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Some phytoregulators to increase the fertility of the olive flowers

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Abstract

This research investigated the flower abortion characteristics in the Kushan olive variety (*Olea europaea* L.). Kushan variety shows forced sterility, ovarian abnormalities and cytogenetic causes in the formation of pollen and the division of chromosomes. The stimultants treatments were done in three trees per treatment as follows: (i) BA 200 ppm, (ii) GA3 200 ppm, (iii) TIBA 200 ppm, (iv) Bor 3g/l (v) Control, Water Treatment. The flowers abortion resulted statistically different. The application of GA3 resulted in 11% fertile flowers more than control group. In conclusion, the ovarian abortion of flowers in the Kushan cultivar depends not only on the anatomical and morphological characteristics of the flower but also on the physiological processes during floral maturation.

Keywords: olive; flower fertility; phytoregulator.

1. Introduction

This research investigated the flower abortion characteristics in the Kushan olive variety (*Olea europaea* L.). This local variety shows forced sterility, ovarian abnormalities and cytogenetic causes in the formation of pollen and the division of chromosomes [1, 2].

The ability of Kushan variety to produce fruit by self-pollination is genetically determined although the genetic expression is highly dependent on climatic and growing conditions (2).

Infertility, as within a plant or variety as well between varieties, is not only affected by the floral anatomic- morphological characteristics, but as well as by physiological processes undergoing during fertilization [2, 3]. Pollen tube preserve the ability to grow normally even when none of the factors which contained there in, are not in the style tissue [4, 5].

Many studies on the use of phytoregulators raported good results on improving the olive flowers fertility. Such hormones are applied as well as in in-vivo and in-vitro olive multiplication. In this contexts, this research aimed to improve the fertility of flowers in the Kushan cultivar.

2. Material and Methods

2.1. Treatments.

he method consisted in the application of four stimulants, as a foliar spray in early blooming of 15 year- old olive trees, grown in Pajo,Tirana. The stimulants treatments were done in three trees per treatment as follows: (i) BA (BA-Benzyl-Adenine) 200 ppm, (ii) GA_3 (Acide Giberelic) 200 ppm, (iii) TIBA (Acide Triiodebenzoic) 200 ppm, (iv) $Na_2B_2O_3 \cdot 4H_2O$ as Solubor DF 3g/l, (v) Control, water treatment.

Spraying is carried out in three phenological phases (i) at the time of full differentiation of cluster, (end of April 10th) (ii) in the complete flower differentiation (May 15th) and (iii) immediately after the fall of the flowers (June 10). The ovarian tests have been conducted in 100 flowers for each variant.

2.2. Studied indicators.

The studied clusters were gathered in the four sides of each tree canopy. The effect of each stimulant was firstly observed in terms of ovarian abortion and flowers position in the cluster. The biometric features, classification, form and cluster symmetry were also analysed. There were also observed the position of flowers and their dynamics at the time of fruit maturity, the number of fruits and their percentage compared to the number of flowers. The fertilized egg cell - the zygote and number of sterile zygotes.

The data analysis was carried out using JMP software, analysis of variance and physiological correlations were performed at a significant level of ($P<0.05$).

3. Results and Discussion

3.1. Flowers morphology:

The final size of inflorescence and flowers is reached just before anthesis from 20 April to 20 May, depending on environmental conditions. The size of the inflorescences and number of flowers varied on the position of the sample, it was in accordance with the physiological state of the tree and climatic conditions [4, 5]. *In the figure 1/1*, are given morphological characteristics of the flower of Kushan olive cultivar, it is uniform and consists of 4 fused green sepals which create a cup at the base of the flower. It has four white petals which are fused at their base and drop as one unit at the end of the blooming. The flower has two stamens with a large, yellow, two-lobed anther on each. The ovary is located in the centre of the sepal cup, it has two carpels with two ovules each. The style is straight, short and thick with a rather large stigma, figure1/1 and 1/2. The number of flowers on each order are given in the figure 1/3 and while the SH Index for quantitative and qualitative traits of the cluster is given in figure 1/4.

