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NEGATIVE EFFECTS OF IRON CHLOROSIS

Irena JUG[1]*, Danijel JUG[1], Vladimir VUKADINOVIĆ[1], Boris ĐURĐEVIĆ[1], Vesna VUKADINOVIĆ[1], Bojan STIPEŠEVIĆ[1], Dusan KOVACEVIC[2]

[1]Faculty of Agronomy, University of Osijek, Kralja Petra Svačića 1d, Croatia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6., Zemun, Serbia
(Corresponding author: ijug@pfos.hr)

Abstract

Three different experiments were conducted to determine the distribution and causes of iron chlorosis at Strategy I (grape vine) and Strategy II plant (maize). In first experiment, during 2007 field research on maize covered 132 sites. Chlorosis was quantified by visual ratings and chlorophyll meter readings. From the chlorophyll meter data collection the relative chlorosis (Kl_rel) were calculated and mapped. Relative chlorosis has averaged 41.42% and increased with increasing pH value of the soil. Second experiment included vegetation pots trial in greenhouse. Maize hybrid OsSK 617 was sown in pots filled by regosoil, with two levels of field water capacity. Iron chlorosis was determined by chlorophyll meter readings, leaf chlorophyll concentration and leaf total Fe. Higher relative chlorosis (43.88%) was recorded in maize grown in wet condition as result of reduced synthesis of chloroplast pigments. Highly significant negative correlation was found between the relative chlorosis and total concentration of chloroplast pigments. The difference in the concentration of iron in the shoots of maize in dry and wet conditions was not statistically justified. Third research was conducted during 2009 on grape vine included field measurements of the intensity of chlorosis and laboratory analysis of soil and plant material. On soils with a high pH values and HCO_3^-, chlorosis was detected frequently, which is a common phenomenon that occurs as iron deficiency. The average value of the relative chlorosis was 36.45%. The concentration of iron in the leaves and petioles of grape vines was not significantly correlated with the relative chlorosis, which can be result of physiological inactivation of iron

Key words: maize, grape vine, iron chlorosis, chlorophyll meter

Intoduction

Iron deficiency chlorosis is one of the major abiotic stresses for plants grown on calcareous and/or alkaline soils because of an extremely low solubility of Fe (Mengel, 1994). Calcareous soils are characterized by high soil pH (between pH 7 and 8.5) (Loeppert and Hallmark 1985). Generally, the total Fe in soils is higher than the soluble Fe required for optimal growth, which is at approximately 10^{-8} mol dm^{-3} in the soil solution (Lindsay 1995). The availability of Fe^{3+} in soils is low; it depends largely on pH and on redox potential and increases with low soil pH and low redox potential. The availability of free Fe^{3+} is higher than in calcareous soil, but even in acidic soil it is usually too low to meet the plant demand for Fe (Lindsay and Schwab 1982). In calcareous soils, the concentration of Fe^{3+} is very low (about 10^{-10} mol dm^{-3}; Lindsay 1995) and thus far too low to sustain optimal plant growth.

