Effect of salicylic acid on the quality of edible oil and fatty acids composition in different regions of sunflower (*Helianthus annuus* L.) heads

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Abstract

To study the effect of salicylic acid (SA) on the amount and quality of oil in different regions of sunflower inflorescence, a factorial experiment was conducted based on randomized complete block design with 3 replications at the University of Mohaghegh Ardabili, Ardabil, Iran. Salicylic acid at concentrations of 0, 0.1, and 0.2 g L^{-1} were sprayed on whole plant at 3 stages (twice before flowering and a third time after flowering) and kernels were collected from different regions of capitulum (inner, middle, and outer layers). Samples were taken fifteen days after flowering with 5-day intervals and the amount of oil and four fatty acids (palmitic, stearic, oleic, and linoleic acids) were measured. Results showed that SA increased content of oil and unsaturated fatty acids but decreased the amount of saturated fatty acids and improved the quality of the kernels obtained from the mother plant. Linoleic acid content increased more than oleic acid by spraying of SA and the concentration of 0.2 g L^{-1} SA was more effective. Kernel position had significant effect on the oil content and fatty acids, so that from the periphery toward the center there was a reduction trend in the oil and saturated fatty acid content.

Keywords: fatty acid; oil; salicylic acid; sunflower

Khani Basiri, H., M. Sedghi and **R. Seyed Sharifi.** 2017. 'Effect of salicylic acid on the quality of edible oil and fatty acids composition in different regions of sunflower (*Helianthus annuus* L.) heads'. *Iranian Journal of Plant Physiology* 8 (1), 2285-2292.

Introduction

Sunflower is an important source of edible oil after soybean, peanut, and canola in the world (Anonymous 2008). The oil contains large amounts of unsaturated fatty acids, which reduce cholesterol and prevent heart disease (Rathore, 2005). The oil has a pleasant taste and contains vitamins A, D, E, and K and is used in making margarine (Hussain et al., 2000). Seed oil content varies between 25-42 percent, but can reach to 65% at very good conditions. Its protein content has been reported in the range of 10 to 25%. The most amounts of lipids accumulate in the sunflower seed cotyledons which contain about 78% of their weight and 7.4% of embryo weight. Oil from the seeds of this plant is drying oil with iodic index about 145-200 (Seyed Sharifi, 2007) which contains four major fatty acids including palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2). Sunflower oil is rich

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Received: March, 2017
Accepted: October, 2017

in linoleic acid and is mainly used in the food industry (Baydar and Erbas, 2005). The optimum temperature for oil synthesis is 18-22 °C (Anderson et al., 1978; Harris et al., 1978).

Environmental conditions and seed position on the mother plant are directly effective on the quality of produced seeds in mustard, sunflower and soybean (Guleria et al., 2007). In sunflower, kernel weight is reduced from the outer layers toward to the centre of capitulum (Goffner et al., 1988; Karadogan et al., 1998). The number of filled kernels in the centre is often low, while significantly reducing the sunflower yield. Low number of filled kernels in the centre (low yield at this area) is associated with vascular absorption (Durrieu, et al., 1985). Kernel filling in annual plants like sunflower is a fundamental process in reproduction and crop yield (Sedghi et al., 2008). Some plants such as sunflower in addition to protein and starch produce and accumulate large amounts of photo-assimilates during seed development in the form of triacylglycerol and store them as lipids which will convert to sucrose during germination through glyoxylate cycle (Taiz and Zeiger, 2006).

Accumulation of lipids during grain filling depends upon the transport of assimilates from the source to sink. In the process of grain filling and dry matter, oils and fatty acids accumulation from the periphery toward the centre of the capitulum have complex patterns (Munshi et al., 2003). Likely, capability of sunflower seeds produced from different positions of inflorescence in germination and emergence is due to the difference in the content of seed reserves (Munshi et al., 2007).

