end of seed filling (mass maturity). Subsequently, seed filling rate (SFR) was determined as:

$$\mathrm{SFR} = \mathrm{MSM}/\boldsymbol{SFD}$$

Where MSM is maximum seed weight and SFD is seed filling duration.

Yield Components

At maturity, plants in 1 m^2 of the middle part of each plot were harvested (with about 15% seed moisture content) in both years and the seeds were separated from the pods and 1000 seed weight and seed yield per unit area were recorded. Seeds per plant were estimated as: seed yield per plant/individual seed weight. Subsequently, the plants were dried at 80℃ for 48 h and plant biomass was determined. Then harvest index was calculated as: (seed yield/plant biomass) × 100.

Oil Accumulation

During seed filling in 2018, five plants were harvested from each plot at 5 days' intervals, beginning 10 days after flowering and then seeds were detached from the pods (with about 10–15% seed moisture content). Percentage of oil for each fresh seed sample was determined by a Soxhlet (multiple unit model with up to 6 extractors). Oil content per seed was calculated as seed weight of each sample × oil percentage.

Fatty Acid Composition

Seed samples of both experiments for the same treatments and replicates at final harvest were separately mixed and powdered. Then, all powder samples were placed in a Soxhlet extraction system at 75℃ for 6 h, using petroleum ether as the solvent (AOCS 1993). Fatty acid composition of the oil samples was tested by gas chromatography. Each oil sample was converted to its fatty acid methyl esters (FAME). Each oil sample (0.2 mL) was dissolved in hexane and trans-esterified with sodium methylate (0.1 M solved in methanol). A microliter of methylated sample was injected into a gas chromatograph (Agilent Technologies, United States), that was equipped with a split injector (split ratio 1: 40) (using nitrogen as the carrier gas) and a flame ionization detector (FID). A fused silica capillary column (HP-88, Cat. No: 112-88A7 Agilent), 100 m × 0.25 mm internal diameter, with 0.2 µm film thickness was used. An initial column temperature was set as 150 °C, then programmed to 190 °C, and finally to 240 °C at a rate of 5 °C/min. The injector and detector temperatures were set at 250 °C and 280 °C, respectively. The peak was identified by comparing the retention time of each fatty acid with standard mixture of FAMEs (FAME Mix RM-6, Product ID O7631-1AMP, Sigma-Aldrich, United States). The fatty acids of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) were estimated, using a computing inte-

Table 1 Means of soluble sugars, soluble proteins, glycine betaine and proline contents of rapeseed leaves for interaction of water supply × growth regulators

Irrigation	Growth regulator	SS (mg g^{-1} DW)	SP (mg g^{-1} DW)	GB (mg g^{-1} DW)	Proline (mg g^{-1} FW)
I$_1$	*W*	27.18 ± 0.96 f	16.89 ± 0.03 j	2.31 ± 0.04 k	1.51 ± 0.04 i
	SA	30.86 ± 1.36 f	16.77 ± 0.05 j	2.78 ± 0.08 i	1.49 ± 0.03 i
	Put	27.26 ± 0.80 f	16.42 ± 0.05 j	2.63 ± 0.07 j	1.44 ± 0.03 i
I$_2$	*W*	30.15 ± 1.72 f	18.02 ± 0.07 i	3.18 ± 0.02 h	2.01 ± 0.11 g
	SA	37.54 ± 1.71 e	21.20 ± 0.04 g	4.92 ± 0.02 f	1.66 ± 0.03 h
	Put	30.99 ± 1.13 f	20.02 ± 0.06 h	3.85 ± 0.04 g	1.73 ± 0.02 h
I$_3$	*W*	46.54 ± 2.40 d	23.04 ± 0.08 f	6.52 ± 0.04 e	3.49 ± 0.06 c
	SA	55.36 ± 3.25 c	27.87 ± 0.04 b	8.22 ± 0.04 c	2.82 ± 0.06 f
	Put	49.25 ± 2.17 d	25.63 ± 0.04 d	7.71 ± 0.05 d	3.02 ± 0.05 e
I$_4$	*W*	57.07 ± 2.80 c	24.73 ± 0.08 e	8.21 ± 0.05 c	4.50 ± 0.07 a
	SA	72.74 ± 1.97 a	30.12 ± 0.04 a	12.76 ± 0.04 a	3.26 ± 0.04 d
	Put	63.96 ± 2.82 b	27.19 ± 0.04 c	10.91 ± 0.04 b	3.94 ± 0.07 b

The values are the means of three replicates ± standard error
Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test)
I_1, I_2, I_3, I_4: irrigation after 70; 70-90-110-130-150; 70-100-130-150; and 150 mm evaporation, respectively
W Water, *Put* Putrescine, *SA* Salicylic acid, *SS* soluble sugars, *SP* soluble proteins, *GB* glycine betaine

grator. The unsaturation index (UI) of each oil sample was calculated as:

$$UI = (\Sigma C_{n:1} + 2\Sigma C_{n:2} + 3\Sigma C_{n:3})/\mathbf{100}$$

Where $C_{n:1}$, $C_{n:2}$ and $C_{n:3}$ are oleic acid, linoleic acid and linolenic acids percentages, respectively.