There are many factors that contribute to chlorosis including genetics, light intensity, ion imbalances, oxygen level, as well as nitrogen, magnesium, manganese, copper, and zinc availability. Iron deficiency induced chlorosis often appears as yellowing along the leaf margin and between the leaf veins. Since the veins themselves typically remain green, this condition is referred to as “interveinal chlorosis.” Iron chlorosis appears on the youngest leaves because the element is very immobile within the plant, and is not translocated from older tissue as needed. Iron is a component of several substances that play key roles in plant physiology. One very important role is in the synthesis and degradation of chlorophyll, although iron itself is not a constituent of the chlorophyll molecule. Chlorophyll in turn plays an important role in plant carbohydrate metabolism (photosynthesis). When iron is lacking in the soil or is in an insoluble form, chlorosis may result. Water availability and temperature regime also influence on chlorophyll production and chlorosis. In other cases, root injury by soil tillage or even drought may cause decreased iron uptake and chlorosis. Sandy soils low in organic matter may also be iron deficient. Iron chlorosis can be identified by visual symptoms (several authors proposed the use of visual scores, from 0 -without symptoms, to 5 -very strong appearance of chlorosis, when more than 10% of leaves chlorotic), by the evaluation of chlorophyll content using chlorophyll meter – indirect method and by plant analysis – direct method. Depending on plants response to iron deficiency, plants can be grouped into Strategy I and Strategy II plants (Römheld and Marschner, 1986). Strategy I plants (dicotyledons and non graminaceous monocotyledons) respond to Fe deficiency by release of reductants - caffeic acid, increased H^+ release, increased transport of Fe to tops and enhancing ferric reduction activity at the root plasma membrane (Chaney et al, 1972, Marschner, 1995). Strategy II plants synthesize and secrete non-proteinogenic amino acids - phytosiderophore. Iron is transported across the plasma membrane as Fe^{3+}phytosiderophore. Due to the different mechanisms of Fe acquisition, Strategy I and Strategy II plants have a different reaction on iron deficiency stress. The aim of this work was to determine the distribution and the causes for the appearance of chlorosis at Strategy I (grape vine) and Strategy II plant (maize).

Materials and methods

Field research - maize

Field research was conducted during 2007 (Jug et al., 2008) on maize crops in the eastern Croatian, and included determination of the crop positions by the GPS, visual estimation of the crop chlorosis (Table 1) and determination of the total concentration of the chloroplast pigments at the chlorotic and nonchlorotic maize plants by indirect method - chlorophyll meter (SPAD, Minolta, Japan). From the chlorophyll meter data collection the relative chlorosis (Kl_rel) were calculated (Formula 1) and mapped at the satellite maps (SRTM - *Shuttle Radar Topography Map).* All data were statistically analyzed by the usual methods.

Table 1. Visual estimation of the crop chlorosis

Fe chlorosis (%)	visual scores
no symptoms	0
less than 20% plants with symptoms	1
20-40% plants with symptoms	2
40-60% plants with symptoms	3
60-80% plants with symptoms	4
more than 80% plants with symptoms	5

Formula 1

$$Kl\_rel=100-(\frac{Kl\_S}{Kl\_N}\times 100)$$

Kl_rel = relative chlorosis (%)
Kl_S = SPAD value – leaves with chorosis symptoms
Kl_N = SPAD value – leaves without chorosis symptoms

Vegetation trial in the pots – maize

The vegetation pots trial has been conducted in the greenhouse. The 40 dm^3 large pots were filled by the regosoil, by maintaining natural stratification of the soil horizons (A-C). Experiment included two levels of field water capacity (50% FWC and 100% FWC) simulating drought and excessively wet conditions. The used maize hybrid was OsSK 617. In the 7-8 leaves stage maize plant material has been collected and analyzed: readings by chlorophyll meter; chlorophyll concentration was determined spectro-photometrically (at wave lengths 662, 644 and 440 nm) from an acetone extract using the methods of Holm and Wettstain and expressed in mg per g of fresh mass (Arsenijević-Maksimović and Pajević, 2002) and plant materials were destruction by wet digestion (mixture of sulfuric and perchloric acid and hydrogen peroxide), (Vukadinovic and Bertić, 1988.). Concentration of the iron was determined directly from the stock solution by atomic absorption spectrophotometry (AAS). All data were statistically analyzed by the usual methods.

Field research - vineyards

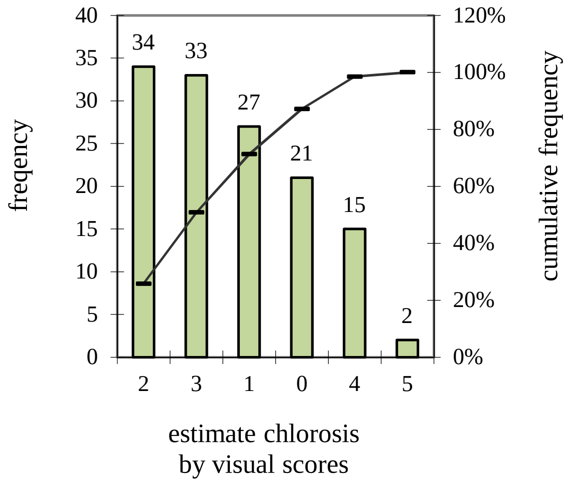
Research was conducted during the grape vine growing season 2009 in the full floral and early ripening grape, in the area of Baranja and Erdut vineyards (Jug et al., 2012).

