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Investigating the State of Flowering and Fruit Formation of Some Olive Cultivars under the Stress of Low Irrigation and Salicylic Acid Foliar Application

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ABSTRACT

In order to investigate the state of flowering and fruit formation of olive Conservalia, Zard and Amphisis cultivars under the influence of salicylic acid foliar application and under-irrigation stress in the Dalaho olive research station (longitude of 45˚, 51΄ E and latitude of 34˚, 30΄ N and the height of sea level 581m) of Kermanshah province, a field factorial experiment based on randomized complete block design with three replications and three factors (salicylic acid concentrations, Irrigation regimes and olive cultivars) was carried out in 2017 and 2018. Two stages of complete spraying of olive trees with salicylic acid at zero concentrations were performed as control (water spraying), 1.5 and 3 mM, before flowering and before the rapid growth of fruit. Irrigation treatments, including irrigation of 100% (control), 75% and 50% of the water requirement of olive trees during the season, were applied with drip irrigation system. The experimental material of this study was 17-year-old trees of three cultivars of olive cultivars. Each experimental unit consisted of two trees. Care and maintenance of trees were applied equally in all treatments. Notes on the characteristics of flowering and fruit formation, including the number of inflorescences in a branch, the number of flowers in an inflorescence, the length of an inflorescence, the number of perfect flowers in an inflorescence, the length of a flowering branch, the number of fruits in a branch, the number of perfect flowers in Branch and percentage of fruit formation (one month before harvest) were calculated based on complete flower in the branch in different treatments and different cultivars. The results of this research showed that foliar spraying of salicylic acid at a concentration of 3 mM in two growth stages before flowering and before the start of rapid fruit growth of the three studied olive cultivars moderated the effects of water stress. On the other hand, due to the decrease in the amount of irrigation water, the number of inflorescences in the branch, the number of perfect flowers in the inflorescence and the percentage of fruit formation decreased.

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1. Introduction

Drought stress is one of the restrictions on agricultural production in arid and semi-arid regions and Iran with average rainfall of 250 mm per year is in this area (Fathian *et al.*, 2015). Drought intensively causes negative consequences on plant water potential, cellular division, photosynthesis and protein synthesis and in this way has effects on plant growth and efficiency (Brito *et al.*, 2019).

Olive tree is a native plant in Mediterranean areas. In these regions, fruit season is summer in olive trees and plants are exposed to water deficiency, high temperature and light intensity. Although olive is a drought-tolerant tree, in stressful conditions, plant’s energy is used in defensive mechanisms and thereby, plant growth and productivity will decline (Fernandes-Silva *et al.*, 2010; Gholami and Zahedi, 2019). Therefore to cultivate olive trees in arid regions, it is necessary to select tolerant cultivars and tolerant cultivars can tolerate water shortage due to larger photosynthetic systems or more capacity for osmotic adjustment (Ennajeh *et al.*, 2006). In drought, the use

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of techniques to save water is so important. One of these techniques is the spraying of anti-transpiration (Abdallah *et al*., 2019).

Salicylic acid is a healthy phenol which is also known as a plant hormone and affects on some of the physiological and metabolic reactions in plants. Salicylic acid due to the increase of TSS (total soluble solid), causes to decrease in fruit water loss and degradation of polysaccharides to monosaccharides (Wang *et al*., 2022). The treatment with salicylic acid causes to delay in ethylene synthesis, photosynthesis system promotion and increase of chlorophyll content and decrease in plant transpiration (Nazar *et al*., 2015). Salicylic acid by activation of antioxidant defense system, the production of secondary metabolites, osmolytes synthesis, and mineral elements balance and keeps of suitable equilibrium between growth and photosynthesis, assists in tolerance of stressful conditions (Khan *et al*., 2015). Salicylic acid has an important role in plant physiological processes. Flowering induction and growth and development and effect on opening and closing mechanisms in stomata and respiration have some of the important roles of salicylic acid (Shaukat *et al*., 2022). Salicylic acid is one of the important molecular signals in plants in response to environmental stresses (Senaratna *et al*., 2000).