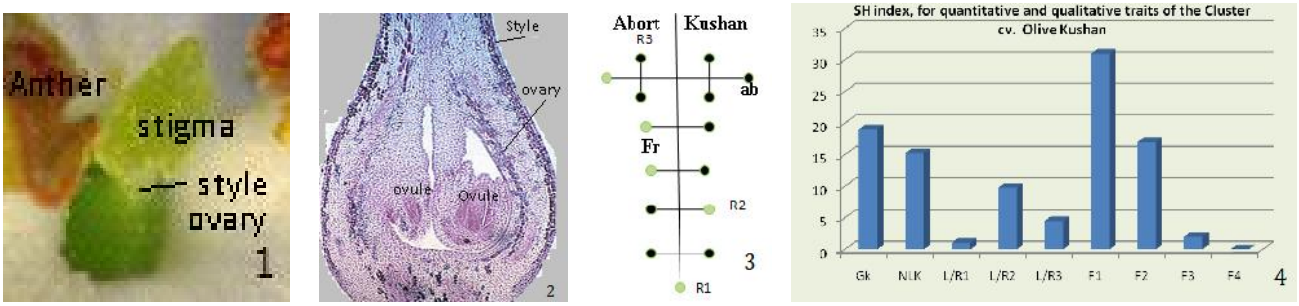

Figure 1. Olive floral aspects of Kushan olive. (1) ovary with stigma, (2) Microscopic aspect of ovarian, (3) Cluster full differentiation, ab- flowers aborted, fr- fertile flowers, (4) SH Index for quantitative and qualitative traits of the cluster.

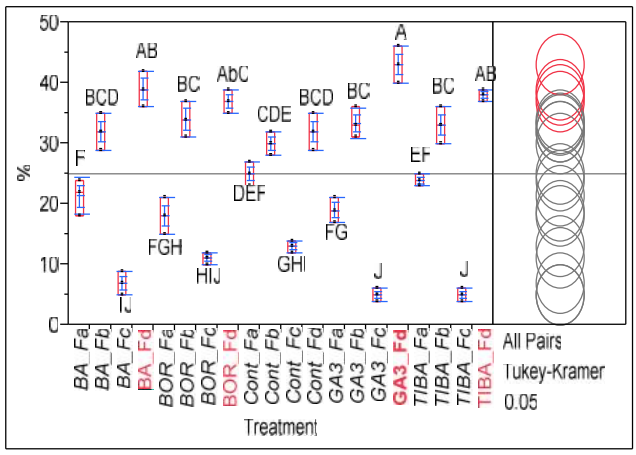

Figure 2: The analysis of variance of flowers fertility in Kushan variety,

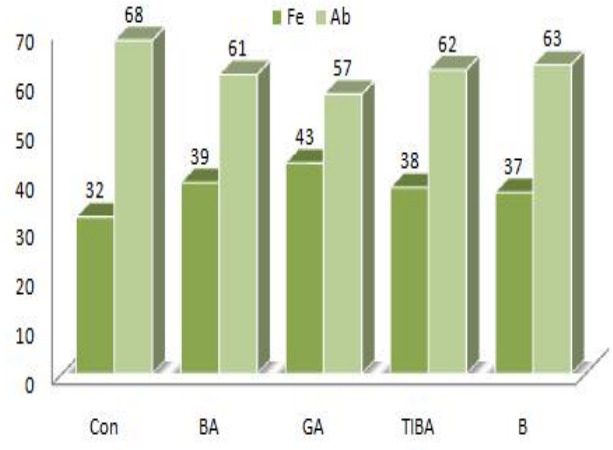

Figura 3 The graphical presentation for the effect of stimulants in ovarian abortion in olive Kushan

3.2. Floral fertility and the level of variation

The analysis of variance (*figure 2)*, showed that the Cluster length, the number of flowers, and the number of fertile flowers were not statistical different compared to the averages. Repeated test for all pairs using Tukey-Kramer, *Average Std. Dev 3,48 and frequency of distribution was 2.20 to 4.82. Tf >Tt* ***Prob > F*** *<.0001**. Level of deviations resulted from 2.6 to 0.19, these values are within the standard deviation limits showing high data reliability. It was proved that phytoregulator treatments have no influence on the mean number of flowers in the analyzed clusters.

The peak of perfect flower dropping resulted to be around two weeks after the bloom. Ten days after full blooming, 20% of the ovaries were fertilised. The percentage of fertilized flowers increased to 60% after 18 days of blooming. The length of the flowering period dependents on the environmental conditions.