The study included field measurements of the intensity of chlorosis and laboratory analysis of soil and plant material. Field measurements of the intensity of chlorosis performed nondestructive method using chlorophyll meter (SPAD 502, Minolta Co., Japan) to a total of 60 locations. In site where chlorophyll meter measurements were peformed, altitude (Alt) and geographic coordinates (Lat and Lon) were recorded. Chlorosis was visually assessed with scores of 0-5 (Table 1). From the chlorophyll meter data collection the relative chlorosis (%) were calculated (Formula 1) and mapped at the vector map.

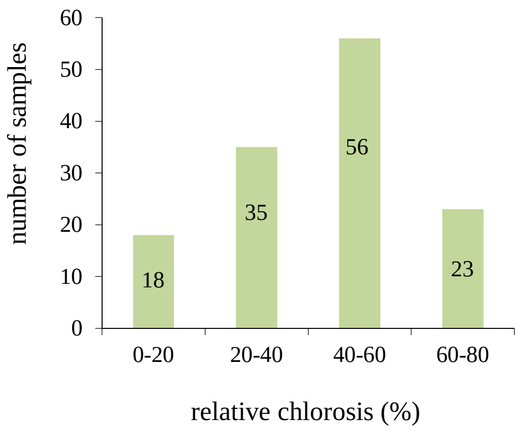
At selected locations from which the chlorophyll meter measured the intensity of chlorosis, soil samples were taken from two depths (0-30 cm and 30-60 cm) as well as samples of plant material (leaf and petiole vines). The air-dried soil samples were determined pH values (pH in H_2O and 1 mol dm^{-3} KCl) (Vukadinovic and Bertić, 1988). The content of available phosphorus and potassium (P_2O_5 and K_2O) was determined using AL method. Humus content in the soil samples was determined by bichromate method (Vukadinovic and Bertić, 1988), and the amount of carbonates in the soil was determined by measuring the volumetric according to Scheibler (Bogdanovic et al., 1966). Samples of leaves and stems of vines were destroyed by wet digestion (mixture of sulfuric and perkloridne acid and hydrogen peroxide), (Vukadinovic and Bertić, 1988). Concentration of the iron was determined directly from the stock solution by atomic absorption spectrophotometry (AAS). All data were statistically analyzed by the usual methods.

Results and discussion

During 2007 field research on maize covered 132 sites on transect Beli Manastir - Osijek – Županja. Visual estimation of the crop chlorosis and determination of the total concentration of the chloroplast pigments by chlorophyll meter were performed on all 132 plant samples. Frequency distribution shown that out of 132 samples only two sites (1.52%) was estimated by grade 5 (occurrence of chlorosis in more than 80% of plants), 15 samples (11.36%), with an estimate of grade 4, 33 samples (25%) with an estimate of grade 3, 34 sample (25.75%), with an estimate of chlorosis grade 2 while 21 samples (15.91%) had no symptoms of chlorosis (Graph 1). The average estimate chlorosis for maize crop was grade 2, while the CV% was very high, reaching 65.09 (Jug et al., 2008). Relative chlorosis has averaged 41.42% with a CV% of 48.84%. Maximum calculate relative chlorosis was 75.25%. Distribution of the relative chlorosis for maize is shown in Figure 1. By surveying from the west toward the east of the Croatia, the soil pH value is increasing, which is leading toward the iron and zinc deficiency and causing the appearance of the chlorosis.