In white seedless grapes, salicylic acid played pragmatic role in maintenance of fruit quality and reduction of water loss and fungal decay in berries (Ardakani *et al*., 2013). Azizifar et al. (2022) evaluated the effects of kaoline and salicylic acid on efficiency and some of the physiological responses in olives in drought stress and they showed that salicylic acid declines ionic leakage and malondialdehyde (MDA) and improved relative water content, chlorophyll, phenol, proline, total carbohydrate and total yield. In another experiment, the effect of Glycine betaine and salicylic acid on fruit characteristics of 3 olive cultivars (Arbequina, Koroneiki and Conservalia) was evaluated. The treatments indicated significant influence on fruit size, seed and flesh /seed ratio (Shojaei *et al*., 2021).

In the present study, the effect of spraying salicylic acid in 3 concentrations was studied on fruit characteristics and flowering in 3 olive cultivars (Conservalia, Zard and Amphisis) exposed to low irrigation regimes.

2. Materials and methods

The present experiment was conducted in the Dalaho Olive Research Station (longitude of 45˚, 51´ E and latitude of 34˚, 30´ N and the height of sea level 581m) of Kermanshah province in 2018 and 2019. The physical and chemical characteristics of soil and water in project region and experimental olive cultivars characteristics are presented in Tables 1-3 respectively.

Table 1. The physical and chemical characteristics of the experimental soil (Kermanshah Laboratory of Soil Science, Agricultural Research and Education Center)

Soil sample	Depth (cm)	Silt (%)	Sand (%)	Potassium (ppm)	Phosphorus (ppm)	Nitrogen (%)	Organic carbon (%)	$CaCO_3$ (%)	pH
1	0-30	44	25	320	11.8	0.17	1.95	33	7.3
2	30-60	37	28	100	6	0.05	1.20	35	7.5

Table 2. Water characteristics of experiment (Kermanshah Laboratory of Soil Science, Agricultural Research and Education Center)

EC ($ds.m^{-1}$)	pH	CO_3^{-2}	HCO_3^-	Cl	So_4	Ca	Na
		(meq/l)					
550	7.28	0	4.60	0.30	1.90	6.60	0.20

Table 3. The characteristics of the studied olive cultivars

Cultivar	Original country	Commercial usage	The full ripening time of fruit
Conservalia	Greece	Conserved	October
Zard	Iran	Dual purpose	October
Amphisis	Greece	Oil production	October

This experiment was carried out from May 2018 until August 2019 as a field factorial experiment based on randomized complete block design with 3 replications and 3 factors (salicylic acid concentrations, irrigation regimes and olive cultivars). 2 steps of complete spraying of olive trees with salicylic acid was done in 0 as a control (spraying by water), 1.5 and 3 mM before flowering and before the beginning of fast growth of fruit, irrigation treatments included 100% (control), 75% and 50% water requirement of olive trees during growth season with drip irrigation system. Plant material was 17-year-old trees from 3 olive cultivars including Conservalia, Zard and Amphisis and every experimental unit consisted of 2 trees. The effects of salicylic acid and water stress were evaluated on flowering characteristics and fruit formation in olive cultivars. Care and maintenance of trees including irrigation, mechanical weeding and manuring were applied equally in all treatments. To evaluate flowering status in olive cultivars in field conditions by salicylic acid spraying, 5 shoots were selected from different sides in May 2019 and accordingly, it was calculated inflorescence number in shoot, flower number in inflorescence, inflorescence length, perfect flower number in inflorescence, flowering shoot length, fruit number in shoot, perfect flower number in shoot and fruit formation percent (one month before harvesting) according to complete flower in shoot (I.O.O.C. 2002). Ultimately, the data analysis was conducted with SAS software (9/1) and mean comparison with multi-dimension Duncan's test.

3. Results and discussion

3.1. Inflorescence number in shoot

Inflorescence number in shoot was affected by cultivar, salicylic acid concentrations and irrigation regimes at the probability level of 1% and interaction effect of cultivar × irrigation regimes was affected at the probability level of 5% (Table 4). In studied olive cultivars, in terms of inflorescence number in shoot, the highest content was observed in Conservalia cultivar and the lowest content was in Zard. By increasing salicylic acid concentration, inflorescence number in shoot is also enhanced. On the other hand, drought stress caused the reduction in inflorescence number in shoot (Table 5). In terms of the interaction effect of cultivar and irrigation, terms of the interaction effect of cultivar and irrigation, the highest inflorescence number in shoot resulted in Conservalia and 100% irrigation regime (Table 6).