The effect of climate on the amount of fruit set is highly significant [2, 6, 7].

The flowers abortion resulted statistically different. In the trees sprayed with water as control group 32% of flowers were fertile. An increase of 7% in fertile flowers was observed in the BA treated trees in comparison with the control group. TIBA application increased the fertile flowers percentage in the same level as boric acid, while the application of GA_3 resulted in 11% fertile flowers more than control group. In the control treatment, 32% of flowers were fertile while 68% of them had aborted flower ovary.

During the stimulant treatments, the fertile flowers resulted at 40% while 60% of flowers were with aborted ovary. The main axis of flowers, has an average length of 19 cm, and the mean number of flowers was 15.24, out of which 6.6% were in the first (R-I), 64.2% in the second (R-II), and 29.2% in the third (R-III) order. In each treatment carried out, the fertility of flowers was higher in the second order.

The form and symmetry of the cluster, as genetic character showed no changes influenced by the environment and stimulants. The average value F^1 (31), F^2 (17), F^3 (2) while F^4 (zero), figure 1/4.

In three orders of the Cluster, analyzed as an average of five treatments the percentage of fertile flowers was 37.8%. The percentage of fertile flowers varied greatly between orders (cv=30%): from 7.4% in the third row and 38.5% in the second one to 67.5%. fertile flowers in the first row.

The analysis of the influence of stimulants in the ovarian abortion, presented graphically in the figure 3, showed that all treatments increased the flower fertility in comparison with control. The highest percentage of fertile flowers resulted from treatmenst was 67.5%.

Table 1. Data on fertility of flowers of cv Kushan treated with BAP, GA3, TIBA and Control according to classification of fertility.

Classification	**Con**	**BA**	**GA_3**	**TIBA**	**B**
Fa- stigma black / brown	25.0±2.0 def	21.3±3.05 f	19.0±2.06fg	24.0±1.2ef	18.0±2.95 h
Fb- Stigma&style black / brown	30.02.2**cde**	32.0±3.0bcd	33.3±2.5bc	33.0±2.7bc	34.0±3.0 bc
Fc-Stigma+Stigma+Ovary black brown	13.0±1.1ghi	7.0±2.00 **ij**	5.0±1.13 **J**	5.0±1.30 **J**	11.0±1.0 hij
Fd-Stigma+Style+Ovary- Green / yellow	32.0±3.0bcd	39.0±2.92ab	43.0±2.8 a	38.0±1.2 ab	37.0±2.1abc
Aborted ovary	68.0±0.71 a	61.0±0.50 c	57.0±0.20 d	62.0±0.3 bc	63.0±0.30 b
Fertility	32.0±0.2 e	39.0±0.3 b	43.0±0.4 a	38.0±0.1 d	37.0±0.3 e
Percentage of fruit at the time of maturity	1.35±0.02 a	1.38±0.02 a	1.39±0.04 a	1.33±0.03 a	1.34±0.04 a

Levels not connected by same letter are significantly different

Fa, Fb, Fc - Aborted Ovary; **Fd**- Fertile Ovary (BA- BA-Benzyl-Adenine), GA_3 - Acide Giberelic, TIBA (Acide Triiodebenzoic, B- $Na_2B_2O_3 \cdot 4H_2O$

Results of this study (treatments) given in *table-1,* and in the *figure-2,* reflects the relationship between floral ontogenesis and flower pollination. The weak links of flowers may be due not only to morphological characteristics, ovarian failure or incompatibility but also to cytological characteristics [*2, 8, 9]*. Considering all factors, it is important to identify the olive cultivars that have low ovarian failure.

After flowering, the needed time for ovule fertilization in this cultivars was 4-5 days. The treatments that gave better results in reduction of the phenomenon of the flower abortion in this cultivar were GA and BA. By *figure-2* and *table-1*, it can be seen that stimulants have differences between each other as regards to ovarian fertility. The effect of stimulants was observed only at the time of flowering, because the number of fruits in relation to mature flowers showed no differences after treatments.