Statistical Analysis

Analysis of variance of the data and comparison of means (Duncan test) at $P \leq 0.05$ were carried out, using SAS 9.4 software. Figures were drawn by the Excel software.

Results

Osmolytes

The interaction of irrigation intervals × growth regulators was significant ($p \leq 0.01$) for osmolytes of rapeseed leaves. The soluble sugars, proteins, glycine betaine and proline contents increased as water stress severed (Table 1). Soluble sugar content was also enhanced by SA spray under stress, but not under normal irrigation. The Put treatment had no significant effect on soluble sugars under normal watering and gradual stress, but had a significant effect on this parameter under moderately gradual and non-gradual water deficits. The soluble proteins of rapeseed leaves were not statistically changed by foliar sprays of SA and Put under normal irrigation, but these proteins were significantly improved by the foliar treatments, particularly by SA spray, under gradual, moderate and severe water limitations. Treatments with SA and Put enhanced glycine betaine content of leaves in all watering levels. In contrast, these growth regulators, especially SA, reduced proline content of rapeseed leaves under different stress levels, but had no significant effect on that in unstressed plants (Table 1).

Rate and Duration of Seed Filling

Individual seed weight was enhanced with progressing seed development up to 29–33 days after flowering, depending on water availability and growth regulator. Thereafter, no changes in seed weight were observed (Fig. 1). The effects of water supply, growth regulators and the interaction of these factors for seed filling duration, seed filling rate and maximum seed weight were significant ($p \leq 0.01$). Water stress decreased seed filling duration and slightly increased seed filling rate. Seed filling duration was significantly increased by SA under all irrigation intervals and by Put under I_3 and I_4 treatments. In contrast, seed filling rate was decreased by SA and Put under different levels of irrigation. Maximum seed weight under severe water deficit was achieved about 3–5 days earlier than that under normal irrigation condition. Exogenous treatment of SA enhanced maximum seed weight of rapeseed under all irrigation intervals by increasing seed filling duration. The Put treated plants only showed significant advantage in maximum seed weight under moderate and severe stresses, compared with untreated plants (Table 2).

Yield Parameters

The effects of water supply and growth regulators and interaction of these factors for seeds per plant, plant biomass