Graph 1. Frequency distribution estimates chlorosis



Graph 2. Intensity of relative chlorosis

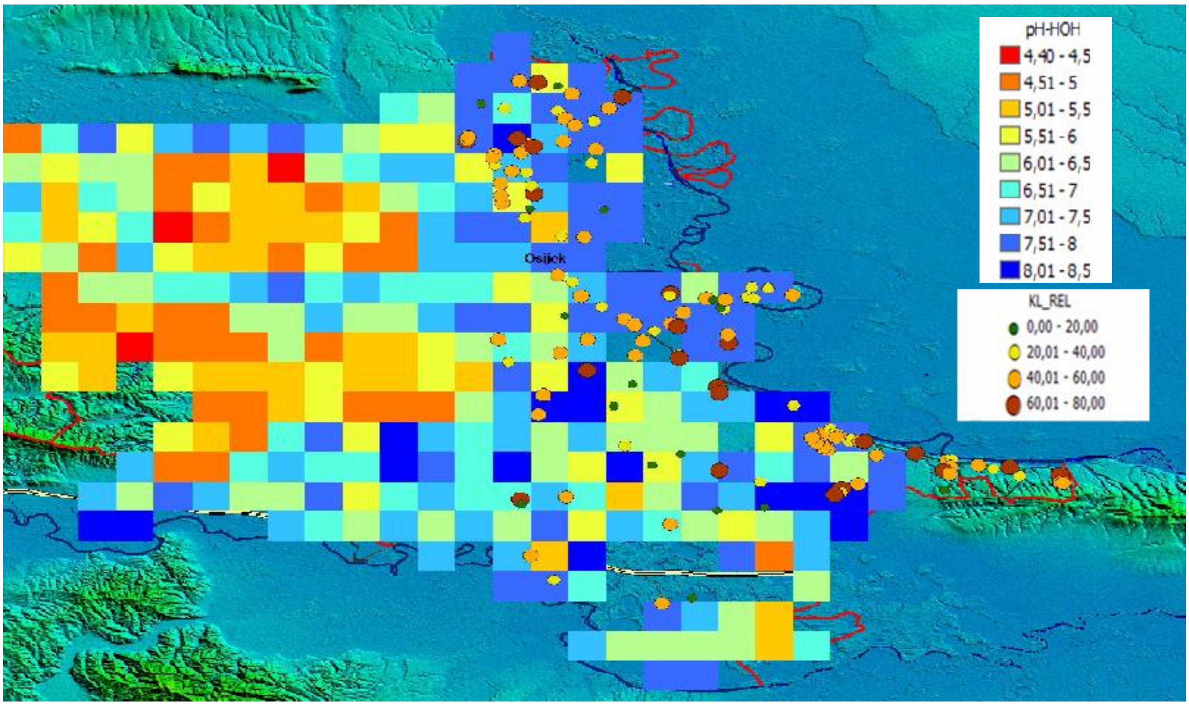


Figure 1 Map of the distribution of relative chlorosis in the east of the line of Beli Manastir - Osijek – Županja and rasterization pH values (4 x 5.5 km)

The highest relative chlorosis (60-80%) was calculated at 17.42% corn crop and the lowest (0-20%) to 13.64% of all samples. Relative chlorosis, which ranged between 40 and 60% was found in 42.42% of the samples, and in 26.52% of the samples relative chlorosis ranged 20-40% (Graph 2).

Maize is very sensitive to zinc deficiency (Mengel and Kirkby, 2001). Zn deficiency was recorded in almost all localities where chlorosis was spotted (Figure 1). In addition to the symptoms of zinc deficiency iron deficiency was also observed (especially in the area of Baranjsko brdo, Figure 2), and less frequent the deficiency of magnesium and manganese. In calcareous soils, the presence of $CaCO_3$, directly or indirectly, in addition to the availability of iron affects the availability of nitrogen, phosphorus, magnesium, potassium, manganese, zinc and copper (Marshner, 1995; Obreza et al., 1993). Acute zinc deficiency on the study area was expected, because the increase in soil pH reaction reduces the bioavailability of zinc in calcareous soils in poorly accessible form.

The aim of vegetation pots trial was investigate the causes of chlorosis in greenhouse conditions on maize depending on soil moisture. The soil used for vegetation trials in pots was calcareous (Table 2). Type of soil was regosols with (A)-C type of soil profile.

