ACCEPTED ARTICLE Investigation of the Relationship between Plant Nutrient Elements and Yellow Color Formation in the Leaf Veins of Watermelon Veysel Aras^{1*}, Ayhan Aydin¹, and Süleyman Yalcin¹ ¹Alata Horticultural Research Institute, Mersin, Türkiye *Corresponding author; e-mail: varas2001@yahoo.com

11 Abstract

1 2

3 4

5 6

7 8

9 10

12 While there is no yellowing in the leaf veins of some watermelons during the seedling stage, veins start to turn yellow after the plant starts to grow, and veins become more yellow as the 13 plant grows. The aim of this study is to investigate whether this yellowing of leaf veins is 14 related to plant nutrients. This study was carried out at the Alata Horticultural Research 15 Institute in Mersin, Turkey, during the spring and summer growing seasons of 2016 and 2018. 16 The S24 line with yellow veins and the Crimson Sweet variety were used as controls. Samples 17 were taken from the leaves below and above the female flower during the female flower 18 period and from the leaves below and above the fruit when the fruits reached the size of a 19 grapefruit. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron 20 (Fe), zinc (Zn), and manganese (Mn) elements were examined in leaf analysis. When the 21 22 places where the leaf samples were taken and the years were evaluated together, there was not a variety that came to the forefront in terms of nitrogen, phosphorus, potassium, calcium, 23 magnesium, and zinc. However, in general, the iron and manganese contents of the S24 line 24 were found to be higher than the control. In line with these results, it is not possible to say that 25 there is a relationship between the yellowing of leaf veins and plant nutrients. 26

27 Key words Watermelon, yellow color leaf veins, plant nutrient elements.

28 29

1. Introduction

A vegetable with a global production of 101.6 million tons, watermelon is very significant
economically. China (60,1 million tons) is the world's greatest producer, followed by Türkiye
(3.5 million tons), India (2.8 million tons), Iran (2.7 million tons), Algeria (2.3 million tons),
Brasil (2.2 million tons), and other nations (FAOSTAT, 2020).

In some areas of Türkiye, watermelons have been farmed extensively for many years. A reduction in yield brought on by consecutive cropping and soil-borne infections, particularly fusarium, are one of the most important issues with watermelon cultivation. It is advised that watermelon not be produced in the fusarium contaminated field for at least five years in the

battle against fusarium (Messiaen, 1974). However, grafting weaker varieties onto stronger 38 rootstocks might aid in the control of some soil-borne diseases and enhance production and 39 quality (Lee, 1994; Oda, 1995; Yetisir et al., 2003). One of the goals of grafting in 40 watermelon was to promote nutritional intake (Ruiz et al., 1997; Pulgar et al., 2000). Organic 41 matter content, soil moisture, CaCO₃ levels, pH and biological properties of the soil affect 42 plant nutrient uptake by plants (Korkmaz and Saltalı, 2012; Marschner, 2012). Greater than 43 30% of the agricultural lands in the world is made up of high pH soils (Chen and Barak, 44 1982). The majority of Turkish soils have low levels of organic matter and relatively high 45 46 CaCO₃ content, which results in soils with a high pH (Erdal et al., 2006). Over 63% of agricultural areas in Türkiye have a pH level higher than 7.5, and 59% of soils have more than 47 5% CaCO₃ (Eyuboglu, 1999). Yellowing on the leaves can sometimes be due to plant 48 nutrients (Turan and Horuz, 2012; Marschner, 2012) and sometimes genetic factors (Guner 49 50 and Wehner, 2004). According to Warid and Abd-El-Hafez (1976), the yellow leaf (Yl) gene produces yellow leaves and is only marginally dominant over green leaves (Guner and 51 52 Wehner, 2004). The first few nodes have light green cotyledons and leaves due to a delay in the formation of green leaves, but following leaves have a more usual green color. Even when 53 the genotype is dgdg, the inhibitor of delayed green leaf (*i*-dg) restores the usual green color 54 of the leaves (Rhodes, 1986). When plants are cultivated under short day environment, the 55 juvenile albino *ja* gene results in decreased chlorophyll in seedling tissues, as well as in leaf 56 edges and fruit rind (Zhang et al., 1996). Zhu et al. (2022) reported in their study to map and 57 functionally verify the leaf yellowing genes in watermelon throughout the entire growth 58 period that leaf yellowing is controlled by a single recessive gene. Xu et al. (2023), in the 59 ethylmethanesulfonate mutagenesis population of the "703" watermelon variety, a 60 chlorophyll-deficient mutant with yellow leaf (Y12) color was identified, and the chlorophyll 61 a, chlorophyll b and carotenoid contents in Y12 leaves were lower than those in wild type 62 (WT) leaves, and the chloroplasts in the leaves were identified. They reported that the 63 ultrastructure revealed the disintegration of chloroplasts in Yl2. Although it is stated that 64 some virus diseases can cause yellow leaf spots on watermelon (Venkataravanappa et al., 65 2020; Jailani et al., 2022; Iriarte et al., 2023), no virus disease that causes yellow veins as in 66 our study has been found. While there is no yellowing in the leaf veins of some watermelons 67 during the seedling stage, veins start to turn yellow after the plant starts to grow, and veins 68 become more yellow as the plant grows (Figure 1). It is generally thought that yellowing of 69 leaves may reduce plant photosynthesis and growth (Gao et al., 2016; Miao et al., 2016; Zhu 70

et al., 2022). The aim of this study is to investigate whether this yellowing of leaf veins is
related to plant nutrients.

73



Figure 1. The S24 line and Crimson Sweet variety used in the study.

2. Material and Method

74

75

76

- This study was carried out in the greenhouses and laboratories of the Alata Horticultural Research Institute in Mersin, Turkey, during the spring and summer growing seasons of 2016 and 2018. The S24 line with yellow veins leaves found in the Alata Horticultural Research Institute gene pool and the Crimson Sweet variety were used as a control (**Figure 1**). The fruit and leaf veins of S24 used in the experiment are a line with yellow. While there is no abnormality in the seedling stage, but veins start to turn yellow after the plant starts to grow, and veins become more yellow as the plant grows.
- 84 The seedlings were planted with a 1.5 m between and 0.4 m within plant distances in
- greenhouses on March 24 in 2016 at latitude 36°37'51.1"N 34°20'43.6"E and on April 24 in
- 86 2018 at latitude 36°37'49.2"N 34°20'41.6"E. Plants were grown on a single stem by being
- 87 suspended on a rope and having their secondary axes removed.