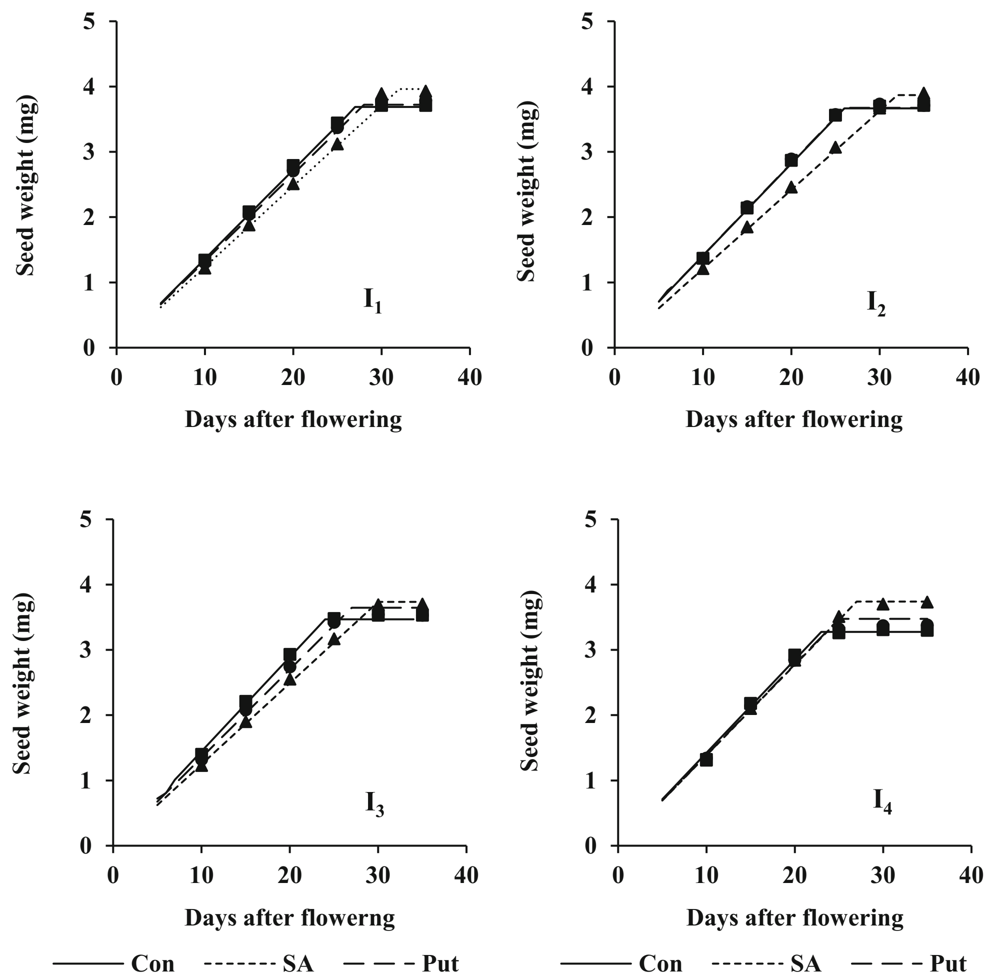


Fig. 1 Changes in seed weight of rapeseed in response to irrigation intervals and growth regulators. I_1, I_2, I_3, I_4: irrigation after 70; 70-90-110-130-150; 70-100-130-150; and 150 mm evaporation. *Con* water, *SA* salicylic acid, *Put* Putrescine

and seed yield were significant (Table 3). Water shortage reduced all of these yield parameters in SA and Put treated and untreated plants. However, these reductions were considerably lowered by gradual water deficit and also by SA and Put treatments. Foliar spray of SA was the superior treatment in improving these traits under all irrigation levels, followed by Put. These superiorities were more evident under moderate and severe water shortages (Table 4).

Oil Accumulation in Seeds

Seed oil content rapidly increased with increasing seed filling up to 30 days after flowering under all treatments. Changes in oil accumulation at later stages of seed development were not considerable. Oil accumulation at early stages of seed development was similar for SA and Put treated and untreated plants under different irrigation levels. However, Put and especially SA treatments caused an increase in rate of oil accumulation and oil content of seeds at later stages of development. This superiority was more pronounced under I_3 and I_4, although maximum oil content of seeds under these irrigation intervals was lower than that under I_1 and I_2 (Fig. 2).

Fatty Acids

Water supply and SA and Put applications had not significant effects on percentage of palmitic and stearic acids (Table 5). However, production of these fatty acids per unit area decreased as water deficit severed, with no significant difference between I_1 and I_2 treatments. Foliar spray of Put and specially SA significantly enhanced palmitic and stearic acids yield, especially under stressful conditions (Table 6).

The impacts of water limitation and foliar treatments and interaction of these factors for oleic acid percentage and yield were significant ($P \leq 0.01$). Oleic acid content of seeds from foliar treated and untreated plants was significantly increased under I_3 and I_4. Percentage of oleic acid in SA and Put treated plants under stress was lower than that in untreated plants (Table 5). In contrast, the highest oleic acid yield under different levels of water stress was recorded for SA treated plants, followed by Put treated plants (Table 6).

Table 2 Means of seed filling duration and rate and maximum seed weight of rapeseed under different irrigation intervals affected by growth regulators

Irrigation	Growth regulator	Seed filling duration (day)	Seed filling rate (mg day^{-1})	Maximum seed weight (mg)
I$_1$	*W*	27.23 ± 0.13 c	0.1362 ± 0.006 c	3.71 ± 0.04 b
	SA	31.74 ± 0.17 a	0.1238 ± 0.002 e	3.93 ± 0.02 a
	Put	28.06 ± 0.09 c	0.1329 ± 0.016 d	3.73 ± 0.04 b
I$_2$	*W*	26.36 ± 0.71 de	0.1410 ± 0.012 ab	3.72 ± 0.11 b
	SA	32.25 ± 0.20 a	0.1209 ± 0.009 f	3.9 ± 0.04 a
	Put	26.25 ± 0.43 e	0.1413 ± 0.014 a	3.71 ± 0.08 b
I$_3$	*W*	24.29 ± 0.91 f	0.1445 ± 0.008 a	3.51 ± 0.07 d
	SA	29.71 ± 0.17 b	0.1245 ± 0.012 e	3.70 ± 0.05 c
	Put	26.74 ± 0.75 d	0.1350 ± 0.012 c	3.61 ± 0.07 cd
I$_4$	*W*	23.17 ± 0.65 g	0.1424 ± 0.015 a	3.30 ± 0.10 ef
	SA	26.62 ± 0.90 d	0.1385 ± 0.007 b	3.73 ± 0.07 c
	Put	24.31 ± 0.42 f	0.1390 ± 0.0014 ab	3.38 ± 0.08 e