Salicylic acid (ortho-hydroxy benzoic acid, SA) is a natural phenolic compound and as a phytohormone plays an important role in the regulation of plant growth and development (Kang et al., 2003). SA stimulates the processes such as flowering, leaf stomatal closure, seed germination, seedling growth, and activation of plant defense systems (Chandra et al., 2007). In addition to its effects on growth and resistance to stress and diseases, SA can increase fruit yield, glycolysis pathway, flowering in thermogenic plants, absorption and transport of ions, photosynthesis rate, stomatal conductance, and transpiration through the stomata (Hayat et al., 2010). The first report on the physiological effects of SA in plants was the *in vivo* induction of flowering and bud growth in cells of tobacco (Eberhard et al., 1989).

Salicylic acid significantly increased oil and protein content in sunflower and with increasing SA concentration from 75 to 100 mg L^{-1} content of oil and protein significantly increased in comparison to control (Hayat et al, 2010). Also, saturated fatty acids decreased but unsaturated fatty acids increased (Dawood et al 2012). It was suggested that SA increases the concentration of auxin, gibberellin, zeatin, and zeatin ribosid while it reduces the abscisic acid contents in plants (Shehata et al., 2000, 2001; Zaghlool, 2002).

To the best of our knowledge there are few reports about the effect of SA on the lipid and fatty acid content in sunflower kernels from different positions, so this experiment designed for such purpose.

Materials and Methods

The experiment was conducted in the research field of the University of Mohaghegh Ardabili, Ardabil, Iran (15' 38° N and 15' 48° E). It is located in the semi-arid and cold climate with the mean precipitation of 400 mm annually. Soil texture was silty-loam with 1350 m altitude. Meteorological characteristics of the research field are shown in Table 1.

The experiment was conducted as factorial based on randomized complete block design with 3 replications. Treatments consisted

Table 1
Meteorological characteristics of the experimental field

	Temperature (°C)			Humidity (%)			Precipitation (mm)
	minimum	maximum	average	minimum	Maximum	average	
22Apr-May	6.6	21.4	14	37.9	92.2	65.0	18.9
May-Jun	10.3	25	17.7	39	90	65	25.6
Jun-Jul	12.7	23.9	18.3	57	95	76	8.7
Jul-17Aug	12.5	27.7	21	40	89	65	2.8

Table 2
Comparison of means for the oil content (%) of different treatments at sampling dates

treatment	N_1*	N_2	N_3	N_4	N_5
S1P1	0.0^{f}	0.0^{g}	0.0^{h}	0.0^{h}	0.600^{g}
S1P2	2.8667^{c}	5.266^{f}	9.700^{f}	12.466^{f}	16.233^{f}
S1P3	4.300^{d}	11.100^{c}	17.600^{c}	22.233^{c}	27.066^{c}
S2P1	0.0^{f}	0.0^{g}	0.766^{g}	0.0^{h}	
S2P2	4.500^{d}	7.333^{e}	18.333^{e}	13.166^{e}	
S2P3	7.600^{b}	13.366^{b}	19.766^{b}	24.066^{b}	29.633^{b}
S3P1	0.0^{f}	0.0^{g}	0.600^{g}	0.800^{g}	0933^{g}
S3P2	5.800^{c}	8.700^{d}	12.333^{d}	17.033^{d}	21.266
S3P3	11.367^{a}	17.500^{a}	22.666^{a}	29.400^{a}	33.433^{a}

P1: inner, P2: middle, P3: outer position, S1: 0, S2: 0.1 and S3: 0.2 g L^{-1} salicylic acid
* N is the samplings with 5 day intervals.

Table 3
Comparison of means for the oleic acid content (%) of different treatments at sampling dates

treatment	N_1*	N_2	N_3	N_4	N_5
S1P1	0.200^{h}	0.533^{h}	0.933^{g}	1.166^{g}	1.533^{f}
S1P2	4.500^{f}	10.800^{f}	16.033^{f}	20.233^{f}	24.533^{e}
S1P3	13.200^{c}	21.100^{c}	28.666^{c}	34.833^{c}	40.700^{b}
S2P1	0.400^{gh}	0.800^{gh}	1.333^{g}	1.366^{g}	1.633^{f}
S2P2	6.933^{e}	13.666^{e}	18.400^{e}	21.333^{e}	25.766^{d}
S2P3	17.133^{b}	24.600^{b}	31.300^{b}	36.800^{b}	41.166^{b}
S3P1	0.733^{g}	0.966^{g}	1.333^{g}	1.433^{g}	1.600^{f}
S3P2	9.900^{d}	16.866^{d}	20.733^{d}	23.366^{d}	27.433^{c}
S3P3	22.033^{a}	27.333^{a}	33.600^{a}	38.133^{a}	43.800^{a}