Table 4. Variance analysis of the effects of cultivar, salicylic acid concentration and irrigation regime on inflorescence characteristics in olive cultivars

Variance resources	d.f.	Infls per shoot	Flowers per infls	Infls length	Perfect flower per infls	Flowering shoot length	Fruit per shoot	Perfect flower per shoot	Fruit formation percent
Cultivar (C)	2	24.23**	651.37**	0.46*	106.15**	1227.83**	167.83**	17249.86**	177.49**
Salicylic acid Con. (S)	2	5.81**	7.38**	0.83**	1.70**	20.47**	1.19**	633.98**	0.46
Irrigation regime (I)	2	46.24**	56.31**	2.30**	6.61**	11.94**	8.58**	3471.41**	1.23
C × S	4	0.39	0.51	0.41*	0.21**	4.62**	0.16	48.23*	0.84
C × I	4	1.77*	5.94**	0.24	0.82**	2.12**	0.04	373.38**	9.71**
S × I	4	0.37	0.93	0.01	0.04	1.17	0.17	21.04	0.71
C × S × I	8	0.07	0.48	0.02	0.03	0.28	0.03	11.04	0.21
Error	52	0.57	0.87	0.12	0.04	0.53	0.10	15.46	0.45
CV (%)		7.19	9.98	15.07	4.43	6.87	12.14	7.44	13.69

*and **: Significant at the 5% and 1% probability levels, respectively. Infls: Inflorescence.

Table 5. Mean comparison of cultivar, salicylic acid concentration and irrigation regime on the characteristics of flower in olive cultivars

Treatment	Infls per shoot	Flowers per infls	Infls length (cm)	Perfect flower per infls	Flowering shoot length (cm)	Fruit per shoot	Perfect flower per shoot	Fruit formation percent (%)
Olive cultivar								
Conservalia	11.35a	14.98a	2.41ab	6.90a	17.52a	5.52a	79.10a	7.15a
Zard	9.51c	7.26b	2.23b	2.94c	10.38b	1.34b	28.69c	4.63b
Amphisis	10.82b	5.86c	2.49a	4.66b	4.04c	1.07c	50.64b	2.03c
Salicylic acid Con.								
0	10.12b	8.82b	2.21b	4.55c	9.67b	2.41b	47.71c	4.46a
1.5 mM	10.53b	9.43a	2.36ab	4.91b	10.94a	2.70a	53.38b	4.71a
3 mM	11.04a	9.86a	2.56a	5.03a	11.33a	2.82a	57.35a	4.63a
Irrigation regimes								
100%	11.67a	10.85a	2.67a	5.35a	11.38a	3.22a	63.76a	4.77a
75%	10.91b	9.29b	2.38b	4.79b	10.49b	2.60b	53.55b	4.36b
50%	9.12c	7.96c	2.08c	4.36c	10.07c	2.10c	41.12c	4.66ab

Different letters in each column show significant differences according to Duncan's multiple-range test. Infls: Inflorescence.

3.2. Flower number in inflorescence

Flower number in inflorescence was affected by cultivar, salicylic acid levels and irrigation regimes and also interaction effect of cultivar × irrigation regimes at the probability level of 1% (Table 4). In studied olive cultivars, in terms of flower number in inflorescence, the highest amount was in Conservalia and the lowest amount was in Amphisis (Table 5). By elevation of salicylic acid concentration, flower number in inflorescence increased and drought stress decreased this character (Table 5). In terms of interaction effect of cultivar and irrigation, the highest flower number in inflorescence was recorded in Conservalia and 100% irrigation (Table 6).