Table 2 Chemical properties of the soil and the concentration of trace elements

layer	pH		OM	mg (100 g)$^{-1}$		$CaCO_3$	IDG	mg kg^{-1}			
	H_2O	KCl	%	P_2O_5	K_2O	%	%	Fe	Cu	Mn	Zn
(A)	8.61	8.01	1.14	3.1	9.72	19.85	7.0	9.11	5.31	12.17	1.52
C	8.76	8.11	0.56	0.7	6.40	17.96	5.5	4.39	0.96	2.28	0.42

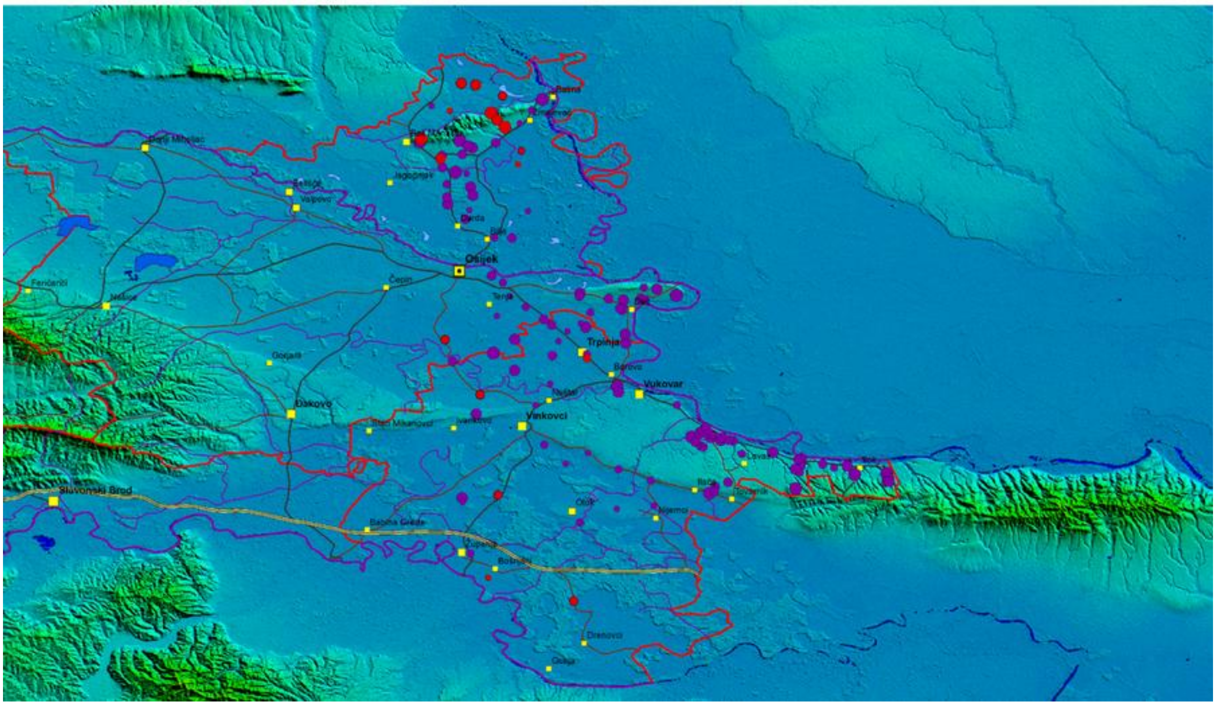


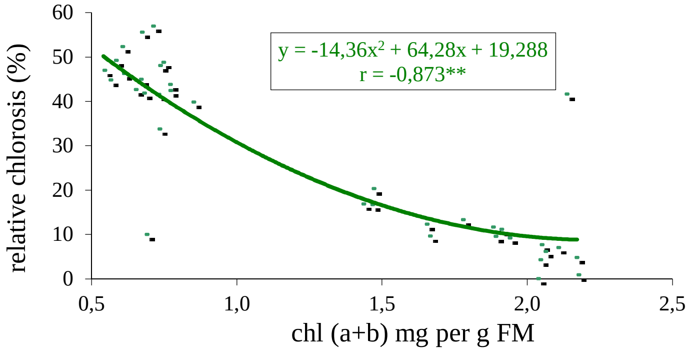
Figure 2 Fe and Zn deficiency on maize

Higher relative chlorosis (43.88%) was recorded in maize on 100% FWC treatments (Table 3) as result of reduced synthesis of chloroplast pigments. Similar results were presented by Tong Yue et al. (1987)

