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## POLLEN MORPHOLOGY of *Prunus mahaleb* L. (Mahaleb) (Rosaceae) and ITS IMPORTANCE in LANDSCAPING of PARKS and GARDENS

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**ABSTRACT.** Mahaleb (*Prunus mahaleb* L.) is a taxon of Rosaceae, which grows in the winter edges are threaded, the lower faces have feathers along the main vein. Fragrant flowers are white in color. When small or 5 -6 mm fruit is ripe, it gets a black color. It is used as the leading tree in the forestation of arid areas on the forest-step border. In this study, (Mahaleb) taxon, one of the trees in the parks and gardens of Eskişehir, was collected and photographed in 2017-2018. During flowering periods, pollens were determined by Wodehouse, Erdtman and SEM methods and they were studied in Light and Scanning Electron Microscopes. Pollen tricolporate, spheroid, exine tectate-striate. *P. mahaleb* is the reflection of people who are shaded under the park and gardens with its beautiful smell, but with a black and sticky state around it, it creates an undesirable view of the street.

**Keywords:** *Prunus mahaleb*, Pollen Morphology, Light Microscope, SEM, Turkey

### INTRODUCTION

Including natural species that grow under the conditions of the region and adapted to the ecosystem of that region in urban landscaping increases the chances of success in plantation and provides important contributions to the urban ecosystem.

The first condition for organizing an aesthetically, economically and biologically optimal environment is the selection of plant species that are optimally compatible with the physical environment. This condition can only be achieved by analyzing the biological and ecological characteristics of the plant species very well. Knowing the aesthetic appearance that plants will exhibit according to the size and shape they will reach, as well as determining their resistance to habitat demands and environmental conditions are important for a successful landscape application. For this reason, it is imperative to make a versatile ecological analysis of the plant species to be used in the field and to choose plant species accordingly [1].

Urban areas where natural species are used can serve to ensure the continuity of natural ecosystems and to rehabilitate degraded ecosystem parts (Ekici, 2010). The use of natural species adds richness to herbal compositions by eliminating the monotonous appearance of the well-known and frequently used exotic species and cultivated plants. Natural species have economical positive contributions to the city, such as low cost and minimum labor due to not requiring too much maintenance [2; 3].

Mahaleb in the natural vegetation in Eskişehir province is also an exemplary taxon adapted to the urban ecology. This taxon has been selected among the natural species suitable for use in vegetation studies in and around the city, to ensure the arrangement of

the city landscape and the enrichment of the urban ecology. In addition, it is aimed to contribute to plant systematics by revealing the pollen morphology.

## MATERIALS AND METHODS

The material of the study is *Prunus mahaleb*, one of the tree species used for herbal design in the parks in Eskişehir city center. Pollen samples of the plant were obtained from the flowers in the trees located on the side of the street next to Eskişehir TEE Park. Pollen samples of the investigated taxa were taken from dried plants found in the Herbarium (OUFE) of Osmangazi University Faculty of Science and Letters. The examination of current pollens under light microscope was done by Wodehouse (1935) method and examination of fossil pollen by Erdtman (1969) method. Morphological examination of the pollens was done under Nikon binocular microscope, oil immersion objective (x100). 50 times measurements were made for all parameters to determine the average values. Standard deviation and variations have been calculated. Each range in the ocular micrometer is 0.98 µm. Microphotographs were taken with a Nikon 80i type microscope and a KAMERAM Digital camera in the Department of Biology, Faculty of Science and Letters, Eskişehir Osmangazi University. The magnification of the photos is x1000. For Scanning electron microscopy (SEM) examinations, unacetholyzed pollen grains were placed on the fixing plate and covered with gold and examined under Jeol 5600 LV Scanning electron microscope (SEM) [11; 12].

Various basic palynological books and various studies have been used for the diagnosis of pollen [4-12].

## RESULTS AND DISCUSSION

Mahaleb (*Prunus mahaleb*) is a shrub from the Rosaceae family or a tree species that reaches 8–10 m and shed leaves in winter. Leaves are circular to broadly ovate, 3–6 cm in length, margins dentate, hairs along the main vein on the lower surface. Its fragrant flowers are white in color. Small or 5-6 mm sized fruit turns black when ripe. It is used as a pioneer tree in the afforestation of arid places on the forest-steppe border (Figs. 1-4).

It is named with different local names such as İdris tree, Yaban Kirazı, Taş Kirazı, Melem, Endez according to the region where it grows. Mahaleb is from the Rosaceae family and can grow up to 10 meters in height and has white flowers. Ripe fruits are black, bitter and sour in taste. It blooms in March, leaves in April, and begins to bear fruit in June.

Mahaleb tree is a cherry tree in the group of trees that can be planted in the Central Anatolia region. This tree is resistant to many ecological factors, especially drought. It is a tree that grows spontaneously in many regions of our country without the need for irrigation, fertilization and spraying. Mahaleb was used frequently as a landscape element in the Ottoman period, but today this tree is not as important as before. Both its tree and its fruit are useful and valuable trees. Today, it is mostly used to inoculate cherries and sour cherries.

The region between France and Germany in central and southern Europe, northern Pakistan and Kyrgyzstan in western and central Asia, and Morocco in northwest Africa is the homeland of the mahaleb. According to the findings of the Light and Scanning Electron Microscopes studies, the pollens were determined as tricolporate, spheroid, and exine tectate-striate (Fig. 5 and Table 1).



**Fig. 1.** General view of *Prunus mahaleb*



**Fig. 2.** Flowers of *Prunus mahaleb*

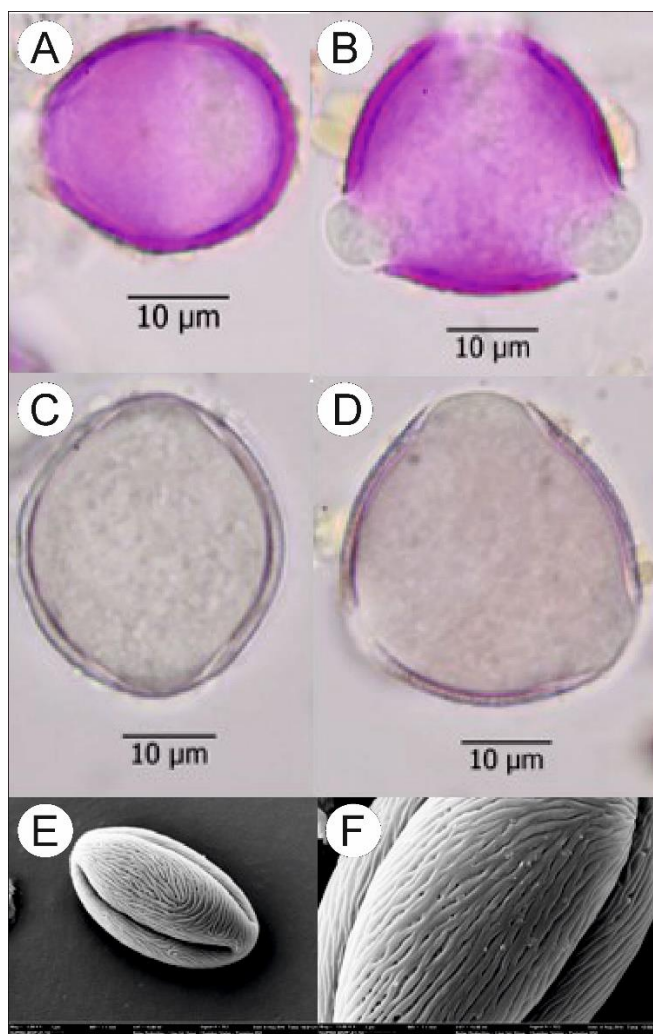


**Fig. 3.** *Fruits of Prunus mahaleb*



**Fig. 4.** *Seeds and seed powder of Prunus mahaleb* [Anon.]





**Fig. 5.** Light microscope **A.** Equatorial (W), **B.** Polar (W), **C.** Equatorial (E), **D.** Polar(E) and SEM **E.** Equatorial, **F.** Exine (Ornamentation) photos of the pollen grains of *Prunus mahaleb*

**Table 1.** Palynological measurements of *Prunus mahaleb*

	Wodehouse		Erdtman		
	M	S	M	S	
<b>P</b>	28,2	± 2,38	28,4	± 2,42	µm
<b>E</b>	27,18	± 3,11	31,68	± 4,56	µm
<b>clg</b>	21,47	± 3,27	18,92	± 2,18	µm
<b>clt</b>	9,56	± 1,32	10,34	± 1,24	µm
<b>plg</b>	8,74	± 1,36	9,90	± 1,44	µm
<b>plt</b>	7,14	± 1,41	7,22	± 3,14	µm
<b>L</b>	28,76	± 2,54	31,60	± 2,66	µm
<b>t</b>	6,68	± 1,28	8,74	± 2,16	µm
<b>i</b>	0,74	± 0,24	-	-	µm
<b>Ex</b>	1,08	± 0,20	1,04	± 0,14	µm

### ***Usage Areas***

- Today, medicine, cosmetics, food, drink, paint, furniture industry benefits from mahaleb tree and fruit.
- Mahaleb tree, which emits a pleasant aromatic scent due to the substance called coumarin in its bark, is also used in pipe making. In addition, people who work in furniture and carving prefer this tree because of its durability.
- Oil obtained by distillation from mahaleb seed is preferred in paint industry and ship paints and varnishes due to its water resistance.
- mahlep in wines made from native to Turkey has an important place in the world.
- The mahaleb seeds contain salicylic acid, the main ingredient of aspirin. Therefore, it is preferred in the production of painkillers and antibiotics in the pharmaceutical industry.
- The mahaleb powder obtained by grinding the seeds of the mahaleb is also an indispensable part of the dough and desserts in our kitchens in patisseries and homes.