The values are the means of three replicates ± standard error
Different letters in each column indicate significant difference at $p \le 0.05$ (Duncan test)
I_1, I_2, I_3, I_4: irrigation after 70; 70-90-110-130-150; 70-100-130-150; and 150 mm evaporation, respectively
W water, *SA* salicylic acid, *Put* Putrescine

Table 3 Combined analysis of variance of the data for seeds per plant, plant biomass and seed yield of rapeseed affected by irrigation intervals and growth regulators

Source	Df	MS		
		Seeds per plant	Plant biomass (g/m^2)	Seed yield (g/m^2)
Year (Y)	1	1835.97	836.81	26.91
Rep/year	4	631.23	1212.38	47.41
Irrigation (I)	3	212,014.60**	780,921.79**	27,280.40**
*Y*I*	3	656.69	1676.01	12.68
I R/Y*	12	116.44	2423.74	12.44
Growth regulator (G)	2	33,517.69**	134,957.56**	4639.87**
*G*I*	6	2668.44**	9903.35**	347.01**
*Y*G*	2	985.91	8050.06	129.79
*Y*G*I*	6	271.58	4029.90	56.70
E	32	314.87	2461.87	35.06
CV	–	3.83	6.58	4.18

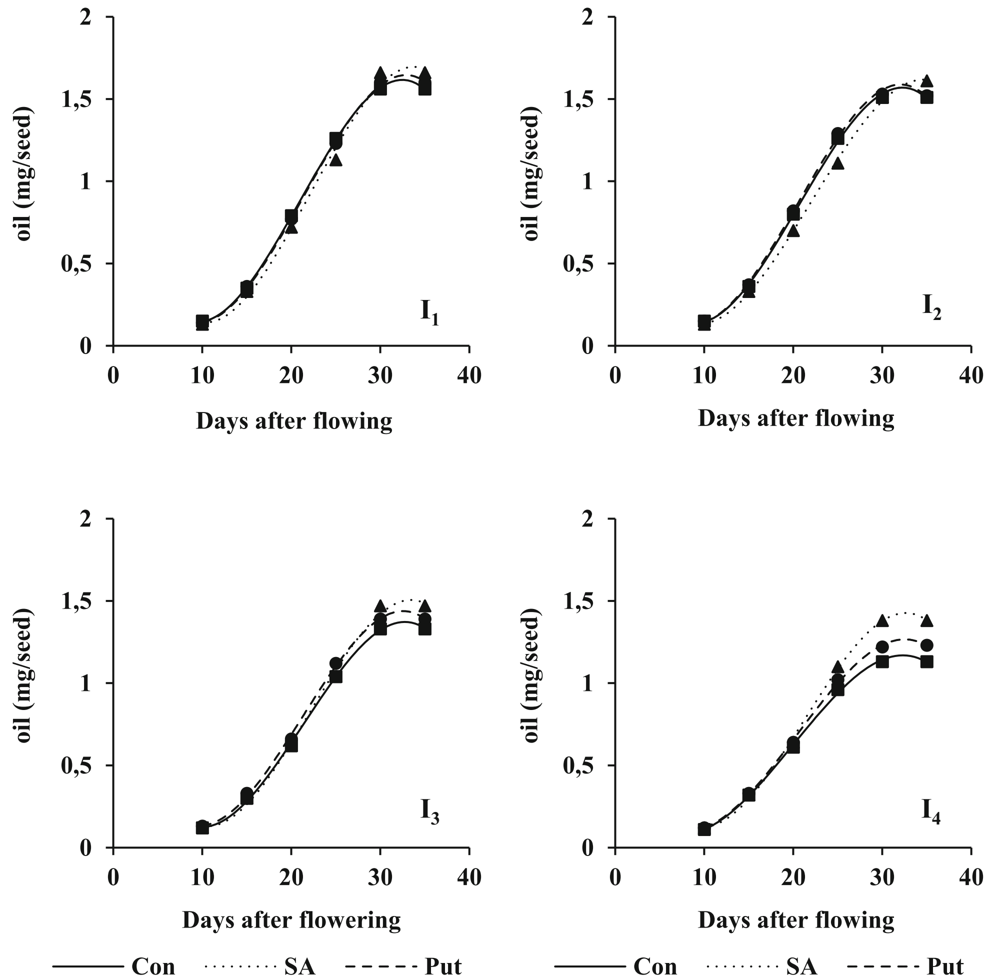
*, **: Significant at $p \le 0.05$ and $p \le 0.01$, respectively