The percentage of fruits at the time of ripping varied from 1.33 to 1.39, with an average of 1.35, std. dev. 0.03, cv= 2.2%. The results showed that the effect of stimulants had no statistical differences compared to control at the time of fruit maturity, confirming that stimulants used had effects only in the flowering stage. Some other factors such as level of nitrogen, water and pathogenic state were considered responsible about the the performance of this phenomenon [10, 11]. Moreover, the critical physical conditions (temperature and precipitation), coincided as unfavorable in this period.

While, in the proccess of pollination there have been pointed out three important factors: stigma ability for the pollen, the growth ability of the pollen tube and physiological ability of the ovule.

However, the connection of different sizes of flowers may be due to sexual incompatibility of floral organs. This phenomenon must be studied regarding the effect of some of the best pollinating varieties.

In one tree with good flowering, fruit set of 1.2 to 2% of the flowers is considered sufficient for a high yield production. Generally, in Kushan olive

cultivar, there were 1-2 perfect flowers per inflorescence, which is sufficient for maximum yield. Weather conditions during flowering time are critical for the yield too. The dry desert winds occurring occasionally during olive flowering cause a reduction in fruit set, although this is mostly due to their effect on the stigma (drying), style (inhibition of pollen tube growth) and ovary (zygote decline). Dry hot winds might also cause the decline of pollinated ovaries even when the tree is no under water stress [2, 5].

Referring to some authors, (*Badr and Hartman*) for the fertility of flowers, the study on Kushan olive cultivar after flower set puted in evidence the importance of nutrition and water, as the responsible factors for further fruit development from the set to their maturity [2, 12].

4. Conclusions

Often the key factor in effective pollination period is the ability of the stigma to support pollen germination. The time required for the pollen tubes to be inserted into ovule. The most important, has been the time that the ovule is capable of being fertilized.

In conclusion, the ovarian abortion of flowers in the Kushan cultivar depends not only on the anatomical and morphological characteristics of the flower but also on the physiological processes during floral maturation.

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6. References

1. Allen A. H. Tankard A. R.. "**The Determination of Boric Acid in Cider, Fruits**, etc". Analyst. **29** (October): 301–304. Bibcode:1904Ana....29..301A. doi:10.1039/an9042900301.
2. Badr, S.A, ; Hartman, H.T ; Margin, G.C. **Endogenous of gibberellins and inhibitors in relation to flower induction and inflorescence development in the olive**. Plant Physiol., 46, 674-679. 1970b.
3. Bini G : **Observations sur l'influence de phyto-regulateurs exogenes sur la fertilite de l'olivier**. OLEA. 1986. n.(17) 51-57
4. Cuevas J, K Pinney y VS Polito. 1999. **Flower differentiation, pistil develop-ment and pistil abortion in olive** (Olea europaea L. 'Manzanillo'). Acta Horti-culturae (ISHS) 474:293-296.
5. Díaz A, A Martín, P Rallo, D Barran-co y R Rosa. 2006. **Self-incompatibi-lity of Arbequina and 'Picual' olive as-sessed by SSR markers.** Journal of the American Society for Horticultural Science **131**:250-255.
6. Lavée S, L Rallo, HF Rapoport y A Troncoso : **The floral biology of the olive: effect of flower number**, type and ditribution on fruit set. Scientia Horticulturae 1996, **66** (3-4): 149-158.
7. Lavée S, L Rallo, HF Rapoport y A Troncoso. 1996. **The floral biology of the olive: effect of flower number, type and ditribution on fruitset**. Scien-tia Horticulturae **66** (3-4): 149-158.
8. Manrique T, HF Rapoport, J Castro y M Pastor. 1999. **Mesocarp cell division and expansion in the growth of olive fruits**. Acta Horticulturae (ISHS) **474**: 301-304.
9. Nigond J. Bioclimatologie de l'olivier. **Protocole d'observations biologiques et phisiques**. FAO, Projet , 70-521
10. Pinney K y VS Polito. 1990. **Flower initiation in 'Manzanillo' olive**. Acta Horticulturae (ISHS) **286**: 203-206.
11. Rallo L y R Fernândez-Escobar. 1985. **Influence of cultivar and flower thinning within the inflorescence on compétition among olive fruit**. Journal of the American Society for Horticultural Science **110** (2): 303-308.
12. Stephenson AG. 1981. **Flower and fruit abortion: proximate causes and ultimate functions**. Annual Rewiew of Ecology and Systematics **12**: 253-279