### ***Benefits of Mahaleb***

- Mahaleb is known to have a lowering effect on blood sugar. For this, it is a spice that helps to lower blood sugar. For this purpose, 2-3 coffee spoons of mahaleb powder can be drunk with water on an empty stomach.
- It has a strengthening effect on the body. Mahaleb, which is powdered as a strengthening and sexual enhancer, can be mixed with honey and eaten 3 dessert spoons a day.
- It is used as a pain reliever.
- It plays a role in reducing prostate enlargement and prostate complaints.
- Mahaleb is also good for the digestive system. It is effective in stomach indigestion and gas problems. It also prevents weight gain because it activates the intestines. Since it accelerates digestion, it helps to reduce the risk of colon cancer in the intestines.
- It protects the body against diseases as it strengthens the immune system. In addition, it has a great effect in reducing abdominal swelling and relieving abdominal pain.
- The calcium contained in mahaleb is good for bone development and bone pain that may occur in the body.
- It is known to be one of the most effective types of spices to eliminate ailments such as asthma and shortness of breath. It removes the phlegm formed in the body and prevents the accumulation of phlegm.
- It balances the sugar level in the blood and prevents the sugar from rising rapidly. It is also good for diabetes due to its balancing feature. If you are at risk of developing diabetes, the insulin hormone it releases eliminates the risk of developing it.

As you can see, mahaleb has many positive effects and benefits for our health. Mahaleb, whose value is decreasing day by day in our country, should be understood again and attention should be paid to mahaleb breeding.

### **CONCLUSION**

The use of natural plant taxa as landscape elements in gardens and parks has many benefits such as easy and good adaptation to environmental conditions, contributing to natural life, requiring less care than foreign origin plants, being more durable and being

a source of nutrition and shelter for wildlife. To ensure ecosystem balance and continuity in cities, it is important to create corridors that will allow transitions between natural vegetation and urban landscape as well as preserving the natural landscape [13-15].

This research aims to raise awareness in terms of the use of plant species suitable for the natural structure of the city in urban green areas by drawing attention to the use of natural plant assets of Eskişehir province in landscape studies. It is thought that the use of natural tree species in landscape designs can increase the success and adaptation of sapling, and will be an appropriate choice against drought and water problems.

In the planting studies carried out in a city, criteria such as the climate of the region, natural vegetation, salinity in the soil, the aesthetic and functional value of the natural species to be used and the ecology of the city should be taken into account in landscape design studies. Besides, higher rates should be given to natural species in terms of maximum shading and water savings. Especially in cities such as Eskişehir where drought and deterioration are seen and felt intensely in the vegetation, these criteria gain great importance. It would be appropriate to show more sensitivity to this issue in the plantation studies to be implemented in the city.





We think that in addition to the systematic characteristics of this taxon in the Rosaceae family, pollen morphologies may also be a distinctive criterion. This study will also shed light on the phylogenetic relationship between the studied taxa. As a result, we believe that pollen studies will be useful for systematic studies since the morphological structures of pollens have distinctive features in determining taxa.

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## EFFECTS OF INTERCROPPING AND CULTIVAR ON SUGAR BEET (*Beta vulgaris* L.) ROOT AND SUGAR YIELD

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**ABSTRACT.** In order to study the effect of the intercropping of different sugar beet cultivars with soybean, Moldavian balm and proso millet on sugar beet yield a factorial experiment was conducted based on randomized complete block design with four replications in 2016 and 2017 in West Azarbayjan, Iran. The results indicated that among the intercropping patterns the highest and lowest LAI were observed in sugar beet–Moldavian balm (4.8) and sugar beet–proso millet (4.2) intercropping patterns, respectively. The highest and lowest root yield was obtained in sugar beet–Moldavian balm (50.21 ton ha<sup>-1</sup>) and sugar beet–soybean (23.70 ton ha<sup>-1</sup>) intercropping patterns, respectively. The highest sugar content was observed in cv. Ghazira and cropping pattern of sugar beet–Moldavian balm (16.12%) that was not significantly different with them in the sugar beet monocropping (16.11%) and sugar beet–proso millet (15.90%). Among the intercropping patterns the sugar beet–Moldavian balm and sugar beet–soybean patterns had the greatest (7.90 ton ha<sup>-1</sup>) and lowest (3.66 ton ha<sup>-1</sup>) gross sugar yield, respectively. The cv. Ghazira had the highest gross and pure sugar yield (12.45 and 10.59 ton ha<sup>-1</sup>, respectively) among the cultivars. We can conclude that the sugar beet–Moldavian balm intercropping pattern could be introduced as sustainable production systems with high productivity and profitability.

**Keywords:** Leaf area index (LAI), sustainable production, sugar content, sugar yield, tuber yield

### INTRODUCTION

Sugar beet (*Beta vulgaris*, L.), a warm season crop, is the second important sugar crop after sugarcane (*Saccharum officinarum* L.), 40% of the sugar is produced from sugar beet [1]. The sugar beet cultivated area in Iran in 2016 was about 110,000 hectares with a production of about 6 million tons [2]. Given the increasing population and the need for sugar, sugar beet is one of the industrial crops that have a major role in production of sugar in Iran. In these conditions the sugar beet growers have focused on the strategies such as development of new cultivars with tolerance to biotic and abiotic factors and high yield potential, modifying the crop row distance and planting arrangement, application of organic fertilizers and also intercropping with other crops to increase the sugar beet root yield and quality [3]. Intercropping is as an example of sustainable systems in agriculture with more resource utilization, quantitative and qualitative increase in yield, reduction of pest damage, diseases and weeds, and reduction in farmers dependence on pesticides, while maintaining product quality and marketability [4], [5]. When two crops with different plant height, vegetation and different growth patterns are cultivated at the same time in intercropping, they create the least competition and this increases the yield of intercropping compared with monocropping [6].

It has been reported that intercropping of proso millet (*Panicum miliaceum* L.) stylo (*Stylosanthes guianensis* L.) maximizes labor efficiency and minimizes the risk in adverse

weather conditions [7]. Usmanikhail et al. [8] evaluated the intercropping of three sugar beet varieties with barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), mustard (*Brassica alba* L.), lentil (*Lens culinaris* L.) and canola (*Brassica napus* L.) concluded that sugar beet yields and economic advantage were the maximum in lentil intercropping compared to other intercropping patterns. El-Fakharany et al. [9] observed that the sugar beet plants intercropped with faba bean (*Vicia faba* L.) had the highest root yield comparing with intercropping with maize (*Zea mays* L.) and cabbage (*Brassica oleracea* var. *capitata* Linneu). These results could be attributed to the least population density of pests infesting sugar beet plants in intercropping of sugar beet with faba bean.

Cultivar (genotype) selection is one of the management solutions to improve crop yield and quality in mono-and intercropping [10]. Refay [11] observed that quality parameters such as sugar content (19.9%), white sugar content (17.3%) and sugar yield ( $19.2 \text{ tha}^{-1}$ ) as well as chemical composition of roots were greater for Samo-2 as compared to those of other two varieties including Univers and Samo-1. Milford et al. [12] reported that leaf area and growth rate in sugar beet varieties differ mainly and selection of suitable cultivar is one of the main factors conferring sugar beet yield and quality traits. In intercropping patterns sugar beet cultivars Cauvery and Shubhra in 1:3 and 1:2 ratios indicated higher tuber and sugar yield than 1:1 ratio [10]. Usmanikhail et al. [8] studied the intercropping of three sugar beet varieties including Kaweterma, Aura and Pamela with different crops and found that among the sugar beet varieties; Kaweterma had the highest performance for growth, tuber yield and quality parameters as intercropped with lentil.

In Iran sugar beet mainly is cultivated as monocropping but in this study our objective is evaluating the 1) effect of intercropping of different sugar beet cultivars with millet, soybean (*Glycine max* L. Merr.) and Moldavian balm (*Dracocephalum moldavica* L.) on sugar beet growth, root and sugar yield.

## MATERIALS AND METHODS

### *Site description*

This experiment was conducted in 2016 and 2017 in the Khoy, West Azerbaijan, Iran ( $38^{\circ} 29' \text{ N}$ ,  $44^{\circ} 51' \text{ E}$ , 1247 m a.s.l.). This region has a hot and dry Mediterranean climate with cold and wet winter and hot and dry summer with 240 mm mean annual precipitation. The mean temperature and total monthly precipitation during the growing season for 2016 and 2017 in Khoy, West Azerbaijan is presented in Table 1. The detail of soil analysis for experimental site is presented in Table 2.

**Table 1.** The mean temperature (°C) and total monthly precipitation (mm) during the growing season for 2016 and 2017 in Khoy, West Azarbayjan, Iran

Months	2016		2017	
	Temperature (°C)	Precipitation (mm)	Temperature (°C)	Precipitation (mm)
January	-0.2	19.7	-7.4	19.0
February	-0.2	21.7	-7.4	10.1
March	8.7	13.1	7.6	17.2
April	13.4	38.3	12.9	52.1
May	19.1	12.8	18.1	49.1
June	21.8	66.7	24.1	5.1
July	25.7	12.8	27.8	1.6
August	27.0	2.5	27.7	1.5
September	20.9	7.2	23.1	0.0
October	13.2	29.7	13.7	19.6
November	5.4	1.5	8.0	39.5
December	-1.8	16.3	4.0	3.8

**Table 2.** The physical and chemical properties of the soil at experimental site

Texture	Sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (mg/kg)	N (%)	OC <sup>1</sup> (%)	pH	EC (dS/m)
Loam	31.4	48.0	20.6	222	9.3	0.09	0.85	8.04	0.81

<sup>1</sup>OC: Organic carbon

### Experimental design and field practice

The experiment was conducted as factorial based on randomized complete block design with 15 treatments and four replications. The first factor was sugar beet cultivar in three levels; Kevin (Strube co., Germany), Ghazira (Kuhn & co. International B.V., Netherlands) and Vaclav (Strube co., Germany), and the second factor was cropping patterns at four levels; monocropping of sugar beet, intercropping of sugar beet and soybean (50:50 ratio), intercropping of sugar beet and Moldavian balm (50:50 ratio) and intercropping of sugar beet and proso millet (*Panicum miliaceum* L.) (50:50 ratio). The intercropping treatments were planted based on replacement design and 50:50 ratios. Also the monocropping of soybean, Moldavian balm and proso millet were considered in the experiment.

During both years of study, field practices consisted of fall mold-board plowing followed by disking and cultivation in spring. The experiment was conducted in the field that was under organic production system in previous eight years as no chemical fertilizers, pesticides and herbicides were used in production systems. Sugar beet was planted on 30 March 2016 and 29 March 2017. Soybean, Moldavian balm and proso millet were planted on 18 April 2016 and 16 April 2017. At all treatments, the area of each plot was 16 (4×4) m<sup>2</sup>, with 8 rows and 4 m length, and 50 cm row space. Planting densities for sugar beet, soybean, Moldavian balm and proso millet was 10, 40, 30 and 30 plants m<sup>-2</sup>, respectively. The sowing depths for sugar beet, soybean, Moldavian balm and proso millet were 3–4, 3–5, 1–2 and 2–3 cm, respectively.