Table 4 Changes in seeds per plant, plant biomass and seed yield of rapeseed affected by irrigation intervals and growth regulators

Irrigation	Growth regulator	Seeds per plant	Plant biomass (g/m^2)	Seed yield (g/m^2)
I$_1$	*W*	547.78 ± 41.85 bc	882.24 ± 28.16 b	171.27 ± 3.85 c
	SA	589.55 ± 39.68 a	986.13 ± 75.75 a	187.17 ± 15.51 a
	Put	554.95 ± 17.82 ab	914.74 ± 55.15 ab	171.05 ± 11.49 c
I$_2$	*W*	516.07 ± 19.32 c	895.66 ± 37.51 b	164.01 ± 4.19 d
	SA	563.54 ± 29.22 ab	966.63 ± 60.53 ab	181.37 ± 10.05 b
	Put	531.80 ± 11.44 bc	899.44 ± 18.35 b	167.16 ± 2.37 cd
I$_3$	*W*	352.31 ± 10.45 ef	539.04 ± 25.12 ef	98.87 ± 4.49 h
	SA	465.10 ± 7.53 d	779.96 ± 16.53 c	142.50 ± 4.48 e
	Put	439.72 ± 8.65 d	681.52 ± 24.82 d	126.92 ± 3.66 f
I$_4$	*W*	279.82 ± 7.16 g	411.60 ± 25.27 g	79.16 ± 3.92 i
	SA	376.25 ± 13.17 e	594.10 ± 33.79 e	113.02 ± 6.35 hg
	Put	333.94 ± 16.12 f	493.58 ± 19.61 f	94.53 ± 7.79 h

The values are the means of three replicates ± standard error
Different letters in each column indicate significant difference at $p \le 0.05$ (Duncan test)
I_1, I_2, I_3, I_4: irrigation after 70; 70-90-110-130-150; 70-100-130-150; and 150 mm evaporation, respectively
W water, *SA* salicylic acid, *Put* Putrescine

Fig. 2 Changes in seed oil content of rapeseed in response to irrigation intervals and growth regulators. I_1, I_2, I_3, I_4: irrigation after 70; 70-90-110-130-150; 70-100-130-150; and 150 mm evaporation. *Con* water, *SA* salicylic acid, *Put* Putrescine



The UI was significantly ($P \leq 0.05$) affected by water deficit, foliar treatments and the interaction of these factors. The UI in SA and Put treated plants under severe stress (non-gradual water deficit) was higher than that in untreated plants (Table 5).

The linoleic and linolenic acids percentages and yields were significantly ($P \leq 0.01$) influenced by water shortage, growth regulators and interaction of these factors. The percentages (Table 5) and yields (Table 6) of these fatty acids were significantly improved by foliar application of SA and Put under limited irrigations, but not under normal irrigation. Treatment of plants with SA showed the highest improvement in linoleic and linolenic acids yields under stress (Table 6).

Discussion

Increasing osmolyte content of plant cells under water stress (Table 1) is a mechanism for maintaining cell water potential in an optimum level (de Sousa et al. 2016). High level of soluble sugars in drought stressed plants is related to rising invertase activity (Hosseini et al. 2015). Enhancing soluble proteins and glycine betaine under different levels of water stress may be associated with degradation of some structural proteins and alteration proteome profile of plants, in order to improve cell water status (Ahanger et al. 2014). Amino acids have an osmo-protective role in different tissues exposed to dehydration (Slama et al. 2015). Proline is an amino acid that increases rapidly when plants are subjected to adverse environmental conditions such as water stress (Zegaoui et al. 2017). According to De Ronde et al. (2000), increasing proline content in leaves under water deficit was related with rising activities of some enzymes such as Pyrroline-5-carboxylate synthase (P5 CS). Enhancing proline synthesis under stressful conditions can certainly reduce chlorophyll synthesis, since both are produced from the same precursor (glutamate). This can ultimately decrease photosynthesis and yield related traits.