88 The experiment was established according to a completely randomized block design with 3

replications and 10 plants per replication. Two periods were determined as the leaf removal

- 90 period. The first was the female flower formation period, the second was the period when the
- 91 fruits reached 11-12 cm size.
- 92 Samples were taken from leaves below (4th and 6th leaves apart from the soil level in plants) 93 and above (10th and 12th leaves apart from the soil level in plants) female flower during the 94 female flower period, and from the leaves below (8th and 9th leaves from the soil level in 95 plants) and above (14th and 16th leaves from the soil level in plants) the fruit when the fruits 96 reached the size of a grapefruit.
- 97 Climatic data (monthly minimum, maximum and average temperature and relative humidity 98 values) for both years in Table 1. In 2016, the highest temperatures were seen in May (31.3 99 °C). In terms of relative humidity, the highest value was taken in May (89.3%) and the lowest 100 value was taken in April (37.0%). In 2018, the highest value was taken in May (34.7 °C) and 101 the lowest (6.7 °C) temperatures were seen in March. In terms of relative humidity, the 102 highest (86.4%) relative humidity was recorded in April, and the lowest (39.6%) relative 103 humidity was recorded in May (**Table 1**).

Table 1. Climate values during the trial months in 2016 and 2	018	3.
--	-----	----

Year	Climate Factor		March	April	May
2016		Minimum	5.2	7.1	10.0
	Temperature (°C)	Maximum	25.7	30.9	31.3
		Average	14.9	18.4	20.3
		Minimum	37.9	37.0	46.1
	Relative humidity (%)	Maximum	77.4	78.6	89.3
		Average	62.8	62.7	73.0
2018		Minimum	6.7	7.2	12.7
	Temperature (°C)	Maximum	27.3	31.9	34.7
		Average	15.6	18.1	22.9

	Minimum	47.7	41.7	39.6
Relative humidity (%)	Maximum	85.4	86.4	82.1
-	Average	72.8	69.7	67.3

105

A drip irrigation system was used for the watering and fertilizing. The irrigation system began with the planting of seedlings of all the accessions utilized in this study, and it was continued as needed based on the greenhouse environment. During the experiment, the soil of old greenhouses was analyzed. During both years of the study, soil was loamy, calcareous, optimum salty, weak inorganic substances, alkali, very poor in potassium and optimum phosphorus (**Table 2**).

Table 2. Soil analysis results for both years.								
Analyzag	Limit	Analysis Results (0-30 cm)						
Analyzes	Values	2016	2018					
Texture (100 g/ml)	30-50	38.00 (loamy)	29.00 (loamy)					
Total Calcitic (CaCO ₃ %)	5-15	28.20 (high calcareous)	27.90 (high calcareous)					
Salinity E.C. ds/m (25 °C)	0-0.8	0.73 (optimum)	0.94 (optimum)					
Organic matter (%)	3-4	2.62 (deficient)	2.90 (deficient)					
pH 1: 2,5	6.0-7.0	7.58 (slightly alkaline)	7.49 (slightly alkaline)					
Available potassium (mg/kg)	244-300	69.80 (very low)	122.80 (very low)					
Receivable phosphorus (mg/kg)	20-40	32.30 (optimum)	28.10 (optimum)					

113

123

112

According to the findings of the soil study, fertilizers were applied in the amounts of 60-80 kg 114 K₂O/ha, 80-100 kg P₂O₅/ha, and 140-160 kg N/ha as pure substances (Gucdemir, 2012). Drip 115 116 irrigation was used to apply fertilizers. By separating the watermelon into three sections according to the three stages of growth, nitrogen, phosphorus, and potassium are provided. Up 117 to the first female blossom, the first stage was defined. The second phase spanned the time 118 between the point at which the first female bloom appears and the time when the fruits were 119 120 the size of 7-8 cm. The third stage included the time between the fruits' apple-size development and harvest. Regular pesticide applications were made for disease and pests that 121 were seen, and mechanical weeding and trimming were also carried out. 122

124 2.1. Plant Nutrient Analysis

Nitrogen (N) determination in dried and ground leaf samples was made according to the modified Kjheldahl method (Kacar, 1972). As reported by Kacar (1972), phosphorus (P) was determined colorimetrically according to the vanadomolybdophosphoric yellow color method in the filters obtained by the nitric-perchloric acid mixture (4:1) wet burning method. Potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and zinc (Zn); It was determined with the help of Inductively Coupled Plasma (ICP) in the filters obtained from the leaf samples as a result of wet burning. Results of K, Ca, and Mg are given aspercentage (%) in dry matter; for Fe, Zn and Mn, it is given as mg/kg of dry matter.

133

134 2.2. Statistical analysis

Statistical analyzes were performed using the JMP 7.0 statistical program (v7.00, SAS Institute Inc., NC 27513-2414, USA) with LS Means Differences Student's t test, according to the randomized block trial design, at the P 0.05 significance level. Statistical analyzes were performed after angle transformation was applied to the percentage data.

139 140

3. **Result and Discussion**

The results related to macro-nutrient (N, P, K, Ca and Mg) contents of accessions were given in Table 3. The most needed element by plants is N. About 1-5% of the total plant dry matter is made up of N, which is a crucial component of proteins, nucleic acids, chlorophyll, coenzymes, phytohormones, and secondary metabolites (Hawkesford et al., 2012).