P1: inner, P2: middle, P3: outer position, S1: 0, S2: 0.1 and S3: 0.2 g L^{-1} salicylic acid
* N is the samplings with 5 day intervals.

of three levels of salicylic acid including 0, 0.1, and 0.2 g L^{-1} and three separate positions for kernels in the head as inner, outer, and middle. Seeds of hybrid oilseed sunflower named Aline 122.1 × R-14 were planted in the field under favorable climatic conditions. Inter and intra row spacing was 65 and 30 cm, respectively. There were 5 rows in each plot. Average planting depth was 5 cm. In order to achieve the desired density four seeds were placed in each hole. Adjusted for density, thinning operation was carried out when the seedlings had two true leaves.

Salicylic acid was sprayed on leaves in all treatments except the control treatment during three stages (two times before flowering and another after flowering) at early morning or evening.

Samplings began after 15 days of flowering with 5 days intervals from the kernels of each head and the amount of the accumulation of lipid reserves was measured. To measure the oil and fatty acids content, 500 mg of seed powder was dissolved in the solvents hexane, chloroform-methanol (2:1 v/v) and chloroform-methanol-water (1:2:0.8 v/v) and extraction was performed. The extracts were dried at low pressure and purified according to Belay and Daier (as quoted in De La Roche et al., 1973). Fatty acid composition was measured by gas-liquid chromatography. Data analysis was performed by SAS 9.1 software. Mean comparisons were done with LSD at 5% probability level.

Results

Oil content

The maximum oil content (33.43%) was observed by spraying 0.2 gL^{-1} of SA at outside region kernels in all samples (Table 2). SA increased the amount of oil 6% in comparison to

Table 4
Comparison of means for the linoleic acid content (%) of different treatments at sampling dates

treatment	N_1*	N_2	N_3	N_4	N_5
S1P1	0.07^{i}	0.10^{i}	0.30^{i}	0.60^{h}	0.800^{g}
S1P2	3.80^{f}	8.20^{f}	11.50^{f}	16.20^{e}	18.633^{e}
S1P3	7.60^{d}	12.70^{d}	16.80^{d}	20.30^{c}	24.300^{c}
S2P1	0.10^{h}	0.30^{h}	0.50^{h}	0.70^{g}	1.033^{gf}
S2P2	6.10^{e}	10.70^{e}	14.60^{e}	17.50^{d}	20.40^{d}
S2P3	10.20^{b}	14.30^{b}	17.90^{b}	22.10^{b}	26.933^{b}
S3P1	0.20^{g}	0.40^{g}	0.60^{g}	0.80^{f}	1.266^{f}
S3P2	9.10^{c}	12.80^{c}	17.40^{c}	20.30^{c}	24.033^{c}
S3P3	11.70^{a}	16.30^{a}	21.60^{a}	28.10^{a}	32.633^{a}

P1: inner, P2: middle, P3: outer position, S1: 0, S2: 0.1 and S3: 0.2 g L^{-1} salicylic acid
* N is the samplings with 5 day intervals.

Table 5
Comparison of means for the palmitic acid content (%) of different treatments at sampling dates

treatment	N_1*	N_2	N_3	N_4	N_5
S1P1	0.0^{e}	0.0^{g}	0.0^{g}	0.043^{g}	0.076^{g}
S1P2	1.500^{b}	2.433^{d}	3.266^{d}	4.200^{d}	5.066^{d}
S1P3	3.400^{a}	5.900^{a}	7.100^{a}	8.033^{a}	9.233^{a}
S2P1	0.0^{e}	0.0^{g}	0.0^{g}	0.033^{g}	0.046^{g}
S2P2	1.100^{c}	1.633^{e}	2.100^{e}	2.933^{e}	3.833^{e}
S2P3	1.500^{b}	3.433^{b}	4.766^{b}	6.233^{b}	7.466^{b}
S3P1	0.0^{e}	0.0^{g}	0.0^{g}	0. 0^{g}	0.050^{g}
S3P2	0.0^{e}	0.933^{f}	1.600^{f}	2.533^{f}	2.600^{f}
S3P3	0.866^{d}	2.933^{c}	4.333^{c}	5.333^{c}	6.200^{c}