Table 3 Value for relative chlorosis (%), chlorophyll (a+b) (mg g^{-1} fresh matter) and concentration of iron (μg g^{-1}) according two different soil water levels

FWC	Kl_rel (%)	chl (a+b)	Fe
50%	11.29^{b}	1.83^{a}	72.67
100%	43.88^{a}	0.73^{b}	72.58
$\bar{x}$	27.58	1.28	72.63

Highly significant negative correlation was found between the relative chlorosis and total concentration of chloroplast pigments (Graph 3). Higher relative chlorosis indicates lower SPAD values (chlorophyll meter readings) which are in accordance with research Bavaresco et al. (2005) and Yadava (1986). Same authors reported positive correlation between SPAD readings and content of chlorophyll.



Graph 3 Correlation between relative chlorosis and concentration of chlorophyll (a+b)

Barwinsky and Remphrey (2008) revealed the presence of highly significant linear correlation between SPAD readings and concentration of chloroplast pigments in the Chinese maple leaves. Plants suffering from lime chlorosis often have a lower concentration of iron in relation to normal plants, although the Fe concentration may be equal to or even greater than in plants without visible symptoms of chlorosis (Marschner, 1995; Pestana et al., 2003). In this study, the difference in the concentration of iron in the shoots of maize in dry and wet conditions was not statistically justified, although visually maize was grown in the wetter conditions had more pronounced symptoms of iron deficiency.

These results suggest the possibility that the concentration of iron may be the same or even higher in chlorotic leaves as a possible consequence of physiological inactivation of iron (Mengel et al., 1984 a, b). Mengel (1994) reported the frequent high concentration of iron in chlorotic leaves compared to normal green leaves, and that this phenomenon indicates the possibility that the occurrence of chlorosis on calcareous soils is not always associated with the adoption of iron by the roots and its translocation to other parts of the plant, but it often depends on the utilization of iron in the leaves. This has been termed the “Fe chlorosis paradox” (Morales et al. 1998; Römheld 2000), and suggests that part of the Fe acquired from the soil by Fe-deficient plants could be immobilized and accumulated in inactive forms somewhere in the leaf (Morales et al. 1998).

Iron chlorosis is a more complex phenomenon in fruit trees than in annual crops (Tagliavini et
al., 2000a). Symptoms of iron chlorosis often start as soon as buds open, likely as a result of insufficient storage of Fe, or develop throughout the vegetative season as a consequence of plant demand being excessive in respect to Fe availability (Tagliavini and Rombolà, 2001). In general, chlorosis occurs more frequently in spring when rainfalls cause a raise in soil bicarbonate concentration in a period of intense Fe demand. If soil conditions after that period improve, new leaves appear green, but those previously chlorotic unlikely re-green. Fruit yield losses caused by leaf chlorosis also depend on the degree and the period the chlorosis develop and, in general, critical periods coincide with blooming and fruit set. Research on grape vine was conducted during the growing season 2009 in 60 locations in Baranja and Erdut vineyards whose average age was 16 years (Jug et al., 2012). Chlorosis appears on higher and slope terrain on loess because of erosion and not adequate applied soil tillage systems. In soils where shallow layers are less rich in $CaCO_3$ than deeper layers, it is likely that grape vines develops chlorosis only when they age and roots explore layers with poor conditions for Fe uptake. Soils which had been subjected to mouldboard ploughing before the plantation may present layers of fine texture, just below the ploughing depth, which could be rich in $CaCO_3$ because of leaching from more shallow layers. Total amounts of iron in cultivated soils, would not justify the development of iron deficiency, which,

nevertheless, often occurs as a result of poor availability of iron for plants. The most expressed problem in the vineyard production is the limiting soil pH value. The highest measured pH value in the water at a depth of 30-60 cm, was 9.03, while pH in KCl was 8.28. The lowest pH values were as follows: pH (H_2O) = 6.92; pH (KCl) = 6.17 (Table 4).