145 In terms of N content, the differences in leaves sampled taken from below the female flower of the S24 and control plants in both years and average of two years were not statistically 146 147 significant. N content of the leaves sampled above the female flower of control plants were higher than S24 plants that of the in both years and average of two years of the experiment. 148 While the N content of the leaf samples taken from the below fruit and the above fruit was 149 found to be insignificant in both control plants and S24 plants in 2016, the content of S24 150 plants was found to be significant compared to the control plants in 2018. While the N content 151 of leaf samples taken from the below fruit was insignificant in terms of the average of two 152 years, the content of S24 plants in leaf samples taken from the above fruit was significant 153 compared to control plants. The N amounts in the samples taken in both years were close to 154 sufficient limit values (2.5-4.5%) (Reuter and Robinson, 1986; Zengin, 2012; Egel et al., 155 2017) and were within these values. N deficiency is one of the most common plant nutrient 156 157 problems for watermelon production. N deficiencies can affect crop yield and quality at any time during the growing season (Doerge et al., 1991). Lack of N causes plants to become 158 159 smaller and have lighter-green leaves than usual. The reduced concentration of chlorophyll is what causes this color effect (Hawkesford et al., 2012). As N is remobilized to younger 160 leaves, chlorosis brought on by N shortage usually starts in the older leaves. Crops lacking in 161 N appear light green or even yellow (Tucker 1984; Taiz and Zeiger, 2006). Since the amount 162 163 of N in leaf samples is mostly at a sufficient level, it is not considered to be a problem caused 164 by N (Reuter and Robinson, 1986; Zengin, 2012; Egel et al., 2017).

In terms of P, no statistical difference was found in the leaf samples taken from the below 165 166 female flower, the above female flower, the below fruit and the above fruit in both S24 and control plants in 2016. In 2018, while the P content of control plants was found to be 167 significant compared to S24 in the leaf samples taken from the below female flower and 168 above the female flower, the P content of S24 plants was higher than that of the control plants 169 in the leaf samples taken from the below fruit and the above fruit. In terms of the average of 170 the two years, the P content of the leaf samples taken from the above female flower was 171 higher in S24 compared to the control plants, while there was no significant difference in 172 terms of P content in the leaf samples taken from the below female flower, the below fruit and 173 the above fruit. P is a significant macronutrient for plants since it is not only a component of 174 175 vital substances (Akhtar et al., 2009; Cetner et al., 2020), but also because it is required for energy transmission and storage during cell metabolism (Jin et al., 2006; Amtmann and Blatt, 176 177 2009). P is an essential plant nutrient for good fruit set and fruit growth, mainly from flowering to final fruit formation. Almost all of the P values in the leaves were found to be 178 insufficient (0.3-0.7%) below the cutoff values (Reuter and Robinson, 1986; Zengin, 2012; 179 Egel et al., 2017). Plants grown in P-deficient soil grow slowly and frequently become scarlet 180 from increased anthocyanin production (Marschner, 1995). Plants with low levels of P 181 frequently have deeper green leaves and stems as well as the development of red and purple 182 hues (Sanchez, 2007). The low amount of P in both years and in different regions of the 183 sample suggests that P will not have an effect on the color difference. 184

In the leaf samples taken from the below female flower in 2016 and 2018 and from the above 185 female flower in 2016, there was no statistical difference between S24 and the control plants 186 in terms of K. In the leaf samples of control plants taken from the above female flower in 187 188 2018 and both from the below fruit and the above fruit in 2016 higher K values were obtained than S24 plants. However, K contents in the leaf samples taken from the below fruit and the 189 190 above fruit were found to be higher at S24 compared to the control in 2018. While there was no statistical difference between S24 and control plants in terms of K in the average of both 191 192 years in terms of leaf samples taken from the below female flower, above the female flower and the above fruit, the leaf samples taken from the below fruit had a higher K content in the 193 194 control plants compared to S24. P is a nutrient needed for watermelon in greater amounts than nitrogen. K is a crucial nutrient for plant growth and development (Schachtman and Liu, 195 1999). It involves important processes in plant cells including osmoregulation, 196 photosynthesis, enzyme activation, the production of carbohydrates, nucleic acids, and 197 198 proteins, as well as the control of water status (Mengel and Kirkby, 2001). K also helps plant

disease control and resistance to heat, cold, and drought (Qian et al., 1997; Fageria, 2009; 199 Rowland et al., 2010). The lowest leaves of the plant usually show the first signs of P 200 deficiency. A drop in plant development rate (resulting in stunted growth) and darker-than-201 normal leaf color are the first signs of K deficiency. As the plant matures, more obvious 202 deficiencies begin to show themselves. The distal end (tip) of the leaf is where symptoms first 203 appear. Normally, the leaf's base is still dark green (Tiwari, 2005). In all of the samples taken 204 in both years, the amount of K was insufficient below the limit values (2.5-3.7%) (Reuter and 205 206 Robinson, 1986; Zengin, 2012; Egel et al., 2017).

207 In terms of Ca, there was no statistical difference between S24 and control in the leaf samples taken from the below female flower in 2018, from the above female flower in 2016, from the 208 209 below fruit in 2018, from the above fruit in 2016 and 2018. Higher Ca values were obtained in leaves the below female flowers than control plants in 2016, while leaf samples taken from 210 211 the above female flower in 2018 and the below fruit in 2016 were higher in S24 than control plants. While the Ca amounts in the leaf samples taken from the above fruit were found to be 212 213 insignificant in terms of the average of both years, Ca was found to be higher than control plants taken from the above female flower, also higher in the leaf samples taken from the 214 female flower and the below fruit in S24 plants. Ca content values obtained from the leaves of 215 both S24 and control plants sampled above the female flower were found to be below the 216 limit values (2.2-5.5%) (Reuter and Robinson, 1986; Zengin, 2012; Egel et al., 2017) range in 217 2016. Living things require Ca to function. It is particularly significant in the physiology of 218 cells because it serves as a signal for a variety of cell processes, including the production of 219 new cell walls in the mitotic spindle during cell division (Taiz and Zeiger, 2006; Shao et al., 220 2008). Ca deficiency results in wilting, deformity, necrosis of fruit and tubers, chlorosis of the 221 222 youngest leaves and shoot apices, weak stems, early flower dehiscence, and failure to set seed in some plants (Bould et al., 1983; Bergmann, 1992; Pilbeam and Morley, 2007). Ca values of 223 224 all samples except these samples (Leaf sampling location, the below female flower, the above female flower, the below fruit and the above fruit) were within the sufficient limit values 225 226 (Reuter and Robinson, 1986; Zengin, 2012; Egel et al., 2017).