Exogenous SA and Put enhanced osmolytes in plant cells (Table 1) by changing gene expression, invertase activity and soluble amino acids (Borsani et al. 2001; Ebeed et al. 2017). This improvement can enhance stress tolerance of rapeseed plants. Proline content was reduced by SA treat-

Table 5 Changes in Fatty acid percentage of rapeseed seeds affected by irrigation intervals and growth regulators

Irrigation	Growth regulators	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	UI (%)
I_1	*W*	5.39 ± 0.08 a	2.65 ± 0.07 a	58.40 ± 0.20 f	21.35 ± 0.07 b	10.46 ± 0.05 c	1.34 ± 0.01 a
	SA	5.23 ± 0.05 a	2.40 ± 0.15 a	58.32 ± 0.10 fg	21.95 ± 0.05 a	10.99 ± 0.02 a	1.34 ± 0.02 a
	Put	5.42 ± 0.09 a	2.58 ± 0.05 a	58.32 ± 0.07 fg	21.78 ± 0.03 a	10.76 ± 0.05 ab	1.30 ± 0.01 abc
I_2	*W*	5.65 ± 0.08 a	2.51 ± 0.08 a	58.39 ± 0.20 f	20.50 ± 0.02 d	10.24 ± 0.02 d	1.31 ± 0.02 abc
	SA	5.73 ± 0.10 a	2.40 ± 0.21 a	57.95 ± 0.24 g	21.12 ± 0.05 c	10.67 ± 0.10 b	1.31 ± 0.01 ab
	Put	5.46 ± 0.18 a	2.49 ± 0.19 a	57.94 ± 0.28 g	21.04 ± 0.07 c	10.60 ± 0.09 bc	1.29 ± 0.01abcd
I_3	*W*	5.25 ± 0.39 a	2.62 ± 0.52 a	62.19 ± 0.10 c	18.45 ± 0.06 g	8.88 ± 0.05 h	1.26 ± 0.02 bcd
	SA	5.60 ± 0.15 a	2.54 ± 0.26 a	60.86 ± 0.12 e	19.79 ± 0.04 e	9.76 ± 0.07 e	1.28 ± 0.01 abcd
	Put	5.40 ± 0.12 a	2.43 ± 0.12 a	61.62 ± 0.17 d	19.45 ± 0.12 f	9.39 ± 0.10 f	1.26 ± 0.02 bcd
I_4	*W*	5.53 ± 0.11 a	2.53 ± 0.09 a	64.79 ± 0.14 a	17.20 ± 0.03 h	8.14 ± 0.03 i	1.24 ± 0.02 d
	SA	5.58 ± 0.23 a	2.43 ± 0.12 a	62.30 ± 0.18 c	18.48 ± 0.09 g	9.20 ± 0.07 fg	1.26 ± 0.01 cd
	Put	5.28 ± 0.09 a	2.45 ± 0.17 a	62.80 ± 0.05 b	18.38 ± 0.04 g	9.06 ± 0.04 hg	1.30 ± 0.01 abc

The values are the means of three replicates ± standard error

Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test)

I_1, I_2, I_3, I_4: irrigation after 70; 70-90-110-130-150; 70-100-130-150; and 150 mm evaporation, respectively

W water, *SA* salicylic acid, *Put* Putrescine

ment (Table 1) in favor of chlorophyll synthesis of plants under drought stress. Decreasing proline content of leaves by Put and SA treatments could be the result of reducing the harmful impacts of water stress on plants via improving the water status of plants (Ghassemi-Golezani et al. 2019).

Increasing rate and decreasing duration of seed filling in rapeseed due to water deficit (Table 2) is the consequence of accelerating seed development and plant senescence by stress (Ghassemi-Golezani and Farhangi-Abriz 2018). This suggests that reduction in maximum seed weight under stress was mainly related with deduction in seed filling duration. Production of larger seeds in SA treated plants under different irrigation intervals was also the result of prolonging filling duration by this treatment (Fig. 1; Table 2). Increasing filling duration by Put and particularly SA sprays is clearly associated with the role of these growth regulators in preventing ethylene synthesis (Cao et al. 2007; Minocha et al. 2014), improving antioxidants activities and chlorophyll content and consequently delaying plant senescence (Gou et al. 2017; Sedaghat et al. 2017; Liu et al. 2017; Mohammadi et al. 2018a; Spormann et al. 2019). Higher seed filling duration and lower filling rate of Put treated plants in comparison with untreated plants were led to statistically similar maximum seed weight in treated and untreated plants (Table 2).