### Data collection

The leaf area index (LAI) of canopy was measured by Accu PAR device (LP-80, Decagon Device INC., USA) at 92 DAP of sugar beet (in 2016) and 91 DAP (in 2017). At maturity stage, the final harvest of sugar beet root was done on 27 October 2016 and 25 October 2017 from central rows of plots (a total area of 6 m<sup>2</sup> from each plot) and the root yield per unit area was determined for different cultivars and cropping systems. Sugar beet root samples were transferred to the Khoy Sugar Beet Laboratory for quality analysis. The sugar content (%), gross and pure sugar yield ha<sup>-1</sup>, were determined.

### Statistical analysis

The SAS Version 9.0.3 was used for ANOVA. The data that were used in ANOVA met the assumptions of homogeneity of variance and normality and did not need transformation. The Duncan's multiple range test was used for mean comparison at 5% probability level.

## RESULTS AND DISCUSSION

### Leaf area index (LAI)

The effect of year and sugar beet cultivar was not significant on LAI. The sugar beet LAI was significantly ( $p \leq 0.05$ ) affected by the cropping pattern. The highest leaf area index (5.492) was belonged to the monocropping of sugar beet (Table 3). Among the intercropping patterns the highest and lowest LAI were observed in sugar beet–Moldavian balm (4.792) and sugar beet–proso millet (4.183) intercropping patterns, respectively (Table 3).

**Table 3.** The mean comparison of sugar beet LAI and root yield affected by the cropping pattern (The means with the same letter in each column are not significantly different at  $p \leq 0.05$ )

Cropping pattern	LAI	Root yield (ton ha <sup>-1</sup> )
sugar beet monocropping	5.492 <sup>a</sup>	98.47 <sup>a</sup>
sugar beet–soybean	4.341 <sup>c</sup>	23.70 <sup>d</sup>
sugar beet–Moldavian balm	4.792 <sup>b</sup>	50.21 <sup>b</sup>
sugar beet–Proso millet	4.183 <sup>c</sup>	28.61 <sup>c</sup>

The decrease in sugar beet LAI in intercropping patterns can be attributed to the reduction of light with lower canopy layer, especially in soybean and proso millet intercropping with sugar beet. Manjunath and Salakinkop [13] reported that in soybean and Proso millets intercropping the LAIs of both crops in monocropping was higher than those in intercropping. Also, Arshad and Ranamukhaarachchi [14] in intercropping of sweet sorghum (*Sorghum bicolor* L.) and mung bean (*Vigna radiata* L.) reported that the LAIs of both crops were higher in monocropping than those of intercropping.

### Root yield

The effect of year and sugar beet cultivar was not significant on root yield. The sugar beet root yield was significantly ( $p \leq 0.05$ ) affected by the cropping pattern. The highest root yield (98.47 ton ha<sup>-1</sup>) was obtained in monocropping of sugar beet (Table 3). Among the intercropping patterns the highest and lowest root yield were obtained in sugar beet–



Moldavian balm (50.21 ton ha<sup>-1</sup>) and sugar beet–soybean (23.70 ton ha<sup>-1</sup>) intercropping patterns, respectively (Table 3).

Among the intercropping pattern the greatest root yield was obtained in sugar beet–Moldavian balm pattern. The reason of decrease in root yield in sugar beet–soybean and sugar beet–proso millet patterns could be attributed to the shadowing of soybean and proso millet on sugar beet canopy and consequently decrease in sugar beet IPAR and root yield. Abou Khadra et al. [15] observed that in the intercropping of sugar beet and wheat (*Triticum aestivum* L.), the highest root yield was related to the monocropping of sugar beet. Similar result was observed by Heba et al. [16] when sugar beet was intercropped with faba bean (*Vicia faba* L.). The effect of intercropping on the root yield of sugar beet mainly depends on the nature and growth habit of the companion crop. Abdel Motagally and Metwally [17] reported that the highest root yield of sugar beet was achieved for monocropping, when sugar beet was intercropped with faba bean [17].

### ***Sugar content (%) and sugar yield***

The interaction effect of year × sugar beet cultivar was significant ( $p \leq 0.01$ ) on sugar content. In 2016 the sugar content of cv. Ghazira (15.89%) was greater than those of cv. Kevin and Vaclav (Table 4). In 2017 the sugar content of cultivars was not significantly different. The sugar contents of cv. Kevin and Vaclav in 2017 were greater than those in 2016. The sugar content of cv. Ghazira was not significantly different in 2016 and 2017 (Table 4). The interaction effect of year × cropping pattern was significant ( $p \leq 0.05$ ) on sugar content (Table 5). In 2016 the sugar content was the greatest in sugar beet–Moldavian balm (15.66%) that was not significantly different with sugar beet monocropping and sugar beet–proso millet patterns. The lowest sugar content (14.89 %) was obtained in sugar beet–soybean intercropping and was not significantly different with sugar beet–proso millet intercropping. In 2017 the sugar content in sugar beet–soybean intercropping (16.03%) was the greatest and was not significantly different with sugar beet monocropping and sugar beet–Moldavian balm intercropping. In 2017 the lowest sugar content (15.48%) was obtained in sugar beet–proso millet intercropping that was not significantly different with sugar beet monocropping. The sugar content of sugar beet–soybean intercropping in 2017 (16.03%) was significantly greater than that of 2016 (14.89%).

The interaction effect of cultivar × cropping pattern was significant ( $p \leq 0.05$ ) on sugar content (Table 5). The highest sugar content was observed in cv. Ghazira and cropping pattern of sugar beet–Moldavian balm (16.12%) that was not significantly different with them in the sugar beet monocropping (16.11%) and sugar beet–proso millet (15.90%). In cv. Kevin the sugar contents were not significantly different among the intercropping patterns. In cv. Vaclav, among the cropping patterns the sugar beet–proso millet intercropping had the lowest sugar content (14.87 %) that was not significantly different with that in the sugar beet mono–cropping (15.28%).

**Table 4.** The means comparison for interaction effect of cultivar × year on sugar content of sugar beet (The means with the same letter in each column are not significantly different at  $p \leq 0.05$ )

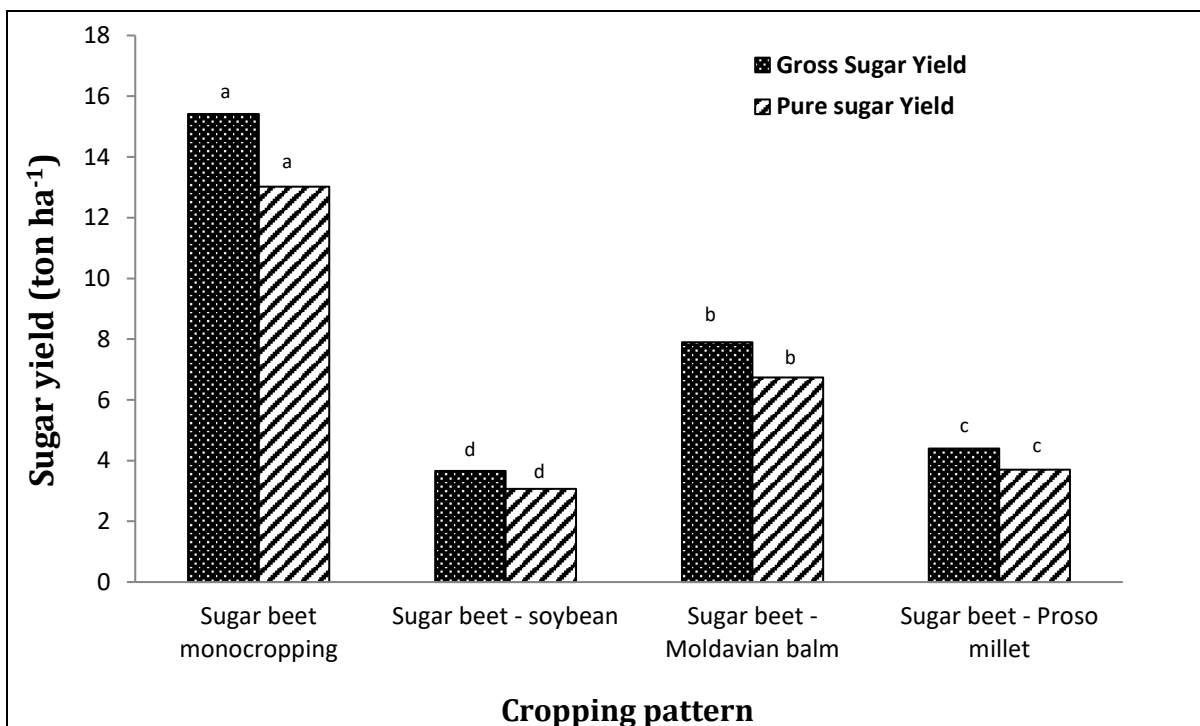
Sugar content (%)		
Cultivar	2016	2017
Kevin	15.00 <sup>b</sup>	15.83 <sup>a</sup>
Ghazira	15.89 <sup>a</sup>	15.89 <sup>a</sup>
Vaclav	15.06 <sup>b</sup>	15.78 <sup>a</sup>

**Table 5.** The means comparison for interaction effect of cropping pattern × year and cropping pattern × cultivar on sugar content of sugar beet (The means with the same letter in each column are not significantly different at  $p \leq 0.05$ )