In terms of Mg, there was no statistical difference in leaf samples taken from the above female flower and the above fruit in 2016. In the leaf samples taken from the below female flower in 2016, higher results were obtained than the control compared to S24. However, Mg content in leaf samples taken from the below fruit in 2016 and the below female flower, the above female flower, the below fruit, and the above fruit in 2018 was higher in S24 compared to the control. In terms of the average data of two years, the Mg content of the leaf samples

taken from S24 was higher than that of the control plants in the leaf samples taken from above 233 the female flower and the below fruit, while no statistical difference was found in the leaf 234 samples taken from below the female flower and the above fruit. Except for the S24 and 235 control plants the above female flower leaf samples in 2016, the Mg contents of the leaves 236 from different regions were within the sufficient limit values (0.4-1.2%)(Reuter and 237 Robinson, 1986; Zengin, 2012; Egel et al., 2017). In order to develop and reproduce, plants 238 need a lot of Mg, an important macronutrient (Gransee and Führs, 2013; Cakmak and Yazici 239 2010). Mg serves as the core atom in chlorophyll molecules, which in turn establishes a 240 biological foundation for the absorption of solar energy and the subsequent creation of 241 oxygen and carbohydrates (Grzebisz, 2015). Mg also contributes to the conversion and 242 preservation of energy (Amtmann and Blatt, 2009). Growth sluggishness and interveinal 243 chlorosis on older leaves are typical signs of Mg shortage (Cakmak and Yazici, 2010). 244 245 Chlorosis often starts in older leaves and spreads to younger leaves (Cakmak and Kirkby, 2008; Farhat et al., 2014). 246

247

248 249

Table 3. Macro element contents of leaves taken from different parts of the plant.

I SI	Variety		N (%)*			P (%)*			K (%)*	
LSL		2016	2018	Av.	2016	2018	Av.	2016	2018	Av.
	S24	2.38	3.47	2.93	0.18	0.18 b	0.18	1.50	1.20	1.35
DEE	Control	2.77	3.52	3.14	0.15	0.22 a	0.19	1.52	1.26	1.39
BFF	CV	0.04	0.02	10.03	0.05	0.02	2.89	0.03	0.02	1.75
	Prob > F	0.0702	0.6361	0.0778	0.0618	0.0020	0.7288	0.8656	0.1596	0.4527
	S24	2.58 b	3.37 b	2.98 b	0.18	0.25 b	0.22 b	1.70	1.46 b	1.58
AFF	Control	3.52 <mark>a</mark>	4.09 a	3.81 a	0.23	0.34 <mark>a</mark>	0.28 a	1.66	1.62 a	1.64
	CV	0.04	0.02	1.78	0.05	0.02	2.61	0.02	0.02	1.05
	Prob > F	0.0084	0.0020	0.0134	0.0643	0.0004	0.0249	0.4447	0.0187	0.1530
	S24	2.55	3.37 <mark>a</mark>	2.96	0.18	0.15 a	0.15	0.81 b	0.87 <mark>a</mark>	0.84 b
DE	Control	2.82	2.89 b	2.85	0.18	0.14 b	0.16	1.37 <mark>a</mark>	0.77 <mark>b</mark>	1.07 a
DГ	CV	0.16	0.02	3.24	0.05	0.02	4.58	0.03	0.02	3.23
	Prob > F	0.6307	0.0047	0.0115	0.0618	0.0188	0.0115	0.0006	0.0106	0.0115
	S24	2.30	4.00 a	3.15 a	0.23	0.20 a	0.21	1.05 b	1.32 a	1.19
	Control	2.43	3.32 b	2.88 b	0.18	0.17 b	0.18	1.36 a	1.07 b	1.21
Аг	CV	0.16	0.02	3.23	0.05	0.02	3.23	0.05	0.02	3.23
	Prob > F	0.8978	0.0024	0.0115	0.0643	0.0044	0.0115	0.0320	0.0015	0.0115

* Angle transformation was applied to the percentage values.(LSL: Leaf sampling location, BFF: Below female flower, AFF: Above female flower, BF: Below fruit, AF: Above fruit)

Table 3 – cont.

I CI	Variate		Ca (%)*		Mg (%)*			
LSL	variety	2016	2018	Av.	2016	2018	Av.	
	S24	4.26 b	3.70	3.98 b	0.70 b	0.77 a	0.73	
DEE	Control	5.12 a	3.71	4.42 a	0.81 a	0.70 b	0.76	
БГГ	CV	0.03	0.02	0.36	0.02	0.02	0.72	
	Prob > F	0.0243	0.9416	0.0032	0.0088	0.0270	0.1180	
	S24	1.27	3.31 a	2.29 a	0.34	0.64 a	0.49 a	
	Control	0.95	2.37 b	1.66 b	0.32	0.40 b	0.36 b	
Агг	CV	0.11	0.02	2.45	0.04	0.02	1.56	
	Prob > F	0.1974	0.0002	0.0152	0.4351	<.0001	0.0068	
BF	S24	7.20 a	3.91	5.55 a	0.95 a	0.87 a	0.90 a	
	Control	3.82 b	3.92	3.87 b	0.60 b	0.77 <mark>b</mark>	0.68 b	

	CV	0.11	0.02	3.23	0.08	0.02	3.23
	Prob > F	0.0205	0.9586	0.0115	0.0193	0.0112	0.0115
	S24	4.72	3.62	4.17	0.68	0.68 a	0.68
	Control	4.00	3.73	3.86	0.63	0.63 b	0.63
Аг	CV	0.07	0.02	3.23	0.07	0.02	3.23
	Prob > F	0.1962	0.3152	0.0115	0.6620	0.0385	0.0115

* Angle transformation was applied to the percentage values. (LSL: Leaf sampling location, BFF: Below female
 flower, AFF: Above female flower, BF: Below fruit, AF: Above fruit)

252

254 The results of micronutrient (Fe, Zn and Mn) contents were given in Table 4. In terms of Fe, 255 no statistically significant difference was found in the leaf samples taken the below female 256 flower in both years, the above female flower and the below fruit in 2016. Higher Fe values were found in leaves of S24, sampled the above female flower, the below fruit and the above 257 fruit in 2018, and the above fruit in 2016 compared to the control plants. While the leaf 258 samples taken from below the and the above female flower were not found to be statistically 259 significant in terms of the average of both years, it was determined that the leaf samples taken 260 from the below fruit and the above fruit were higher in Fe in the S24 plant than in the control. 261 Fe content values related to S24 plants from all samples except for the samples the above fruit 262 in 2018 were insufficient and below the limit values (120-335 mg/kg) in both years (Reuter 263 264 and Robinson, 1986; Zengin, 2012; Egel et al., 2017). 265 In terms of zinc (Zn), there was no statistically significant difference in leaf samples taken from the below female flower, the below fruit and the above fruit in 2016. Leaf samples of 266

control plants taken from the below female flower in 2018 and the above female flower in
268 2016 gave higher Zn values than S24. However, the leaf samples of S24 taken from the above
269 female flower and both the below and above fruit in 2018 gave higher leaf Zn content values
270 than the control plants.