Seeds per plant is closely related with flower and pod number. Water deficit can enhance abscission of flowers via stimulating synthesis of ethylene (Arraes et al. 2015), leading to a deduction in pods and seeds per plant (Table 4). Enhancing seeds per plant by application of SA and Put under different watering levels, especially under stressful conditions (Table 4), is strongly associated with the roles of these growth regulators in enhancing osmo-regulators (Table 1), inhibition of ethylene synthesis and flower abscission (Hayat and Ahmad 2007; Anwar et al. 2015). The PA content of flowers is more than other plant organs, and exogenous PA treatment can increase the flowering of poor flowering plants (Applewhite et al. 2010) and consequently the number of seeds per plant (Table 4).

Water deficit reduces plant biomass (Table 4) through decreasing growth (Eziz et al. 2017) and accelerating senescence (Zhang et al. 2011). Foliar application of SA and Put enhanced plant biomass under all irrigation intervals (Table 4) via improving chlorophyll content and plant growth (Anwar et al. 2015; Ghassemi-Golezani et al. 2018a). This could be achieved through enhancing the antioxidant capacity of plants and preventing ethylene synthesis and chlorophyll degradation by these growth regulators (Radhakrishnan and Lee 2013; Sedaghat et al. 2017). Reduction of seed yield under water stress (Table 4) is the consequence of reducing seed filling duration, seed weight (Tables 1 and 2) and particularly seeds per plant (Table 4). Water stress decreases leaf expansion rate, impairs photosynthetic machinery and accelerates leaf senescence (Farooq et al. 2009a), that reduce seed yield (Table 4) and oil accumulation per seed (Fig. 2). In general, the impact of drought stress on seeds per plant, plant biomass, seed yield and oil content considerably reduced, when water limitation imposed gradually (Table 4; Fig. 2). This is a clear indication of drought acclimation in plants subjected to a gradual water deficit. Application of SA and Put improved seed yield under different irrigation intervals via increasing seed filling period, seed weight and seeds per plant. Seed yield of rapeseed under different levels of watering and foliar treatments was largely affected by number of seeds per plant, compared with 1000 seed weight (Table 4).

Table 6 Changes in Fatty acid content of rapeseed seeds affected by irrigation intervals and growth regulators

Irrigation	Growth regulator	Palmitic acid (g/m^2)	Stearic acid (g/m^2)	Oleic acid (g/m^2)	Linoleic acid (g/m^2)	Linolenic acid (g/m^2)
I_1	*W*	9.31 ± 0.19 b	4.13 ± 0.11 abc	100.83 ± 0.84 a	36.93 ± 0.21 b	18.06 ± 0.19 b
	SA	9.34 ± 0.17 b	4.64 ± 0.22 a	103.31 ± 0.97 a	38.79 ± 0.50 a	19.34 ± 0.20 a
	Put	9.46 ± 0.12 b	4.55 ± 0.11 a	101.54 ± 0.63 a	37.97 ± 0.21 ab	18.80 ± 0.18 a
I_2	*W*	9.15 ± 0.26 b	3.89 ± 0.18 bc	94.61 ± 1.31 c	33.22 ± 0.47 d	16.59 ± 0.25 c
	SA	10.22 ± 0.25 a	4.48 ± 0.32 a	103.46 ± 2.16 a	37.71 ± 0.88 ab	19.05 ± 0.59 a
	Put	9.20 ± 0.35 b	4.20 ± 0.33 ab	97.73 ± 0.23 b	35.49 ± 0.12 c	17.88 ± 0.26 b
I_3	*W*	5.23 ± 0.37 e	2.41 ± 0.04 ef	61.97 ± 0.45 g	18.38 ± 0.04 h	8.85 ± 0.02 g
	SA	7.97 ± 0.22 c	3.61 ± 0.38 cd	86.69 ± 0.19 d	28.19 ± 0.13 e	13.90 ± 0.13 d
	Put	6.80 ± 0.23 d	3.31 ± 0.11 d	77.64 ± 0.93 e	24.51 ± 0.45 f	11.83 ± 0.25 e
I_4	*W*	4.35 ± 0.10 f	1.93 ± 0.07 f	51.04 ± 0.54 h	13.54 ± 0.11 i	6.41 ± 0.06 h
	SA	6.26 ± 0.33 d	2.73 ± 0.23 e	69.88 ± 0.69 f	20.73 ± 0.36 g	10.31 ± 0.21 f
	Put	5.10 ± 0.25 e	2.44 ± 0.23 ef	60.60 ± 2.31 g	17.73 ± 0.72 h	8.74 ± 0.37 g

The values are the means of three replicates ± standard error

Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test)