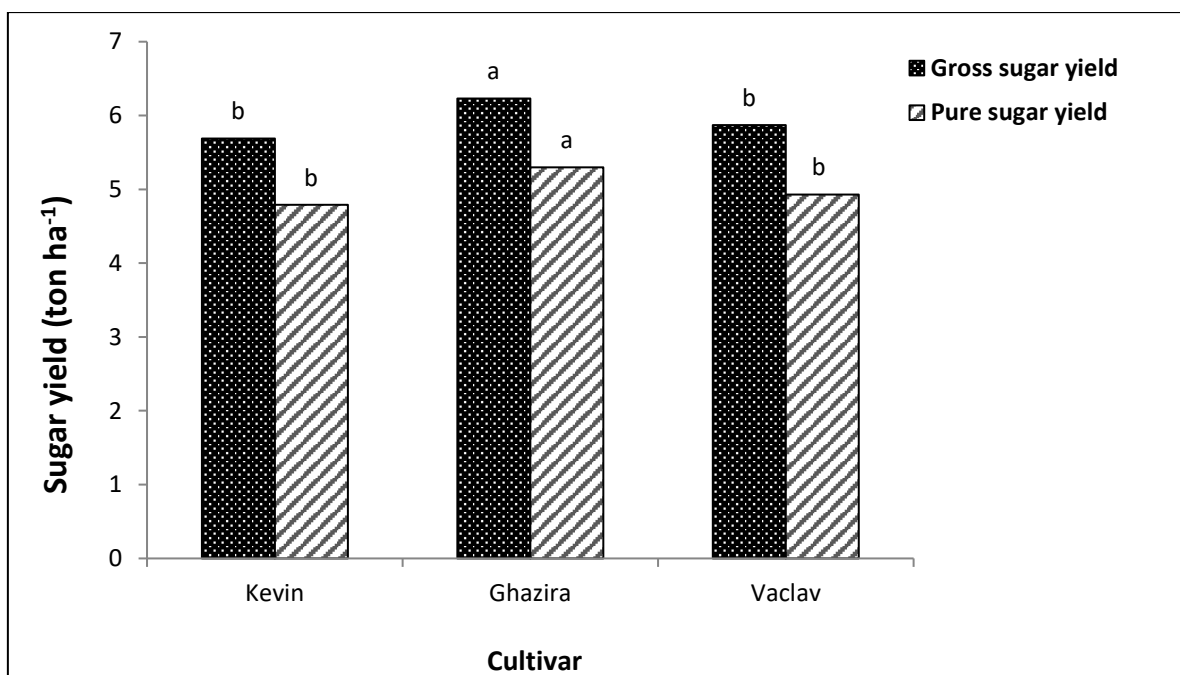
Cropping pattern	Sugar content (%)				
	Year		Sugar beet cultivar		
	2016	2017	Kevin	Ghazira	Vaclav
sugar beet monocropping	15.45 <sup>bc</sup>	15.84 <sup>ab</sup>	15.56 <sup>abc</sup>	16.11 <sup>a</sup>	15.28 <sup>cd</sup>
sugar beet–soybean	14.89 <sup>d</sup>	16.03 <sup>a</sup>	15.19 <sup>cd</sup>	15.44 <sup>bc</sup>	15.75 <sup>abc</sup>
sugar beet–Moldavian balm	15.66 <sup>abc</sup>	15.98 <sup>a</sup>	15.57 <sup>abc</sup>	16.12 <sup>a</sup>	15.77 <sup>abc</sup>
sugar beet–Proso millet	15.26 <sup>cd</sup>	15.48 <sup>bc</sup>	15.34 <sup>cd</sup>	15.90 <sup>ab</sup>	14.87 <sup>d</sup>

Gross and pure sugar yield was significantly affected by cropping pattern ( $p \leq 0.05$ ). The greatest gross sugar yield (15.41 ton ha<sup>-1</sup>) was observed in sugar beet monocropping (Figure 1). Among the intercropping patterns the sugar beet–Moldavian balm and sugar beet–soybean patterns had the greatest (7.90 ton ha<sup>-1</sup>) and lowest (3.66 ton ha<sup>-1</sup>) gross sugar yield, respectively. The results for pure sugar yield were similar to the gross sugar yield, and only the values of pure sugar yields were lower than those of gross sugar yield (Figure 1).

The effect of sugar beet cultivar was significant on gross sugar yield ( $p \leq 0.05$ ) and pure sugar yield ( $p \leq 0.01$ ). The cv. Ghazira had the highest gross and pure sugar yield (12.45 and 10.59 ton ha<sup>-1</sup>, respectively) among the cultivars (Figure 2). The gross and pure sugar yield in cv.s Kevin and Vaclav were not significantly different.



**Figure 1.** The mean comparison for effect of cropping pattern on pure and gross sugar yield (The means with the same letter are not significantly different at  $p \leq 0.05$ )



**Figure 2.** The mean comparison of gross sugar yield and pure sugar yield affected by the cultivar (The means with the same letter in each column are not significantly different at  $p \leq 0.05$ )

The sugar content was affected by interaction effects of year, sugar beet cultivar and cropping pattern. In 2017 the sugar contents of cv.s Kevin and Vaclav were greater than those in 2016. This may be attributed to the lower precipitation in April-September for 2017 than 2016 (Table 2). The different response in term of sugar content could be due

to various environmental factors such as temperature and precipitation in two years of the study [18], [19]. Mahrokh and Khajehpour [20] also reported that in drought stress condition the sugar content of sugar beet increased. Generally the sugar beet–Moldavian balm intercropping had the greatest sugar content in both years and all sugar beet cultivars. It could be concluded that cv. Ghazira is the best cultivar for sugar beet intercropping specially with higher crops. Previous studies [11], [12] also confirmed that the sugar beet cultivars are different in sugar content. According to reports, all three cv.s Kevin, Ghazira and Vaclav, have high sugar content and low sugar impurities [21].

## CONCLUSIONS






We observed that in sustainable production system of sugar beet the intercropping with other crops could increase the productivity of cropping system. The differences in tuber and sugar yield for intercropping patterns between two years could be explained by differences in average temperature and precipitations in growth season. The sugar beet–soybean and sugar beet–Moldavian balm intercropping patterns could be recommended in sustainable production systems in order to increase crop production per unit area without chemical fertilizer and pesticide application that is consistent with environmentally-friendly agriculture.

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## THE EFFECTS OF ESKİŞEHİR CEMENT PLANT ON AIR POLLUTION, AGRICULTURAL POLLUTION, HUMAN AND ENVIRONMENT

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**ABSTRACT.** With the industrial revolution, the establishment of large factories, industrialization and the increase in the human population have increased the human pressure on natural resources significantly. Major mining and processing activities are carried out worldwide in order to meet the increasing raw material need with the accelerated production processes. The effects on the nature of the mining activities carried out in many countries rich in natural resources, especially Turkey unfortunately is often devastating. The short and long-term effects of unconsciously established facilities on the biotic and abiotic environment without taking into account the short and long-term environmental impact assessments, especially in the regions where the old enterprises are located, appear noticeably and dramatically. Unfortunately, sometimes serious irreversible deterioration occurs in habitats and ecosystems in the areas where these facilities are located. There are also many different industrial facilities within the borders of Eskişehir province. Eskişehir Cement factory, which is the subject of this study, was chosen because it has been the subject of complaints by the local people for years and unfortunately, environmentally friendly solutions to solve these complaints were not brought. The negative effects of mining activities on human health and the environment can be minimized by scientific measures. The most important aim of our study is to draw attention to facilities experiencing similar environmental problems to the Eskişehir Cement factory.

**Keywords:** *Eskişehir cement plant, air pollution, agricultural pollution, human and environment*

### INTRODUCTION

The industrial revolution has had many effects on humanity and the environment. Between these effects, especially the prolongation of average human life and the increasing population indirectly affected the environment and nature. The increase in the human population brings with it increasing demands. On the other hand, the impacts of the factories established unconsciously in order to meet the increasing demands of the human population on the environment can reach destructive levels. The best example of this situation is global warming, which we visibly feel today as a result of the increasing atmospheric concentrations of greenhouse gases that are unconsciously released into the atmosphere. Undoubtedly, the biggest factor in the emergence of global warming is the relentless increase in the carbon footprint per individual in the last 200 years with the industrial revolution. Considering the permanent damage to natural vegetation and their habitats in order to carry out industrial activities, we come across the fact that we are very close to the irreversible line [1, 2].

As in all the world in recent years, environmental pollution is one of Turkey's most important problem. As mentioned above, the effects of industrial activities on the

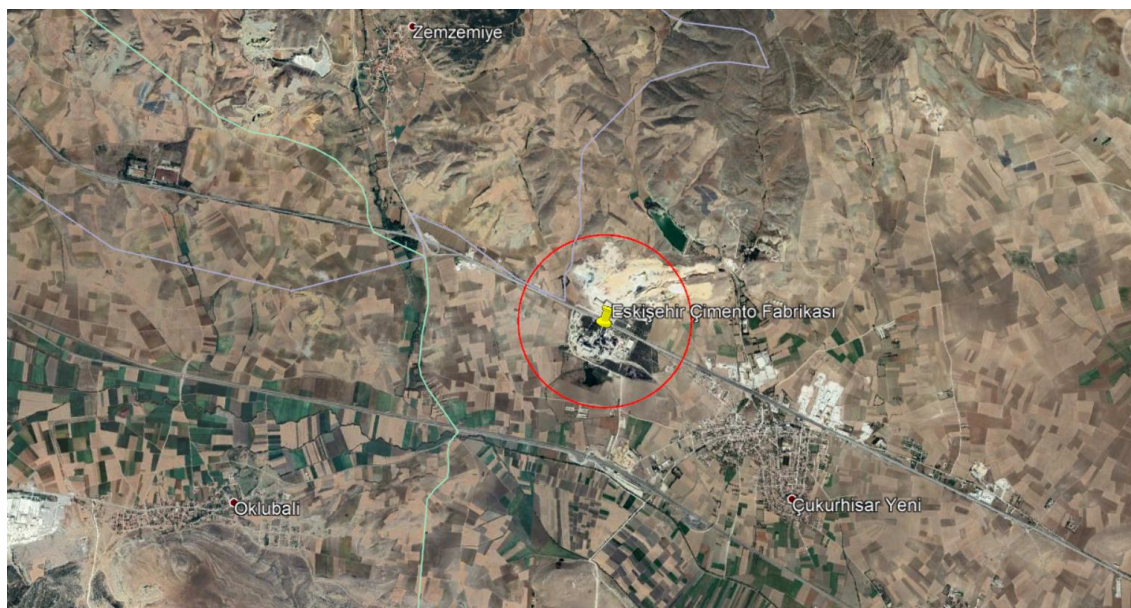
environment are on a global scale, but every facility built unconsciously damages the biotic and abiotic environment in their region depending on the production process they perform. The effects of unplanned facilities that are not nature and environment friendly, and waste disposal processes are not carried out properly affect human health directly or indirectly with the environment. Moreover, these impacts can cause permanent damage or fatal consequences depending on the amount and type of environmental pollutants created in the long term. One of the activities known to cause the aforementioned damages when carried out in unconscious facilities is mining activities. Undoubtedly, mining activities have been an activity that has been tried to facilitate human life from past to present. However, the realization of very large-scale mining and operation activities in order to meet the raw material needs arising with the industrial revolution resulted in more pollution of the environment [3,4, 5].