271 While the Zn contents in the leaf samples were not found to be significant in the samples 272 taken from the below female flower, the below fruit and the above the fruit, in terms of the 273 average of two years, they were higher in the leaf samples taken from the below fruit than in S24 in control plants. In 2016 and average of two years, Zn contents of all samples except for 274 the sample taken from the above female flower, were insufficient and below the limit values 275 (20-60 mg/kg) (Reuter and Robinson, 1986; Zengin, 2012; Egel et al., 2017). Numerous 276 macromolecules, including hundreds of enzymes, depend on Zn for their structural and 277 functional integrity (Alloway, 2009; Broadley et al., 2012; Coleman, 1998). Zn is essential for 278 279 the metabolism of auxins, proteins, and carbohydrates, among other processes (Marschner, 280 1995; Reddy, 2006; Broadley et al., 2007). Interveinal chlorosis (mottling), a lighter green to pale yellow tint that occurs between the midrib and secondary veins, is the earliest symptom 281

of Zn deficiency in all plants. The internodes are short and the developing leaves are smaller
than usual. These conditions are sometimes referred to as tiny leaf and rosetting (Storey,
284 2007).

In terms of Mn, no statistically significant difference was found in the leaf samples taken 285 from the below female flower, the above female flower and the above fruit in 2016. Higher 286 Mn results were obtained in S24 compared to the control in leaf samples taken from below 287 fruit in 2016, and the below female flower, the above female flower, the below fruit, the 288 above fruit in 2018. Average of two years, Mn contents were higher in leaf samples from the 289 290 below female flower and the below fruit in S24 than in control plants, while samples taken from the above female flower and the above fruit were not found to be statistically significant. 291 292 The Mn amounts of the leaf samples taken from the above female flower in both 2016 and 2018 were below the limit (60-240 mg/kg) values (Reuter and Robinson, 1986; Zengin, 293 294 2012). Due to its impacts on photosynthesis, plant hormone activity, carbohydrate synthesis, and disease resistance, Mn plays important roles in plant growth and development and, 295 296 therefore, in crop production and quality (Eaton, 2015). The breadth and scope of the issue, 297 which limits agricultural yield in many parts of the world due to Mn shortage, are sometimes 298 hidden by the absence of visible leaf symptoms (Schmidt et al., 2016). Plants typically don't show symptoms of Mn shortage until growth rate and production are severely stunted. Mn 299 deficiency-related foliar symptoms often manifest as diffuse interveinal chlorosis on young, 300 enlarged leaf blades (Memon et al., 1981). On the leaves of plants with severe deficiencies, 301 significant necrotic patches or streaks can also appear. The center leaves are frequently where 302 symptoms initially appear (Humphries et al., 2007). 303

304

Table 4. Micro element contents of leaves taken from different parts of the plant.

TST	Voriety	_	Fe (mg/kg	g)	7	Zn (mg/kg		Ν	Mn (<mark>mg/k</mark> g	g)
LSL	variety	2016	2018	Av.	2016	2018	Av.	2016	2018	Av.
	S24	68.57	85.06	76.82	20.83	18.39 <mark>b</mark>	19.61	72.53	82.95 <mark>a</mark>	77.74 a
BFF	Control	70.81	83.33	77.07	13.26	20.41 a	16.83	86.73	51.77 <mark>b</mark>	69.24 b
	CV	0.07	0.03	3.61	0.35	0.03	17.97	0.11	0.03	3.04
	Prob > F	0.7849	0.4907	0.9211	0.1932	0.0186	0.4083	0.1285	<.0001	0.0431
	S24	95.98	75.58 <mark>a</mark>	85.78	25.81 b	16.67 <mark>a</mark>	21.24 b	37.77	45.34 <mark>a</mark>	41.55
AFF	Control	104.29	56.17 b	80.23	34.45 <mark>a</mark>	14.98 <mark>b</mark>	24.72 a	30.88	29.27 b	30.07
	CV	0.13	0.03	6.10	0.08	0.03	1.41	0.19	0.03	9.30
	Prob > F	0.4742	0.0004	0.3116	0.0097	0.0172	0.0058	0.2733	0.0001	0.0518
	S24	80.99	78.79 <mark>a</mark>	79.89 a	14.93	17.00 <mark>a</mark>	15.96	133.31 a	115.15 a	124.23 a
DE	Control	78.21	63.10 b	70.65 b	21.93	12.05 b	16.99	79.71 <mark>b</mark>	78.46 <mark>b</mark>	79.08 b
ЫΓ	CV	0.06	0.03	2.72	0.34	0.03	18.40	0.20	0.03	7.25
	Prob > F	0.5033	0.0013	0.0312	0.2398	0.0002	0.7183	0.0394	0.0002	0.0173
	S24	88.39 <mark>a</mark>	155.16 <mark>a</mark>	121.77 a	15.72	14.38 <mark>a</mark>	15.05	99.81	70.92 <mark>a</mark>	85.36
AF	Control	77.88 <mark>b</mark>	61.32 b	69.60 b	20.86	10.89 <mark>b</mark>	15.88	82.64	59.04 <mark>b</mark>	70.84
	CV	0.04	0.04	0.80	0.28	0.03	12.97	0.15	0.03	8.35
	Prob > F	0.0249	<.0001	0.0001	0.2794	0.0005	0.6637	0.1888	0.0026	0.1122