I_1, I_2, I_3, I_4: irrigation after 70; 70-90-110-130-150; 70-100-130-150; and 150 mm evaporation, respectively

W water, *SA* salicylic acid, *Put* Putrescine

Reduction of seed filling duration under water stress is the main cause of decreasing oil content per seed (Fig. 2). The oil accumulation in seeds at later stages of development may be related with the photosynthetic activity of the pods (Hua et al. 2012). High oil accumulation in the seeds is also partly the result of rising the expression of genes related with fatty-acid biosynthesis (Liu et al. 2014). Foliar spray of SA enhances chlorophyll content, photochemical efficiency of PSII (Fv/Fm) and leaf area (Ghassemi-Golezani et al. 2018a), leading to a higher seed yield (Table 3) and oil content per seed (Fig. 2). SA also increases the accumulation of oil by increasing the proteins involved in the transcription of synthetic oil genes (Wu et al. 2014). Put improves leaf area and photosynthetic capacity of plants (Çaliskan et al. 2017), thereby enhances seed yield (Table 4) and oil content per seed (Fig. 2).

Rapeseed fatty acids might be influenced by environmental stresses and genetic factors (Khan et al. 2018). No significant effect of water limitation on percentages of palmitc acid and stearic acid (Table 5) indicates that saturated fatty acids in rapeseed oil were not significantly changed by this stress. This result was also supported by the reports of Ghassemi-Golezani and Farhangi-Abriz (2018) for soybean and Pritchard et al. (2000) for canola. Acceleration of lipid accumulation and lowering the activity of enzymes such as D_{12} desaturase due to water deficit were led to an increase in percentage of oleic acid and a decrease in percentages of linoleic and linolenic acids (Table 5). Damage to the leaves under stressful conditions may deactivate these enzymes which can be harmful for lipid metabolism. Water limitation can also limit the transport of fatty acids in plants (Joshan et al. 2019), leading to an increment in oleic acid and a decrement in linoleic and linolenic acids percentages, since the latter fatty acids are produced from the former one. Declining the yields of all fatty acids under water deficit (Table 6) was the result of decreasing seed yield in drought stressed plants (Table 6).

Decreasing oleic acid and increasing linoleic and linolenic acids as a result of SA treatment (Table 5) reflecting an increase in the activity of oleoyl-phosphatidylcholine D_{12} desaturase and the fluidity of membrane lipids (Kachroo et al. 2003). Enhancing seed yield of rapeseed by SA spray (Table 4) was led to an increase in yields of all fatty acids (Table 6). Increasing linoleic and linolenic acids production from oleic acid due to Put application (Table 5) may be achieved by up or down regulation of some proteins and enzyme phospholipases in plant carbohydrate metabolism (Zarza Cubero 2017). The fatty acids yield of the put treated plants was less than SA treated plants (Table 6), due to the lower seed yield (Table 4).

The UI reduction of rapeseed oil with increasing water deficit was mainly the result of decreasing linoleic and linolenic acids under stress (Table 5). Foliar sprays of SA and Put enhanced the synthesis of these unsaturated fatty acids, leading to a high UI and oil quality of rapeseed. Improving the UI and oil quality by SA treatment was supported by a previous report on soybean under salt stress (Ghassemi-Golezani and Farhangi-Abriz 2018).

Conclusion

Water limitation increased soluble sugars, soluble proteins, glycine betaine and proline contents of rapeseed plants. However, most of the yield related traits decreased under moderate and severe drought stress. In general, gradual water deficit considerably reduced the impact of drought stress on seeds per plant, plant biomass, seed yield and oil content per seed, which is an indication of drought acclimation in rapeseed plants. Foliar sprays of Put and SA caused a further rising of the osmo-regulators that preserve the osmotic potential of leaf cells and reduce the injurious effects of drought stress on plants. These treatments, particularly SA, improved seed yield of rapeseed under different irrigation intervals via enhancing plant biomass, filling duration and yield components. Exogenous SA and Put also increased oil content per seed through increasing seed filling duration, especially under stressful conditions. Water limitation had no significant effect on percentages of saturated fatty acids of palmitic acid and stearic acid, but enhanced oleic acid and reduced linoleic acid and linolenic acid as unsaturated fatty acids. The SA and Put treatments under severe water deficit improved UI by reducing oleic acid and enhancing linoleic acid and linolenic acid contents. Foliar spray of SA was the superior treatment in improving seed and oil production of rapeseed under normal and limited irrigation conditions.

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Conflict of interest H. Mabudi Bilasvar, K. Ghassemi-Golezani and A.D. Mohammadi Nassab declare that they have no competing interests.

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