The effects of environmental pollution emerging as a result of increasing industrial activities have reached noticeable levels in our country, especially in recent years. The increasing number of factories established has led to the need for new lands where these factories will be established. When selecting the locations of these facilities, it is imperative to select suitable areas, taking into account many important issues such as the damage they may cause to the environment and ecosystem in the short and long term, and their effects on local people. In addition, it is necessary to determine and control the necessary waste disposal processes in the regions where the facilities will be established, to determine the species and ecosystem diversity of the region where biodiversity studies will be carried out in the relevant areas. Also, the creation of the species conservation action plans is important to establish the nature friend facilities. However, keeping commercial concerns at the forefront during the location selection process and therefore establishing facilities by making the wrong location unfortunately causes serious problems in human, animal and plant life. In the ongoing process, the fact that these factories continue their activities inadequate or without taking any precautions against the pollution they create can sometimes create irreversible problems in terms of health [6, 7, 8].

There are also many different industrial facilities within the borders of Eskişehir province. Eskişehir Cement Factory, which is the subject of this study, was chosen because it has been the subject of complaints by the local people for years and unfortunately, environmentally friendly solutions to solve these complaints were not brought. The negative effects of mining activities on human health and the environment can be minimized by scientific measures. The most important aim of our study is to draw attention to this problem and offer solutions to facilities experiencing similar problems through the relevant sample facility.

## **ESKİŞEHİR CEMENT FACTORY**

Eskişehir Cement Factory was put into service in 1954. The factory is 23 km away from Eskişehir center, on the Eskişehir-Bursa highway, around Çukurhisar village in the center (Fig. 1). The location of the factory established on the side of the highway is located at a point united with the city center. Due to the fact that the city center and the fields, gardens and vineyards are side by side, the dust from the factory chimneys threatens the health of the people living in the village, as well as agricultural activities.



**Fig. 1.** Location of Eskişehir Cement Factory

Eskişehir Cement Factory creates a serious visual pollution for Çukurhisar neighborhood center and its immediate surroundings. The main reason for this pollution is the air released from the factory chimneys without sufficient infiltration into the atmosphere. Cement particles (dust) suspended in the smoke coming out of the chimneys when the factory is operating can even be seen in the atmosphere around the factory. Moreover, these particles discharged into the atmosphere are transported to the agricultural areas and settlements around the factory by atmospheric air movements. The distance between the center of Çukurhisar neighborhood, which is the closest to the factory, and the factory center is approximately 2 km. Apart from Çukurhisar district, the distance between Oklubalı and Zemzemiye districts and the factory is approximately 4.5 km. Although other settlements are relatively affected by the factory, it is seen that the residents of Çukurhisar district are the most exposed to factory wastes. Especially the trees, agricultural areas and residences in the areas close to the factory are often exposed to a light ash-colored sediment and those living here are constantly breathing these dust.

During our works, it was stated by the local people that after the Eskişehir Cement Factory was put into operation, there was a loss of yield in the fields and gardens of the people of the region, especially the fruit trees close to the factory were dried and their fruits fell. It has been determined that the dust emitted to the environment during the cement production process, in particular, covers the plants in lands closer than 1 km to the cement factory, reducing the amount of light on the plants and clogging their pores. It is seen that this situation has clearly caused the local people who have lands in the region to lose economically, especially when compared with the areas not affected by these dusts. Due to the prevailing wind direction, especially in the south and southeast of the district, dust accumulation on the upper surface of the plant leaves is higher than in other directions, and dust accumulation decreases as it moves away from the factory.

Falling of leaves, dimensional shrinkage, and chlorosis was detected in fruit trees affected by the flue dust of Eskişehir Cement Factory. Again, dwarfing has been observed in plants. Also, there is a serious decrease in resistance to plant pathogens in plants exposed to pollution. The reason for the shrinkage of the leaves can be considered as the



adaptation of the plant to the negative external environment conditions created by cement powders.

As a result, photosynthesis and transpiration in the plant are partially prevented by the effect of cement particles accumulating on the plants. It is of vital importance to keep the stomata open and to provide air circulation between the cells for both oxidative phosphorylation and photosynthesis to occur in plants. Besides, another important condition for photosynthesis is that sunlight can reach the leaves without any obstacle. Microscopic cement powders limit this vital activity. The most obvious and serious consequence of this situation is that it causes a decrease in the growth and yield of plants.

## **WASTE MATERIALS ARISING IN THE CEMENT PRODUCTION PROCESS AND ITS POSSIBLE ENVIRONMENTAL EFFECTS**

Cement production is a process where each stage has separate damage to nature. For example, fossil fuels are used extensively as an energy source from the extraction and transportation of raw materials required for cement production and the cement production process from these materials. One of the important and indispensable stages in cement production is the firing of the cement raw material at 1350-1450 ° C. During this process, fossil fuels are used as fuel in incinerators. The wastes of these fuels are left in the atmosphere. It is known that 5-6% of the carbon dioxide released into the atmosphere with the human effect is generated by cement factories. In the process of firing cement, the use of industrial wastes and waste sludge as alternative fuels has started to be used as the cost is lower than fossil fuels. Although this method seems to be a way to get rid of waste, the burning of this industrial waste in furnaces causes the release of intensive toxic metals into the air [9, 10, 11, 12].

Cement itself is a very serious contaminant due to the toxic chemicals in its structure. For example, cement powders contain elements such as antimony, arsenic, lead, cadmium, chromium, cobalt, copper, manganese, nickel, thallium, tin, vanadium, zinc, beryllium, selenium, tellurium and mercury, some of which are highly toxic. Considering this situation, the severity of the pollution caused by cement dust spray, which we observe intensively in the environment within the borders of Çukurhisar district of Eskişehir province, becomes clearer [9, 10, 11].

Defined as one of the major environmental risk factors, air pollution is cited as the cause of many premature deaths every year. It is observed that many diseases, especially respiratory system diseases due to air pollution, occur at higher frequencies in populations where people are exposed to pollutants [3, 13].

Cement factories are one of the most important sources of atmospheric pollution in their regions. Sulfur oxides, nitrogen oxide, carbon monoxide, carbon dioxide, dust and particulate matter, volatile organic compounds (VOCs), dioxin, furan, methane and heavy metals are the main pollutants released into the atmosphere from cement factories. Using industrial wastes instead of fossil fuels in cement kilns is the most important source of heavy metals, dioxin and furan. Heavy metals are metals or semi-metals that enter the body by inhalation and mouth and have toxic effects even in small amounts. Since heavy metals cannot be removed from the body, side effects occur when they exceed the toxic limit by biomagnification. The main clinical symptoms that occur as a result of long-term exposure to heavy metals are depression, headache, skin, digestive, hormone, prostate, cardiovascular, troitis and immune system problems and serious diseases such as cancer,

Alzheimer's, Parkinson's, ALS, MS. It is a fact that today everyone knows that aromatic compounds such as dioxin and furans are toxic and carcinogenic [14, 15].

In the areas where cement factories are located, the most important factor responsible for the transport of heavy metals to the lungs by respiration is the particles released into the atmosphere. Particle size is directly related to the amount of heavy metal taken into the body. Therefore, the amount of diseases related to toxic by-products is visibly higher in areas where toxic particles are released into the atmosphere and where factories do not have proper particle filters in their chimneys [13].

One of the most important factors affecting the chemical properties of the soil of a region is the pollutant elements in that region. Cement factories discharge heavy metals into the soil in particulate form, especially in their immediate surroundings. This affects the whole ecosystem in the region due to the physicochemical properties of the soil [16, 17, 18, 19, 20].

Another pollutant released into the atmosphere from cement factories is nitrogen and sulfur oxides. These turn into acidic components in the atmosphere and mix with soil and water with precipitation. Changes in acid rain and soil and water pH directly cause adverse effects on aquatic organisms and vegetation living in these habitats. In soils exposed to acid rain for a long time, productivity decreases with the decrease in biological activity and the change in physicochemical structure [21].



As a result; Cement is one of the building materials that are difficult to produce due to the many toxic substances released during the production process. For this reason, production activities carried out without taking necessary measures cause serious irreversible damage to nature and humanity. The important thing here is to consider the profits and losses of cement production. Turkey is among the countries from producing more cement needs. It is a very sad situation that a building material whose production is so harmful to nature and living beings is chosen as an export product. With this article, it is aimed to raise awareness about the harms of cement on nature and the environment.

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## THE EFFECTS OF IRRIGATION ON CHLOROPHYLL CONTENT OF POMEGRANATE (*Punica granatum* L.) TREES

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**ABSTRACT.** Pomegranate (*Punica granatum* L.) is a characteristic species of the Mediterranean area. It is well adapted to the growth conditions in Turkey and is frequently found growing in wild or semi wild conditions. Pomegranates can tolerate long duration of drought once the plant is established but regular irrigation is mandatory in commercial production. In this research the effect of different irrigation on content of leaf pigments of four different pomegranate cultivars was determined. Three different water amounts were applied by drip irrigation system. First leaf samples were taken a week before starting the irrigation treatments and continued until the end of the harvest season with four weeks interval and they were taken five times. Results showed that all chlorophyll components (a, b and total) were affected by the water and they were higher in the irrigated trees than non irrigated trees.

**Keywords:** Pomegranate, drought stress, irrigation, chlorophyll a, chlorophyll b

### INTRODUCTION

The pomegranate (*Punica granatum* L.) is one of the oldest known edible fruits. Its history dates to very ancient times [5, 13]. This fruit tree is one of the species mentioned in the holy books and is often associated to fertility [16].

In Turkey, the number of pomegranate orchards started to increase rapidly since the last decade of the 1900s. Pomegranate grows mainly in tropics and subtropics climates; also in warm temperature zone. It adapts to all kinds of soils and climate and tolerant to salinity and drought conditions. Besides, it is tolerant to iron chlorosis and limestone in the soil [8].