* Angle transformation was applied to the percentage values. (LSL: Leaf sampling location, BFF: Below female flower, AFF: Above female flower, BF: Below fruit, AF: Above fruit) 306 307

4. Conclusion 308

305

When the places where the leaf samples were taken and the years were evaluated together, 309 there was not a variety that came to the forefront in terms of N, P, K, Ca, Mg and Zn. 310 However, in general, Fe and Mn contents in the S24 line were found to be higher than the 311 control. In terms of the average of two years, the Ca content in leaf samples taken from the 312 below female flower was higher in control plants than in S24, while in terms of Mg content, 313 314 the content of S24 was higher than in control plants, and there was no difference in the contents of other elements. In terms of the average of two years, the N, P and Zn contents in 315 leaf samples taken from the above female flower was higher in control plants than in S24, 316 while in terms of Ca and Mg content, the content of S24 was higher than in control plants, and 317 there was no difference in the contents of other elements. In terms of the average of two years, 318 the N and Fe contents in leaf samples taken from the above fruit was higher in S24 than in 319 control plants, while there was no difference in the contents of other elements. In line with 320 these results, it is not possible to say that there is a relationship between yellowing of leaf 321 322 veins and plant nutrients. It has been concluded that the yellowing of these leaf veins may be due to genetic factors, not plant nutrients. Dou et al. (2018), in their study for the genetic 323 324 mapping of watermelon yellow skin color, used the watermelon with yellow leaf veins as in our study and stated that the genetic material of wild watermelon consists mainly of green 325 skin; different skin colors emerged as a result of the progress of evolution, artificial selection, 326 and gene mutations, including yellow skin. Mutation of the gene can occur in the anterior 327 region of chromosome 4 in the watermelon genome, and there may be two or three closely 328 related genes in this range that control the phenotype of yellow skin, yellow veins, and yellow 329 330 petioles. Mutation in this region not only causes the skin color of the fruit to change but also the veins and leaves. They concluded that leaf color mutations may affect plant 331 332 photosynthesis, growth, and development, but yellow veins and petioles did not show any effect in their experiments, so this candidate region plays a very important role in the control 333 334 of plant photosynthesis. After that, it will be possible to illuminate it with a mapping study to 335 be carried out on this subject.

Acknowledgements

The authors are thanks to Alata Horticultural Research Institute for leaf nutrient analysis. 338

340 References

336

337

339

- 341 Akhtar, M.S., Oki, Y., Adachi, T. 2009. Mobilization and Acquisition of Sparingly Soluble P-
- 342 Sources by Brassica Cultivars under P-Starved Environment I. Differential Growth Response,
- P-Efficiency Characteristics and P-Remobilization. Journal of Integrative Plant Biology 51:
 1008–1023.
- Alloway, B.J. 2009. Soil Factors Associated with Zinc Deficiency in Crops and Humans.
- Environ. Geochem. Health 31:537–548.
- Amtmann, A. Blatt, M.R. 2009. Regulation of Macronutrient Transport. New Phytologist 181,
 35-52
- 349 Bergmann, W. 1992. Nutritional Disorders of Plants: Development, Visual and Analytical
- 350 Diagnosis. Jena, Germany: Gustav Fischer.
- Bould, C., Hewitt, E.J. Needham, P. 1983. Diagnosis of Mineral Disorders in Plants, Vol. 1:
- 352 Principles. London, U.K.: HMSO.
- Broadley, M., Brown, P., Cakmak, I., Rengel, Z. Zhao, F. 2012. Function of Nutrients:
- 354 Micronutrients. In Mineral Nutrition of Higher Plants, 3rd edn., ed. P. Marschner, pp. 191-
- 355 248. London, U.K.: Academic Press.
- Broadley, M.R., White, P.J., Hammond, J.P., Zelko, I., Lux, A. 2007. Zinc in Plants. New
 Phytologist 173, 677-702.
- 358 Cakmak, I., Kirkby, E.A. 2008. Role of Magnesium in Carbon Partitioning and Alleviating
- 359 Photooxidative Damage. Physiol. Plant. 133, 692–704. doi: 10.1111/j.1399360 3054.2007.01042.x.
- 361 Cakmak, I., Yazici, A.M. 2010. Magnesium: A Forgotten Element in Crop Production. Better
 362 Crop. 2010, 94, 23–25.
- Cetner, M.D., Kalaji, H.M., Borucki, W., Kowalczyk, K. 2020. Special issue in honour of
 Prof. Reto J. Strasser-Phosphorus Deficiency Affects the I-Step of Chlorophyll a
 Fluorescence Induction Curve of Radish. Photosynthetica, 58(SPECIAL ISSUE), 671-681.
 doi: 10.32615/ps.2020.015
- Chen, Y., Barak, P. 1982. Iron Nutrition of Plants in Calcareous Soils. Advances in
 Agronomy Volume 35, 217–240. doi:10.1016/s0065-2113(08)60326-0
- Coleman, J.E. 1998. Zinc Enzymes. Curr. Opin. Chem. Biol. 2:222–234.
- Doerge, T., Roth, R., Gardner, B. 1991. Nitrogen Management Guide for Watermelon. In
 Nitrogen Fertilizer Management in Arizona. The University of Arizona.
- 372 Dou, J., Lu, X., Ali, A., Zhao, S., Zhang, L., He, N., Liu, W. 2018. Genetic mapping reveals a
- marker for yellow skin in watermelon (*Citrullus lanatus* L.). Plos One, 13(9), e0200617.
- doi:10.1371/journal.pone.0200617