Table 6. Mean comparison of interaction effect of cultivar and irrigation regimes on flower characteristics in olive cultivars

Cultivar	Irrigation regimes	Infls per shoot	Flowers per infls	Perfect flower per infls	Flowering shoot length (cm)	Perfect flower per shoot	Fruit formation percent (%)
Conservalia	100%	12.56a	16.81a	7.61a	18.48a	95.84a	6.39b
	75%	11.88ab	13.73b	6.87b	17.23b	81.72b	6.73b
	50%	9.61c	13.39b	6.21c	16.86b	59.75c	8.32a
Zard	100%	10.72bc	9.34c	3.61e	11.38c	38.89e	4.86c
	75%	10.03c	7.26d	2.87f	10.23d	28.88f	4.29c
	50%	7.77d	5.19e	2.35g	9.43d	18.29g	4.74c
Amphisis	100%	11.71ab	6.39de	4.82d	4.17e	56.56c	3.06d
	75%	10.80bc	5.88de	4.63d	4.02e	50.04d	2.06d
	50%	9.97c	5.31e	4.52d	3.93e	45.33de	0.93e

Different letters in each column show significant differences according to Duncan's multiple-range test. Infls: Inflorescence.

3.3. Inflorescence length

Inflorescence length was affected by salicylic acid levels and irrigation regime at the probability level of 1% and cultivar effect and interaction effect of cultivar × salicylic acid concentration at the probability of 5% (Table 4). In studied olive cultivars in terms of inflorescence length, the highest content was in Amphisis and the lowest content was in Zard cultivar (Table 5). By increasing of salicylic acid concentration, the inflorescence length increased and drought stress caused to reduction of inflorescence length (Table 5). In terms of interaction effect of cultivar and salicylic acid concentration, the highest inflorescence length resulted in Amphisis and 3 mM salicylic acid (Table 7).

Table 7. Mean comparison of interaction effect of cultivar and salicylic acid concentration on flower characteristics in olive cultivars

Cultivar	Salicylic acid Con.	Infls length (cm)	Perfect flower per infls	Flowering shoot length (cm)	Perfect flower per shoot
Conservalia	0	2.34ab	6.64b	16.10b	73.99b
	1.5 mM	2.42ab	7.06a	17.89a	80.29ab
	3 mM	2.47ab	6.99a	18.57a	83.01a
Zard	0	2.17b	2.71f	8.91d	25.47f
	1.5 mM	2.25b	3.06e	10.89c	29.55f
	3 mM	2.27b	3.05e	11.33c	31.04f
Amphisis	0	2.11b	4.30d	3.99e	43.65e
	1.5 mM	2.42ab	4.60d	4.04e	50.29d
	3 mM	2.94a	5.06c	4.09e	57.97c

Different letters in each column show significant differences according to Duncan's multiple-range test. Infls: Inflorescence.

3.4. The perfect flower number in inflorescence

The perfect flower number in inflorescence was affected by cultivar, salicylic acid levels and irrigation and also interaction effect of cultivar × salicylic acid and cultivar × irrigation regimes at the probability level of 1% (Table 4). In studied olive cultivars, in terms of perfect flower number in inflorescence, the highest number was in Conservalia and the lowest one was in Zard cultivar (Table 5). By considering salicylic acid concentration, the perfect flower number in inflorescence increased while drought stress decreased the perfect flower number in inflorescence (Table 5). In terms of interaction effect of cultivar × salicylic acid, the highest perfect flower number in inflorescence resulted in Conservalia and salicylic acid 1.5 and 3 mM (Table 7). In terms of cultivar × irrigation regime, the highest perfect flower number in inflorescence was observed in Conservalia and 100% irrigation (Table 6).

3.5. Flowering shoot length

The effect of cultivar, salicylic acid level and irrigation regime and interaction effects of cultivar × salicylic acid concentration and cultivar × irrigation regime at the probability level of 1% on the flowering shoot length was significant (Table 4). The highest flowering shoot length was in Conservalia and the lowest length was in Amplisis. By enhancement of salicylic acid concentration, flowering shoot length increased while drought stress caused the reduction of the flowering shoot length (Table 5). In terms of the interaction effect of cultivar × salicylic acid concentration, the highest flowering shoot length was in Conservalia and 1.5 and 3 mM salicylic acid (Table 7). In terms of interaction effect of cultivar and irrigation regime, the highest flowering shoot length was in Conservalia and 100% irrigation (Table 6).

3.6. The fruit number in shoot

The fruit number in shoot was affected by cultivar, salicylic acid levels and irrigation regimes at the probability of 1% significantly (Table 4). In the studied olive cultivars, the highest fruit number in shoot was in Conservalia and the lowest number was in Amphisis.