Regular irrigation and nutrition are required for obtaining high quality and quantity. Fruit cracking and splitting is an important problem in the pomegranate cultivation. Fruit splitting occur because of irregular irrigation practices or excessive rain during the maturation period and it is accepted a major fruit defect [6, 7, 19]. Also, it is due to extreme fluctuations in day and night temperatures, soil moisture and relative humidity, dry wind, heavy rain or irrigation following a dry condition in developed fruits [8]. Especially excessive nitrogenous fertilization increases fruit cracking [10]. Trees take up the plant nutrients from the soil by their roots as dissolved in water. For this reason, even if there are enough nutrients around its root, because of inadequate water supply it is not possible to take up the nutrient by the plants. It is necessary to provide irrigation without causing hydric stress in the plants for optimum growth and quality fruit production. Optimal irrigation duration, frequency and amount depend on some factors. These factors are soil type, temperature and relative humidity, tree size, age, rootstock, specie, variety, growing phase of tree and evaporation [10]. The applied water affects vegetative and generative growth, yield and fruit quality of trees. The harmful effect of water stress on plant growth is attributed to the decreased osmotic potential of the growing medium and nutrient ions shortage.

The chlorophylls are virtually essential pigments for the photosynthesis. Solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments. Therefore low chlorophyll concentrations can directly limit photosynthetic potential and hereby primary production [9]. Chlorophyll amount gives an indirect estimation of the nutrient status because much of leaf nitrogen is incorporated in chlorophyll [18]. In addition, leaf chlorophyll amount is closely related to plant stress [17].

The goal of this study is to examine effects of different irrigation amounts on leaf chlorophyll contents of pomegranate trees.

## MATERIALS AND METHODS

This study was done with ‘İzmir 1’, ‘İzmir 2’, ‘İzmir 1499’ and ‘İzmir 1513’ pomegranate varieties in Bornova County of İzmir province, Turkey.

Three different irrigation water amounts ( $S_0$  = no irrigation,  $S_1$  = 50% and  $S_2$  = 100% of the water quantity evaporating from class A pan) were applied by drip irrigation system. Evaporation was measured with class A-pan. The crop pan coefficients ( $K_{cp}$ ) for  $I_1$  and  $I_2$  irrigation treatment was taken 0.50 and 1.00, respectively. Irrigations were applied with 7-day interval. The applied quantity of irrigation water was calculated as given below formula.

$$I = E_{pan} \times K_{cp} \times A \times P$$

Where:

I: The quantity of irrigation water (liters);

$E_{pan}$ : The quantity of evaporation in class A pan (mm);

$K_{cp}$ : Crop pan coefficient;

A: Tree area (18 m<sup>2</sup> per tree);

P: Wetted area percentage (30%).

Irrigation was started 16<sup>th</sup> June and finished 8<sup>th</sup> September. Applied water quantities are given in Table 1.

First leaf samples were taken a week before beginning irrigation and continued until harvest season with four weeks interval.

Analysis of Chlorophyll a (Chl- a), Chlorophyll b (Chl- b), and total chlorophyll (Total chl) in leaf was performed by Arnon [2]. Calculation was done following formulas.

$$\text{Chl a} = [(0.0127 * A_{663.0}) - (0.00269 * A_{645})] * 100$$

$$\text{Chl b} = [(0.0229 * A_{645.0}) - (0.00468 * A_{663})] * 100$$

$$\text{Total chl} = [(0.0202 * A_{645.0}) - (0.00802 * A_{663})] * 100$$

The experiment was designed in a randomized complete block design with four replications. In each replication one tree was used. Statistical analyses of all data were performed with SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA). Differences between the means were compared by Duncan test at a significance level of  $P < 0.05$ .

**Table 1.** Applied amount of irrigation water (L/tree)

Irrigation No	Water quantity <sup>1</sup>		Irrigation No	Water quantity	
	S <sub>1</sub>	S <sub>2</sub>		S <sub>1</sub>	S <sub>2</sub>
1	140	281	8	173	346
2	105	210	9	174	348
3	165	329	10	158	317
4	140	280	11.	188	377
5	153	306	12	127	254
6	169	337	13	115	230
7	176	352	Total	1984	3968

<sup>1</sup>S<sub>1</sub> and S<sub>2</sub>: = 50 and 100% of the water quantity evaporating from class A pan, respectively.

## RESULTS

Leaf average chl-a, chl-b and total chlorophyll were affected depend on irrigation water amount and varieties. These differences were statistically significant. Average leaf chl-a, chl-b and total chlorophyll of irrigated (50% or 100%) trees were higher than non-irrigated trees. All average leaf chlorophyll parameters of İzmir 2 pomegranate variety's trees were higher than other trees of varieties. All the other varieties were statistically similar and formed in the second group. The leaf chlorophyll content was the lowest at the beginning of growing season. The highest values were found in the end of season.

Under non-irrigation treatment, Chl-a content of İzmir 1, İzmir 2, İzmir 1499 and İzmir 1513 varieties were 5.350, 3.882, 3.610 and 3.731 mg/L at the early of season, respectively. It was increased during the summer period and reached 6.701, 7.925, 6.466 and 8.092 mg/L in the October. Especially, it increased too much in the leaf of İzmir 1513. In I<sub>1</sub> treatments, Chl-a content of İzmir 1, 2, 1499 and 1513 varieties were 4.629, 3.393, 3.537 and 4.310 mg/L at the beginning of season, respectively and these values increased during the season and reached 9.172, 8.659, 8.987 and 7.403 mg/L in the October. Similar changes occurred at full (I<sub>2</sub>) irrigation treatment.

Chl-b content showed similar trend to chl-a. It was lower in the beginning of the irrigation season than end of season. The highest chl-b contents were obtained from 100% (I<sub>2</sub>) treatments. But there were not differences between I<sub>1</sub> and I<sub>2</sub> treatments statistically. Chl-b content of İzmir 1, 2, 1499 and 1513 varieties were found as 1.526, 1.105, 1.018 and 0.977 mg/L at the beginning of season, respectively in the non-irrigation treatment. It was increased during the summer period (except İzmir 1) and reached 1.175, 1.557, 1.481 and 1.375 mg/L in the October.

In I<sub>1</sub> treatments, leaf chl-b contents were found as 1.309, 0.942, 1.104 and 1.223 mg/L of İzmir 1, İzmir 2, İzmir 1499 and İzmir 1513 varieties at the beginning of season, and these values increased during the season and reached 2.623, 2.474, 2.753 and 2.131 mg/L in the October. It increased too much in the leaf of İzmir 2 variety. Similar changes found at 100% (S<sub>2</sub>) irrigation.

**Table 2.** Leaf chlorophyll –a content of pomegranate varieties (mg/L)

Irrigation	Variety	Sampling Date
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		05/06	03/07	31/07	28/08	05/10	Average
0 %	İzmir 1	5,350	5,718	6,821	5,229	6,701	<b>5,964</b>
	İzmir 2	3,882	7,700	6,563	7,366	7,925	<b>6,687</b>
	İzmir 1499	3,61	3,487	3,580	5,629	6,466	<b>4,554</b>
	İzmir 1513	3,731	5,817	5,322	5,927	8,092	<b>5,778</b>
	<b>Mean</b>	<b>4,143</b>	<b>5,681</b>	<b>5,5715</b>	<b>6,038</b>	<b>7,296</b>	<b>5,746</b>
50 %	İzmir 1	4,629	6,657	5,887	5,546	9,172	<b>6,378</b>
	İzmir 2	3,393	8,773	6,149	7,77	8,659	<b>6,949</b>
	İzmir 1499	3,537	7,361	6,049	6,985	8,987	<b>6,584</b>
	İzmir 1513	4,31	7,531	6,453	7,514	7,403	<b>6,642</b>
	<b>Mean</b>	<b>3,967</b>	<b>7,581</b>	<b>6,135</b>	<b>6,954</b>	<b>8,555</b>	<b>6,638</b>
100 %	İzmir 1	3,793	8,7	5,497	7,607	-	<b>6,399</b>
	İzmir 2	5,049	9,695	7,668	7,913	-	<b>7,581</b>
	İzmir 1499	4,433	7,465	5,192	7,549	-	<b>6,160</b>
	İzmir 1513	4,892	6,881	5,62	7,616	-	<b>6,252</b>
	<b>Mean</b>	<b>4,542</b>	<b>8,185</b>	<b>5,994</b>	<b>7,671</b>	-	<b>6,598</b>
LSD <sub>0,05 irr</sub>	0,376**		LSD <sub>0,05 irr</sub>		0,586**		
LSD <sub>0,05 vart</sub>	0,530**		LSD <sub>0,05 vart</sub>		1,051**		
LSD <sub>0,05 irr*var</sub>	0,903*		LSD <sub>0,05 irr*var</sub>		1,186*		
LSD <sub>0,05 irr*var *date</sub>	ns						
		*p<0,05	**p<0,01	ns non-significant			

**Table 3.** Leaf chlorophyll –b content of pomegranate varieties (mg/L)

Irrigation	Variety	Sampling Date					Average
		05/06	03/07	31/07	28/08	05/10	
0 %	İzmir 1	1,526	1,664	2,223	1,463	1,863	<b>1,748</b>
	İzmir 2	1,105	2,254	1,927	2,173	2,367	<b>1,965</b>
	İzmir 1499	1,018	1,911	1,118	1,626	2,062	<b>1,547</b>
	İzmir 1513	0,977	1,762	1,534	1,733	2,286	<b>1,658</b>
	<b>Mean</b>	<b>1,157</b>	<b>1,898</b>	<b>1,7005</b>	<b>1,745</b>	<b>2,1445</b>	<b>1,730</b>
50 %	İzmir 1	1,309	2,028	1,708	1,608	2,623	<b>1,856</b>
	İzmir 2	0,942	2,709	1,796	2,329	2,474	<b>2,050</b>
	İzmir 1499	1,104	2,289	1,886	2,061	2,753	<b>2,019</b>
	İzmir 1513	1,223	2,272	1,876	2,169	2,131	<b>1,934</b>
	<b>Mean</b>	<b>1,1445</b>	<b>2,3245</b>	<b>1,8165</b>	<b>2,04175</b>	<b>2,495</b>	<b>1,965</b>
100 %	İzmir 1	1,175	2,624	1,649	2,142	-	<b>1,898</b>
	İzmir 2	1,557	2,970	2,256	2,365	-	<b>2,287</b>
	İzmir 1499	1,481	2,390	1,555	2,370	-	<b>1,949</b>
	İzmir 1513	1,375	2,144	1,592	2,327	-	<b>1,860</b>
	<b>Mean</b>	<b>1,397</b>	<b>2,532</b>	<b>1,763</b>	<b>2,301</b>	-	<b>1,998</b>
LSD <sub>0,05 irr</sub>	0,153*		LSD <sub>0,05 irr</sub>		0,100**		
LSD <sub>0,05 vart</sub>	0,171**		LSD <sub>0,05 vart</sub>		0,338**		
LSD <sub>0,05 irr*var</sub>	ns		LSD <sub>0,05 irr*var</sub>		ns		
LSD <sub>0,05 irr*var *date</sub>	ns						
		*p<0,05	**p<0,01	ns non-significant			