- Eaton, T.E. 2015. Manganese. In Handbook of Plant Nutrition, ed. A.V. Barker and D.J.
- 376 Pilbeam, pp. 427–486. Boca Raton, FL: CRC Press.
- Egel, D., Foster, R., Maynard, E., Weller, S., Babadoost. M. 2017. Midwest Vegetable
 Production Guide for Commercial Growers.
- 379 Erdal, I., Kepenek, K., Kizilgöz, I. 2006. Effect of Elemental Sulphur and Sulphur Containing
- 380 Waste on the Iron Nutrition of Strawberry Plants Grown in a Calcareous Soil. Biological
- 381 Agriculture & Horticulture, 23(3), 263–272. doi:10.1080/01448765.2006.9755328.
- 382 Eyuboglu, F. 1999. Fertility Status of Turkish Soils. Publications of the Prime Ministry
- 383 General Directorate of Rural Services, Soil and Fertilizer Research Institute, p.122.
- Fageria, N.K. 2009. Nutrient Uptake in Crop Plants. Boca Raton, FL: CRC Press.
- FAOSTAT, 2020. https://www.fao.org/faostat/en/#data/QCL (date: 19 December 2022).
- 386 Farhat, N., Rabhi, M., Krol, M., Barhoumi, Z., Ivanov, A. G., McCarthy, A., Abdelly, C.,
- 387 Smaoui, A., Hüner, N. P. A. 2014. Starch and Sugar Accumulation in Sulla Carnosa Leaves
- Upon Mg²⁺ Starvation. Acta Physiologiae Plantarum, 36(8), 2157–2165. doi:10.1007/s11738014-1592-y
- Gao, M., Hu, L., Li, Y., Weng, Y. 2016. The chlorophyll-deficient golden leaf mutation in
- 391 cucumber is due to a single nucleotide substitution in CsChII for magnesium chelatase I
- subunit. Theoretical and Applied Genetics, 129(10), 1961–1973. doi:10.1007/s00122-016-
- 393 2752-9
- 394 Gransee, A., Führs, H. 2013. Magnesium Mobility in Soils as a Challenge for Soil and Plant
- Analysis, Magnesium Fertilization and Root Uptake Under Adverse Growth Conditions. Plant
 and Soil, 368(1-2), 5–21. doi:10.1007/s11104-012-1567-y
- 397 Grzebisz, W. 2015. Magnesium. In Handbook of Plant Nutrition, eds. A.V. Barker and D.J.
- 398 Pilbeam, pp. 199-260. Boca Raton, FL: CRC Press.
- Gucdemir, İ.H. 2012. Plant Nutrition Recipe Preparation Technique Based on Soil Analysis
 and Practical Examples. In Plant Nutrition (Ed. Karaman M R) pp. 961-1066.
- 401 Guner, N., Wehner, T.C. 2004. The Genes of Watermelon. Hortscience, 39(6):1175–1182.
- 402 Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Skrumsager, I., White,
- 403 P. 2012. Functions of macronutrients. In Marschner's Mineral Nutrition of Higher Plants, 3rd
- 404 ed.; Marschner, H., Ed.; Academic Press: London, UK, pp. 135–189.
- Humphries, J.M., Stangoulis, J.C.R., Graham, R.D. 2007. Manganese. In Handbook of Plant
 Nutrition, eds. A.V. Barker and D.J. Pilbeam, pp. 352–374. Boca Raton, FL: CRC Press.

- 407 Iriarte, F., Jailani, A.A., Paret, M.L. 2023. First report of Watermelon crinkle leaf-associated
- virus 1 (WCLaV-1) on Cucurbita pepo in the United States. New Disease Reports 47(2),
 e12167. https://doi.org/10.1002/ndr2.12167
- 410 Jailani, A. A. K., Iriarte, F., Hochmuth, B., Willis, S. M., Warren, M., Dey, K., Velez-
- 411 Climent, M., McVay, J., Bag, S., Paret, M.L. 2022. First report of Cucurbit chlorotic yellows
- 412 virus affecting watermelon in the United States. Plant Disease. 106 (2), 774-774.
- 413 doi:10.1094/PDIS-03-21-0639-PDN
- Jeong, J., Connolly, E. L. 2009. Iron uptake mechanisms in plants: Functions of the FRO
 family of ferric reductases. Plant Science, 176(6), 709–714.
 doi:10.1016/j.plantsci.2009.02.011
- 417 Jin, J., Wang, G., Liu, X., Pan, X., Herbert, S. J., Tang, C. 2006. Interaction Between
- 418 Phosphorus Nutrition and Drought on Grain Yield, and Assimilation of Phosphorus and
- 419 Nitrogen in Two Soybean Cultivars Differing in Protein Concentration in Grains. Journal of
- 420 Plant Nutrition, 29(8), 1433–1449. doi:10.1080/01904160600837089
- 421 Kacar, B. 1972. Chemical Analysis of Plant and Soil. Plant Analysis. Volume II. Ankara
 422 University, Faculty of Agriculture. Publication No: 453, Ankara.
- 423 Korkmaz, A., Saltalı, K. 2012. Factors Affecting Plant Nutrient Availability. In Plant nutrition
- 424 (ed. Karaman, M. R.). p.93-122.
- Lee, J.M. 1994. Cultivation of Grafted Vegetables. I. Current status, grafting methods and
 benefits, HortScience, 29, 235-39.
- Ma, J.F. 2005. Plant root responses to three abundant soil minerals: Silicon, aluminum and
 iron. Critical Reviews in Plant Sciences 24, 267-281.
- 429 Marschner, H. 2012. Mineral Nutrition of Higher Plants. 3nd ed. Academic Press; San Diego,
 430 U.S.A.
- 431 Marschner, H. 1995. Mineral Nutrition of High Plants (2nd Edn), London Academic Press,
 432 London, 889 pp.
- 433 Memon, A.R., Chino, M., Hara, K., Yatawawa, M. 1981. Microdistribution of Manganese in
- the Leaf Tissue of Different Plant Species as Revealed by X-Ray Microanalyser. Physiol.
 Plant. 53, 225–232.
- 436 Mengel, K., Kirkby, E.A. 2001. Principles of Plant Nutrition (4th Edn), International Potash
 437 Institute, Switzerland, 687 pp.
- Messiaen, C.M. 1974. Le Potager Tropical (1- Généralités). Agence de Coop., Culturelle et
 Technique Publisher, Paris.