By increasing of salicylic acid concentration, fruit number in shoot increased while drought stress decreased fruit number in shoot (Table 5).

3.7. The perfect flower number in shoot

The effects of cultivar, salicylic acid levels and irrigation regime and interaction effect of cultivar and irrigation regime at the probability of 1% and the interaction effect of salicylic acid concentration and cultivar at the probability of 5% on the perfect flower number in shoot are significant (Table 4). In the studied olive cultivars, the highest perfect flower number in shoot was in Conservalia and the lowest number was in Amphisis, by increasing salicylic acid concentration, the perfect flower in shoot increased, but drought stress declined the perfect flower number in shoot (Table 5). In terms of the interaction effect of cultivar and salicylic acid concentration, the highest number of perfect flower in shoot was observed in Conservalia cultivar and 1.5 and 3 mM salicylic acid (Table 7). In terms of the interaction effect of cultivar and irrigation, the highest perfect flower number in shoot was in Conservalia and 100% irrigation (Table 6).

3.8. Fruit formation percent

The effect of cultivar and the interaction effect of cultivar and irrigation regime on the fruit formation percent was significant at the probability of 1% (Table 4). In the studied olive cultivar, the highest fruit formation percent was in Conservalia and the lowest percent was in Zard cultivar (Table 5). In terms of the interaction effect of cultivar and irrigation regimes, the highest fruit formation percent was in Conservalia and 50% irrigation (Table 6).

By considering the obtained results, the flower characteristics and fruit formation were different in 3 olive cultivars, flower induction in olive is related to internal and external factors. The studies showed that flower induction in olive is in June-July and before the beginning of seed hardening. The formation, growth and development of flowers start with flower induction which is a kind of modification in the available genes in the buds to form reproductive buds. Flower initiation is the next step when the structure of reproductive bud can be visible with microscope in autumn. Eventually, in the differentiation phase, the bud grows to form the flower organs at the late of winter (Rapoport *et al.*, 2012). Water Supply and irrigation is an important factors in obtaining production with high quality and quantity. It is also impossible to absorb nutrients and transfer them to different parts of the tree through water. Although slight stresses prevent vegetative growth in olives to reduce abortion, it is recommended that 3 times including before flowering, during flowering and after that, irrigation would be conducted. Meanwhile, to prevent fruit fall at the time of fruit ripening, during maturation and before harvesting, considering olive water requirement is necessary (Santos, 2018; Alcaras *et al*., 2021).

Low irrigation during winter has no effect on flower or inflorescence formation in olive trees. But water shortage during changing of the flower and inflorescence development, affects on flowering parameters and causes no formation of inflorescence, the reduction of flower number in inflorescence and decline of perfect flower number (Rapoport *et al.*, 2012). Alaey et al. (2011) reported that the application of 200 mg.L^{-1} salicylic acid caused the enhancement of fresh weight in Rose cutting flowers. Salicylic acid increases cell division in plant apical dominance and plant height (Mandhanis *et al*., 2006). Also, salicylic acid as a pseudohormone, increases protein synthesis and induces the flower buds (Martin-Mex *et al*., 2005).

By considering the recording of flower characteristics data this experiment was carried out at second year while flower induction was in the before year (the first experimental year) with the beginning pf low irrigation and salicylic acid spraying. Therefore, flower characteristics are affected by low irrigation and salicylic acid spraying in the second year. The irrigation level and salicylic acid spraying are effective in flower and inflorescence formation, so flower parameters including inflorescence number in shoot, flower number in inflorescence, inflorescence length, perfect flower number in inflorescence, flowering shoot length, fruit number in shoot, perfect flower number in shoot and fruit formation percent indicated the significant reduction compared with control in this investigation.

4. Conclusion

Spraying anti-transpiration such as salicylic acid is one of the techniques to maintain water in the soil. The use of salicylic acid 1.5 and 3 mM assisted in mitigating the adverse effects of drought stress on olive trees in this experiment. Despite olive is a drought-tolerant tree, the most tolerant cultivars outperform in stressful conditions. Conservalia exposed to water stress indicated the highest flowering status compared with other studied cultivars in this experiment and as a promising cultivar, it is recommended in the stressful environment.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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