**Table 4.** Leaf total chlorophyll content of pomegranate varieties (mg/L)

Irrigation	Variety	Sampling Date					Average
		05/06	03/07	31/07	28/08	05/10	
0 %	İzmir 1	6,877	7,382	9,044	6,693	8,564	<b>7,712</b>
	İzmir 2	4,987	9,954	8,490	9,539	10,293	<b>8,653</b>
	İzmir 1499	4,628	5,398	4,698	7,255	8,528	<b>6,101</b>
	İzmir 1513	4,708	7,580	6,856	7,660	10,378	<b>7,436</b>
	<b>Mean</b>	<b>5,300</b>	<b>7,579</b>	<b>7,272</b>	<b>7,78675</b>	<b>9,441</b>	<b>7,476</b>
50 %	İzmir 1	5,938	8,685	7,594	7,154	11,795	<b>8,233</b>
	İzmir 2	4,335	11,483	7,945	10,100	11,133	<b>8,999</b>
	İzmir 1499	4,641	9,651	7,935	9,046	11,739	<b>8,602</b>
	İzmir 1513	5,533	9,803	8,329	9,683	9,534	<b>8,576</b>
	<b>Mean</b>	<b>5,112</b>	<b>9,905</b>	<b>7,951</b>	<b>8,996</b>	<b>11,050</b>	<b>8,603</b>
100 %	İzmir 1	4,968	11,324	7,146	9,749	-	<b>8,297</b>
	İzmir 2	6,607	12,664	9,924	10,278	-	<b>9,868</b>
	İzmir 1499	5,914	9,856	6,747	9,919	-	<b>8,109</b>
	İzmir 1513	6,267	9,026	7,212	9,942	-	<b>8,112</b>
	<b>Mean</b>	<b>5,939</b>	<b>10,718</b>	<b>7,757</b>	<b>9,972</b>	-	<b>8,596</b>
LSD <sub>0,05 irr</sub>		0,517**		LSD <sub>0,05 irr</sub>		0,762**	
LSD <sub>0,05 vart</sub>		0,690**		LSD <sub>0,05 vart</sub>		1,367**	
LSD <sub>0,05 irr*var</sub>		ns		LSD <sub>0,05 irr*var</sub>		ns	
LSD <sub>0,05 irr*var *date</sub>		ns					

\*p&lt;0,05 \*\*p&lt;0,01 ns non-significant

## DISCUSSION AND CONCLUSION

Under drought conditions, plants reduce water loss by stomatal closure which results both in a reduction of transpiration and the inhibition of photosynthesis coupled with reducing CO<sub>2</sub> uptake. The amount of available metabolites required for the development of plants decreases because of decreasing photosynthesis. The photosynthetic activity of the crops is one of the important factors influencing the yield that can be observed by the measurement of physiological traits such as leaf chlorophyll content, net photosynthetic rate, and stomatal conductance. In this study, the effect of irrigation on leaf chlorophyll content was investigated. The chlorophyll content of a leaf is the indicator of a plant's physiological condition. A large part of the total chlorophyll was determined to be Chl a. *Chlorophyll a*, is the primary photosynthetic pigment in green plants for the transfer of light energy to a chemical acceptor [11] and chlorophyll a was more resistant to dehydration than other chlorophyll components [14]. In higher plant leaves, chlorophyll content changes along the different stages of plant development. Thus, all chlorophyll parameters changed during the season. Chl-a, chl-b and total chlorophyll of all varieties' were low at early June. They started to increase throughout development periods and reached the highest level at the harvest period. Chlorophyll is the basic unit of plant energy systems during the photosynthesis event [4]. Besides light quality, chlorophyll production and activity are influenced by nutrition and chemical metabolites produced in the plant system. Therefore, chlorophyll amounts increased as the leaves got their full size [9]. Water deficiency has an indirect effect on photosynthesis. Total chlorophyll content was higher in the irrigated trees than non-irrigated trees, in this study.

As the nutrients uptake from roots increased by watered [10], the amount of leaf chlorophyll of the irrigated trees increased. Increased uptake of Fe in these treatments also plays important role in chlorophyll formation [15]. Basiouny reported that chlorophyll content of peach leaves from irrigated trees was higher than that of tree







leaves from nonirrigated trees [3]. It was found that chlorophyll a, b and total reduced under drought stress conditions in the pear [12] and fig [1]. Furthermore, leaf chlorophyll content is closely related to plant stress and senescence. The results of this experiment showed that irrigation had effect on leaf chlorophyll content of pomegranate trees.

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## EFFECT OF ROOTSTOCKS ON POLLEN PRODUCTION, VIABILITY AND GERMINATION IN GRAFTED TETRAPLOID AND DIPLOID WATERMELON

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**ABSTRACT.** The two years study was conducted to investigate the effects of graft combinations on pollen production, pollen germination, pollen viability and normal pollen development in grafted tetraploid and diploid watermelons. Two scions (tetraploid watermelon line ‘ST 101’ and diploid watermelon line ‘WL 92’) were grafted onto three rootstocks (*Cucurbita* interspecific hybrid ‘Nun-9075’; *Lagenaria siceraria* ‘Argentario’ and citron watermelon ‘PI 296341’) and non-grafted ST 101 and WL 92 were used as control. Cultivation was conducted in spring seasons of 2016 and 2017 at horticultural experimental fields; flower and pollen analysis were done at the cytological laboratory of the Department of Horticulture, Cukurova University in Turkey. All graft combinations were grown and received the same management practices and flowers for analysis were collected randomly from every plot. There was a significant difference in pollen germination among graft combinations. In 2016, the highest pollen germination percentages were 94.13% and 89.85% obtained in non-grafted ST 101 and PI 269342/WL 92. In 2017, the highest pollen germination percentage value (79.75%) was obtained in Nun-9075/ST 101. No significant difference was found among graft combinations in percentage of pollen viability and normal pollen development in both years. Although no significant difference was observed in 2016 in number of pollen, the highest value was recorded in Argentario/WL 92 (509719.61 pollen per staminate flower) and the lowest value was obtained in Nun-9075/ST 101 (279494.76 pollen per staminate flower). In 2017, Nun-9075/WL 92 graft combination resulted in the highest number (260682.61 pollen per staminate flower) of pollen compared to other graft combinations. WL 92 diploid scion resulted in a higher number of pollen compared to tetraploid ST 101 scion. This study indicates that grafting increases production and development of normal pollen and improve pollen germination and viability.

**Keywords:** Germination percentage<sup>1</sup>, graft combination<sup>2</sup>, pollen<sup>3</sup>, pollen viability<sup>4</sup>, rootstock<sup>5</sup>

### INTRODUCTION

Watermelon production increases day by day in both open field cultivation and in protected cultivation such as greenhouses and high tunnels. Production under protected cultivation is carried out two to three times a year [9]. The production during winter influences anther dehiscence which results in poor fruit set due to low temperature and high humidity [1].

Pollen is very important for fertilization and seed formation especially in triploid seed production due to low quality of triploid seeds formed. Good pollen viability and germination are useful in explaining the lack of fertility that determines fruit set and total fruit yield. In the production of triploid fruits, tetraploid female flowers must be pollinated by pollen from diploid plants this is because triploid watermelon plants do not produce sufficient viable pollen to pollinate themselves [11]. Besides triploid watermelon, the quality and quantity of pollen are very important in the production of diploid and tetraploid fruits.

Recently different techniques have been used in watermelon production where some of them have caused low pollen production. Mapping populations developed from crosses between cultivars have been observed to cause diminishing pollen viability, high levels of marker segregation and low fruit set [14, 15]. Also, the performance of pollenizers is species and or variety dependent; some watermelon varieties produce more viable pollen than others. Fiacchino and Walters [4] showed that ‘Crimson Sweet’ was more effective than ‘Fiesta’. Freeman et al. [5] observed triploid plants pollinated by ‘Companion’ variety yielded less than those pollinated by ‘Jenny’, ‘Patron’, ‘SP-1’ and ‘Sidekick’. The pollen quality and quantity in watermelon genotypes are very important in breeding programs because the superior genotypes can be used to increase the success of crosses [6]. Therefore, this study aimed at determining the best rootstock with the highest pollen viability, pollen germination and normal pollen production capacity so that they can be used in diploid crosses and triploid watermelon production.

## MATERIALS AND METHODS

A two-year study was conducted in the open field of the Department of Horticulture of Cukurova University in Adana, Turkey during 2016 and 2017.

### *Plant material*

Three rootstocks, ‘Nun-9075’ the *Cucurbita* interspecific hybrid rootstock (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) which is widely used for grafting watermelons in Turkey, the bottle gourd ‘Argentario’ (*Lagenaria siceraria* (Mol.) Standl., and citron watermelon ‘PI 296341’ (*Citrullus lanatus* var. *citroides*), which have high grafting combination ability and high resistance to *Fusarium* were used as rootstocks. Seeds of ‘PI 296341’ were obtained from the watermelon genetic resources collection of the Department of Horticulture, Cukurova University and other seeds were obtained from the Antalya Tarim Company.

Two watermelon lines, *Citrullus lanatus* var. *lanatus*, were used as scions, the tetraploid ‘ST 101’ was used as the female parent and the diploid ‘WL 92’ was used as male parent. All rootstocks were grafted with ST 101 and WL 92 scions, therefore, the following graft combinations were obtained (Table 1).