P1: inner, P2: middle, P3: outer position, S1: 0, S2: 0.1 and S3: 0.2 g L^{-1} salicylic acid
* N is the samplings with 5 day intervals.

Table 6
Comparison of means for the stearic acid content (%) of different treatments at sampling dates

treatment	N_1*	N_2	N_3	N_4	N_5
S1P1	0.0^{f}	0.0^{g}	0.0^{g}	0.0^{g}	0.050^{g}
S1P2	0.900^{d}	1.766^{d}	2.733^{d}	3.266^{d}	4.100^{d}
S1P3	2.500^{a}	3.600^{a}	5.100^{a}	6.400^{a}	7.533^{a}
S2P1	0.0^{f}	0.0^{g}	0.0^{g}	0.0^{g}	0.030^{g}
S2P2	0.733^{e}	1.566^{e}	2.100^{e}	2.833^{e}	3.533^{e}
S2P3	1.933^{b}	3.00^{b}	4.666^{b}	5.766^{b}	7.100^{b}
S3P1	0.0^{f}	0.0^{g}	0.0^{g}	0.0^{g}	0.026^{g}
S3P2	0.0^{f}	0.600^{f}	1.300^{f}	1.866^{f}	2.366^{f}
S3P3	1.233^{c}	2.733^{c}	3.700^{c}	4.900^{c}	5.833^{c}

P1: inner, P2: middle, P3: outer position, S1: 0, S2: 0.1 and S3: 0.2 g L^{-1} salicylic acid
* N is the samplings with 5 day intervals.

the control. None of SA concentrations had any effect on the oil content of central kernels (Table 2).

In the case of fatty acids, oleic and linoleic acids had a similar trend with oil content in different samplings (Tables 3 and 4); however, palmitic and stearic acids showed the opposite trend with oleic and linoleic acids (Tables 5 and 6). The highest percentages of palmitic (9.23) and stearic acid (7.53) were observed at outer kernels without SA treatment and with increasing the SA concentration from 0 to 0.2 g L^{-1} there was a reduction in the content of theses fatty acids.

Salicylic acid increased the linoleic acid content more than oleic acid. Linoleic acid content increased approximately 8% (from 24.3 to 32.6%), but oleic acid content increased only 3% (from 40.7 to 43.8%) at the final sampling (Table 3 and

4). Therefore, SA was found to promote the edible quality of sunflower oil (by increasing linoleic acid content) and the frying quality (by increasing oleic acid content) of oil, while it was more effective on the edible quality.

Kernel position had significant impact on oil and fatty acids content so that from the periphery toward the center of the head, oil and saturated fatty acids decreased (Tables 2, 5 and 6).

Discussion

In general, SA increased oil percentage and oleic and linoleic acids content (unsaturated fatty acids) and decreased palmitic and stearic acids (saturated fatty acids). In this case with increasing unsaturated fatty acids, quality of oil also increased.

Noreen et al. (2009) reported that high concentrations of SA significantly increased seed oil content and essential unsaturated fatty acids, but it decreased stearic acid significantly. It seems that SA causes flowers to open early in all regions of the head and in this way kernels accumulate more dry matter which finally converts to more oil. Dawood et al. (2012) reported that SA induces flowering, increases flower life, delays aging, and increases cell metabolism.

Spraying of SA at the concentration of 0.2 gL^{-1} increased kernel weight in all regions of head in sunflower, but was more effective on outer kernels, followed by middle position and then central kernels. SA is effective on dry matter accumulation in sunflower and it can be used to increase the yield of this plant (Sedghi et al., 2013).