- 440 Miao, H., Zhang, S., Wang, M., Wang, Y., Weng, Y., & Gu, X. (2016). Fine Mapping of
- 441 Virescent Leaf Gene v-1 in Cucumber (*Cucumis sativus* L.). International Journal of
 442 Molecular Sciences, 17(10), 1602. doi:10.3390/ijms17101602
- 443 Oda, M. 1995. New Grafting Methods for Fruit–Bearing Vegetables in JAPAN. Japan
 444 Agricultural Research Quarterly, 29, 187-198.
- 445 Pilbeam, D.J., Morley, P.S. 2007. Calcium. In Handbook of Plant Nutrition, eds. A.V. Barker
- and D.J. Pilbeam, pp. 121–144. Boca Raton, FL: CRC Press.
- 447 Pulgar, G., Villora, G., Moreno, D.A., Romero, L. 2000. Improving the Mineral Nutrition in
- 448 Grafted Watermelon Plants: Nitrogen Metabolism. Plant Biology, 43, 607-609.
- 449 doi:10.1023/A:1002856117053
- 450 Qian, Y.L., Fry, J.D., Upham, W.S. 1997. Rooting and Drought Avoidance of Warm-Season
- 451 Turfgrasses and Tall Fescue in Kansas. Crop Sci. 37:905–910.
- 452 Reddy, K.J. 2006. Nutrient Stress. In: Rao KVM, Raghavendra AS, Reddy KJ (Eds)
- 453 Physiology and Molecular Biology of Stress Tolerance in Plants, Springer, Netherlands, pp454 187-217.
- 455 Reuter, D.J., Robinson, J.B. 1986. Plant Analysis: An Interpretation Manual. Melbourne,
 456 Sydney: Inkata Press. p.218.
- 457 Rhodes, B.B. 1986. Genes Affecting Foliage Color in Watermelon. Journal of Heredity,
 458 77(2), 134–135. doi:10.1093/oxfordjournals.jhered.a110190.
- 459 Römheld, V., Nikolic, M. 2007. Iron. In: Barker AV, Pilbeam DJ (Eds) Handbook of Plant
 460 Nutrition (1st Edn), CRC Taylor and Francis, NY, pp 329-350.
- 461 Rowland, J.H., Cisar, J.L., Snyder, G.H., Sartain, J.B.A., Wright, L., Erickson, J.E. 2010.
- 462 Optimal Nitrogen and Potassium Fertilization Rates for Establishment of Warm-Season
- 463 Putting Greens. Agron. J. 102:1601–1605.
- 464 Ruiz, J.M., Belakbir, A., López-Cantarero. I., Romero, L. 1997. Leaf-Macronutrient Content
- and Yield in Grafted Melon Plants. A model to evaluate the influence of rootstock genotype.
- 466 Scientia Horticulturae, 71(3-4), 227–234. doi:10.1016/s0304-4238(97)00106-4.
- 467 Sanchez, C.A. 2007. Phosphorus. In: Barker AV, Pilbeam DJ (Eds) Handbook of Plant
 468 Nutrition (1st Edn), CRC Taylor and Francis, NY, pp 411-435.
- 469 Schachtman, D., Liu, W. 1999. Molecular Pieces to The Puzzle of The Interaction Between
- 470 Potassium and Sodium Uptake in Plants. Trends in Plants Science. Volume 4, Issue 7, 1 July
- 471 1999, Pages 281-287. doi:10.1016/S1360-1385(99)01428-4.

- 472 Schmidt, S.B., Jensen, P.E., Husted, S. 2016. Manganese Deficiency in Plants: The Impact on
- 473 Photosystem II, Trends in Plant Science, Volume 21, Issue 7, Pages 622-632,
 474 https://doi.org/10.1016/j.tplants.2016.03.001.
- 475 Shao, H.B., Song, W.Y., Chu, L.Y. 2008. Advances of Calcium Signals Involved in Plant
- 476 Anti-Drought. Comptes Rendus Biologies 331, 587-596.
- 477 Storey, J.B. 2007. Zinc. In Handbook of Plant Nutrition, ed. A.V. Barker and D.J. Pilbeam,
- 478 pp. 411–435. Boca Raton, FL: CRC Press.
- Taiz, L., Zeiger, E. 2006. Plant Physiology (4th Edn), Sinauer Associates, Massachusetts, 690
 pp.
- 481 Tiwari, K.N. 2005. Diagnosing Potassium Deficiency and Maximizing Fruit Crop
 482 Productivity. Better Crops/Vol. 89, No. 4.
- 483 Tucker, T.C. 1984. Diagnosis of Nitrogen Deficiency in Plants. In Nitrogen in Crop
- 484 Production, R.D. Hauck (Ed.). doi:10.2134/1990.nitrogenincropproduction.c16.
- 485 Turan, M., Horuz, A. 2012. Basic principles of plant nutrition. In Plant nutrition (ed.
 486 Karaman, M. R.), p.123-346.
- 487 Venkataravanappa, V., Ashwathappa, K. V., Reddy, C. N. L., Shankarappa, K. S., & Reddy,
- 488 M. K. 2020. Characterization of Tomato leaf curl New Delhi virus associated with leaf curl
- 489 and yellowing disease of Watermelon and development of LAMP assay for its detection. 3

490 Biotech, 10(6). doi:10.1007/s13205-020-02245-x

- Xu, B., Zhang, C., Gu, Y., Cheng, R., Huang, D., Liu, X., Sun, Y. 2023. Physiological and
 Transcriptomic Analysis of a Yellow Leaf Mutant in Watermelon. Scientific Reports,
 13:9647. 10.1038/s41598-023-36656-6
- 494 Yetisir, H., Sari, N., Yücel, S. 2003. Rootstock Resistance to Fusarium Wilt and Effect on
- 495 Watermelon Fruit Yield and Quality. Phytoparasitica, 31, 163-169. doi:10.1007/BF02980786.
- 496 Zengin, M. 2012. Basic Principles in the Interpretation of Soil and Plant Analysis Results. In
- 497 Plant Nutrition (ed. Karaman, M. R.), p.837-959.
- Zhang, X.P., Rhodes, B.B., Bridges, W.C. 1996. Phenotype, Inheritance and Regulation of
 Expression of a New Virescent Mutant in Watermelon: Juvenile Albino. Journal of American
 Society Horticulture Science, 121(4):609–615. doi:10.21273/JASHS.121.4.609.
- Zhu, Y., Yuan, G., Wang, Y., An, G., Li, W., Liu, J., Sun, D. 2022. Mapping and functional
 verification of leaf yellowing genes in watermelon during whole growth period. Frontier in
 Plant Science. 13:1049114. doi: 10.3389/fpls.2022.1049114