**Table 1.** Graft combinations used in the experiment

<b>ST 101 (Tetraploid female parent scion)</b>	<b>WL 92 (Diploid male parent scion)</b>
Argentario/ST 101	Argentario/WL 92
Nun-9075/ST 101	Nun-9075/WL 92
PI 296341/ST 101	PI 296341/WL 92
Control - ST 101 (Non grafted)	Control - WL 92 (Non grafted)

### *Seed sowing, Grafting and Transplanting*

Seed sowing, grafting and management practices of the grafted seedlings were conducted at Antalya Tarim Company in Antalya, Turkey. Seeds were sown on 26<sup>th</sup> January 2016 and grafting was done on 23<sup>rd</sup> February 2016. Seed sowing and grafting practices for year 2017 were performed on 03<sup>rd</sup> February 2017 and 22<sup>nd</sup> February 2017 respectively. Seedlings were transplanted to the open field in Adana on 30<sup>th</sup> March 2016

and on 07<sup>th</sup> April 2017. Grafted and control (non-grafted) plants were transplanted at a spacing of 3 m × 0.75 m. In every plot, 16 plants grafted with tetraploid ST 101 female parent scion followed by 4 plants grafted with diploid WL 92 male parent scion were transplanted with four replications in a Latin Square design. Soon after transplanting plants were covered with low plastic tunnels to protect from cold weather and heavy rain; wide open holes on the plastic tunnels were made to allow air exchange and the tunnels were completely removed after 3 weeks. Plants were irrigated with drip irrigation once after every two days for the first two months. Fertilizer was applied through a drip irrigation system at a rate of 15:15:20 kg per 1000 m<sup>2</sup> with pure nutrients as N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O. Monoammonium Phosphate (MAP) and Potassium Nitrate (KNO<sub>3</sub>) at a ratio of 18:18:18 and 20:20:20 respectively were used. Weeds, insect pests and diseases were regularly controlled whenever the signs of presence were observed.

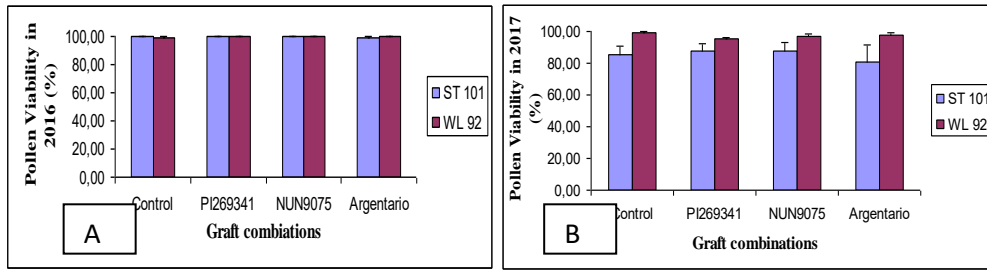
To determine pollen production capacity, in vitro pollen viability and pollen germination of the rootstocks, 10 flowers were used in every replication. Five mature flowers before opening when pale yellow color started to develop (approximately one day before anthesis) were selected and closed by using clips in the afternoon for pollen germination and viability. In the morning, the 5 flowers closed by clips were picked from each replicate and other 5 flowers that were not closed by clips but unopened near to anthesis were picked and sent to the laboratory for anther counting and determination of pollen production capacity. In vitro pollen viability, pollen germination and normal pollen production assays were conducted according to Norton [12], Eti and Stosser [3], Ozkan and Eti, [13].

### ***Statistical Analysis***

Data were subjected to analysis of variance (ANOVA) to determine the effects of different rootstocks in two different years. A significant difference among means was calculated by using Tukey Multiple Range Test at a significance level of  $P \leq 0.05$ . Statistical analysis was performed using JMP (v8.00, SAS Institute Inc., NC 27513-2414, USA) statistics software.

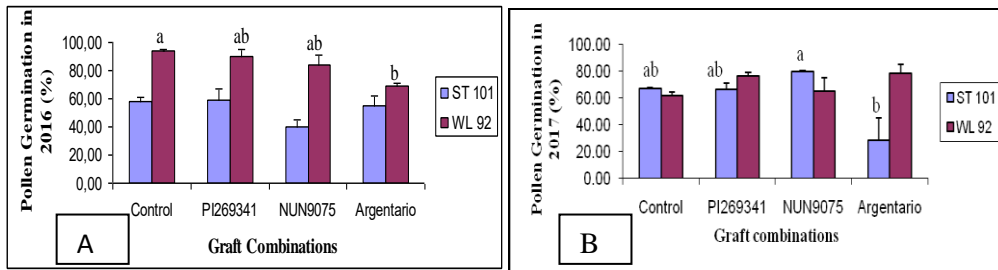
## **RESULTS AND DISCUSSION**

Results of pollen viability percentage of the grafted and non grafted watermelon for both diploid (WL 92) and tetraploid (ST 101) scions are presented in Fig.1. There was no significant difference between rootstocks and scions in pollen viability for 2016 and 2017 growing seasons. The percentage pollen viability for 2016 ranged between 99.16 - 100.00% for WL 92 and 99.37 - 100.00% for ST 101. In 2017 the pollen was somehow lower compared to that of 2016, also WL 92 scions resulted in a higher pollen viability percentage value compared to ST 101. The pollen viability percentage value for ST 101 ranged from 81.00 - 87.40% obtained in Argentario and Nun-9075 respectively while that of WL 92 ranged between 95.53 - 99.16%.



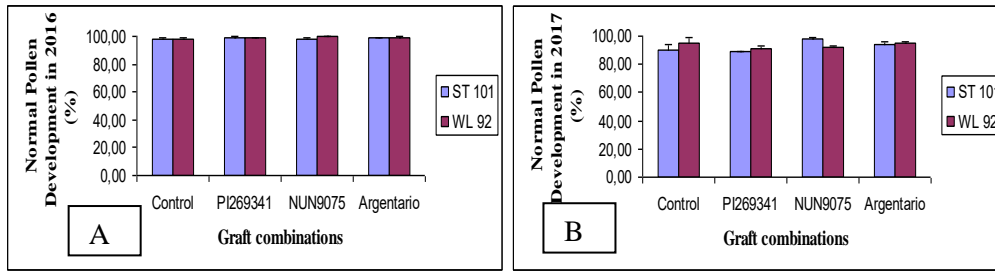
**Fig. 1.** Pollen viability percentage of 3 different rootstocks and control for 2016 (A) and 2017 (B) growing seasons

Significant differences were observed between rootstocks and also between scions in pollen germination percentage (Fig. 2). In 2016 (Fig. 2 A), significant difference was only observed in WL 92 scion with the highest percentage obtained in control (96.13%) and in grafted plants the highest value was obtained in PI 269341 rootstock (89.85%). No significant difference in pollen germination percentage was observed between graft combinations in ST 101, the values ranged between 39.78% - 58.80% obtained in Nun-9075 and PI 269341 rootstocks respectively. In 2017 (Fig. 2 B), the highest pollen germination percentage was obtained in Nun-9075/ST 101 (79.75%) and in Argentario/WL 92 (78.70%). However, Argentario/ST 101 resulted in the lowest pollen germination value (28.60%). The lower pollen germination percentage in ST 101 may be due to the few and immature flowers. The flowers in ST 101 were very few in such away necessitated picking of those available which also were immature. Generally the flower production and pollen content in ST 101 group were very poor compared to WL 92.



**Fig. 2.** Pollen germination percentage of 3 different rootstocks and control for 2016 (A) and 2017 (B) growing seasons

There was no significant difference between rootstocks in normal pollen development in both scions and years (Fig. 3). However, In ST 101 scion, PI 269341 rootstock resulted in the highest average normal pollen percentage value of 99.34% and the lowest value was obtained in control (97.84%). In WL 92 scion, the highest normal pollen percentage value was obtained in Nun-9075 (99.51%) and the lowest average value was obtained in control (98.22%). The percentage pollen viability and pollen germination obtained in this current study are higher compared to those reported by Kombo and Sari [8].



**Fig. 3.** Normal pollen development of 3 different rootstocks and control for 2016 (A) and 2017 (B) growing seasons

There was a significant difference among rootstocks in the number of pollen per anther in both years and the number of pollen per staminate flower in the 2017 season (Table 2). Argentario/WL 92 resulted in the highest average number of pollen per anther in the first year (169906.54), while Nun-9075/WL 92 resulted in highest number of pollen per anther (82863.59) in the second year. The highest number of pollen per staminate flower was found in the first whereby Argentario/WL obtained 509719.61 number of pollen per staminate flower, two times higher than the highest number of pollen found in the second year in Nun-9075/WL (260682.61).

**Table 2.** Pollen production in different graft combinations

Graft Combination	Number of pollen per anther		Number of pollen per flower	
	2016	2017	2016	2017
Control (ST 101)	90885.857 b	58034.59 ab	326077.22	186320.88 ab
PI 269341/ST 101	112328.37 ab	39194.77 b	368180.60	125423.27 b
Nun-9075/ST 101	91391.95 b	58060.86 ab	279494.76	193496.37 ab
Argentario/ST 101	132431.54 ab	40973.23 b	425512.85	133485.62 ab
Control (WL 92)	101525.87 ab	76834.74 ab	311744.15	239183.02 ab
PI 269341/WL 92	143330.65 ab	76256.38 ab	429991.94	233808.12 ab
Nun-9075/WL 92	122664.29 ab	82863.59 a	402221.63	260682.61 a
Argentario/WL 92	169906.54 a	72027.01 ab	509719.61	221230.85 ab
Prob>f	0.014	0.01	0.06	0.02
LSD 5%	68951.89	40283.77	ns	131687.47

Few flowers and low number of pollen per staminate flower in the second year may be due to the stress caused by heavy rainfall at the beginning of the spring season. Several studies have shown that stress adversely affects pollen production per flower [16]. Virus infection [7], amount and timing of leaf damage [2] and soil fertility [10] affect staminate flower production and pollen production per flower. Thus, less number of flowers and low number of pollen found in this current study also reveals that stress caused by rainfall at flowering affects pollen production.

## CONCLUSION

This study indicates that grafting highly increases the production and development of normal pollen and improves pollen germination and viability. ‘Nun 9075’ and ‘Argentario’ are the best rootstocks that provide more number of pollen per staminate flower with higher percentage viability, hence, these rootstocks can directly increase fruit yield and quality in triploid watermelon.

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