Changes in the composition of seed reserves such as lipids, fatty acids, and other metabolites are biochemical changes in seed reservoirs (Chung et al., 1995). Generally, there are two major changes during seed maturation. First, changes in the seed volume and the second, biochemical and physiological changes which interfere in cell division and enlargement. SA affects many physiological and biochemical processes and thereby enhances growth and mobility (transport) of photosynthetic active substances from the source repository to the sinks (Abd El-Wahed et al., 2006; Al-Hakimi and Hamada, 2001). Assimilate direction to the seeds and flowers in different positions related to the relationship between the sources (leaf and stem carbon stocks, Hall et al., 1990) and sinks and their phloem conjunctions (Farrar and Gunn, 1996). The increase in oil and protein content can be due to the increase in vegetative growth and the transport of materials in response to SA application (Dawood et al., 2012).

Weiss (1983) reported that during the development of sunflower kernels, the amount of oleic acid decreases while linoleic acid content increases. In addition, SA may be required for auxin and cytokinin synthesis (Metwally et al., 2003; Gharib, 2006). Ibrahim et al. (2001) also reported that cytokinins are likely to be necessary for synthesis of fatty acids, especially unsaturated and long-chain fatty acids compared with saturated fatty acids.

Competition for assimilates and space on the receptacle has been observed among the peripheral kernels and weak central kernels (Keiller and Morgan, 1988; Steer et al., 1988). The number of filled seeds in the center position of head is often low while it significantly reduces the total kernel quality. Low number of filled seeds in the center (i.e., low performance area) is associated with vascular absorption of this area (Durrieu et al., 1985).

Zimmerman and Fick (1973) observed that linoleic acid increases from periphery toward center and oleic acid shows an inverse trend. On the other hand, Baydar and Erbas (2005) in a two-year study reported that in the first year, palmitic, stearic, and linoleic acids decreased while oleic acid increased linearly from peripheral toward central regions, but in the second year there was an increase in palmitic, stearic, and oleic acids and a reduction in linoleic acid content.

Accumulation of fatty acids is strongly influenced by environmental conditions and follows a specific pattern. Certainly, one cannot conclude that with increase in one fatty acid content, others increase or decrease. With kernel growth and maturation all fatty acids are increased but the rate of increase is different for each acid. In other words, genetic differences in oil content and fatty acid composition at development stage needs to find some reasons (Ishikawa et al., 2001).

Kernel oil and fatty acids content in the peripheral and middle regions of head were much

higher than central kernels and the observed results may be due to SA effect (Tables 2 to 6). However, Baydar and Erbas (2005) and Karadogan et al. (1998) reported that the kernels of the middle region had the highest oil content (45.6%).

The average temperature during the growing season was 14-21 °C, relative humidity was 65-70%, and total rainfall was 119 mm (Table 1). In this study the effect of environmental factors should not be ignored. The maximum oil yield achieves in the altitudes less than 1,500 meters. The percentage of unsaturated fatty acids in oilseeds which are grown in high-latitude and temperate regions is higher than oilseeds which are grown in low-latitude and tropical places (Seyed Sharifi, 2007). Anderson et al. (1978) and Harris et al. (1978) reported that the best temperature for oil synthesis is 18-22 °C. This is consistent with our experimental conditions (Table 1).

Miralls et al. (1997) reported that high temperatures and low relative humidity in the growing season caused poor pollination and increased the number of hollow kernels. In general, favorable environmental conditions were presented in this study for growth and synthesis of unsaturated fatty acids and oils. So the results obtained from the use of SA should be considered besides the presence of favorable environmental conditions.

Conclusion

In this experiment, spraying of 0.2 g L^{-1} SA increased the oil and fatty acid content of sunflowers which subsequently increased edible and frying quality of oil by increasing unsaturated fatty acid content. On the other hand, we observed that remaining kernels around the sampling area even in the central region had greater growth. From the literature and observations of this experiment it is concluded that the presence of more hollow kernels in the center of head may be due to the lack of enough space for growth and late opening of florets in this area. This leads central kernels be weak sinks for absorption of photo assimilates. So, it is suggested that breeders attempt to decrease the density of florets in central region of head and let the kernels fill efficiently.

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