Table 4 Average of chemical analyzes (60 samples), coefficient of variation (CV%), minimum and maximum values

	pH KCl	pH H_2O	AL-P_2O_5 mg 100 g^{-1}	AL-K_2O mg 100 g^{-1}	% OM	% $CaCO_3$
average	7.62	8.45	19.60	20.17	1.55	7.70
CV%	6.28	5.52	64.11	19.44	20.74	79.92
Min	6.17	6.92	3.40	8.13	0.85	0.00
Max	8.28	9.03	63.80	31.84	2.21	21.21

At total of 34 locations very alkaline soil reaction was measured, pH>8.51; at 17 locations moderately alkaline soil reaction (8.01-8.5) were measured, while slightly alkaline reaction was determined at 5 locations. Neutral to slightly alkaline soil solution was measured at 3 sites, while only one site had a pH between 6.5-7.0. Soils with high pH value had mainly high carbonate content (Figure 3), while the largest content of $CaCO_3$ was 21.21%. On soils, which have a high pH in the presence of HCO_3^- ions, chlorosis is a common phenomenon that occurs as iron deficiency is confirmed by research Ksour et al. (2005).

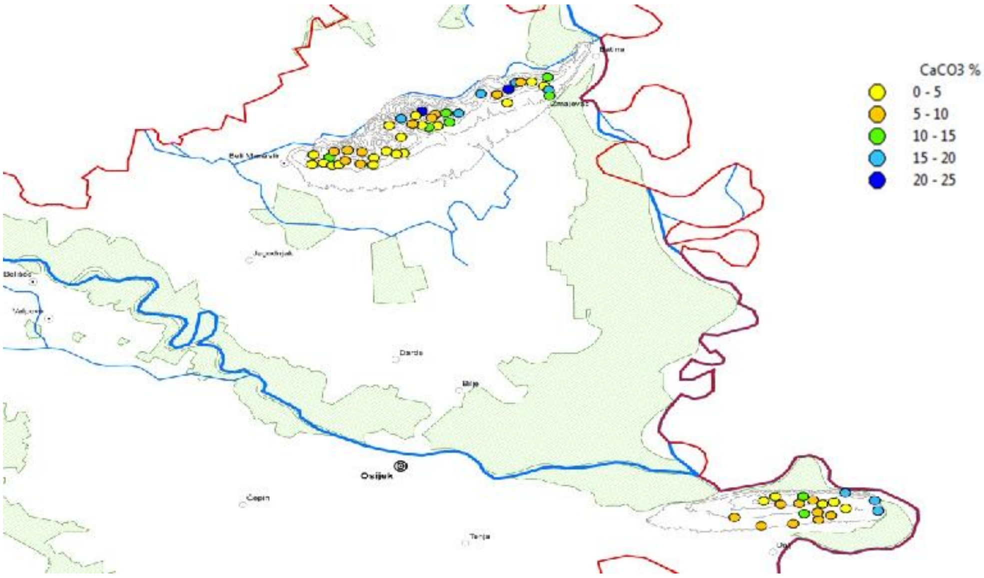


Figure 3. Distribution of $CaCO_3$ in Baranja and Erdut vineyards

The average visual chlorosis rating of grapevine was 2, while CV was very high (75.19%). Frequency distribution showed that only one site had 80-100% of grape vinesbeing chlorotic (0.60%). At 17 sites, there was no appearance of chlorosis (28.33%), while the three sites (5.00%) to 20% of grapevine leaves appeared chlorotic. At 16 locations vines had 20-40% of leaves with symptoms of chlorosis (26.67%). At 15 sites assessment chlorosis was 3, which means that 40-60% of vines had symptoms of chlorosis (25.00%). In 8 localities chlorosis of grape vine is estimated to 4, which means that 60-80% of the vine had chlorosis symptoms.
The average value of the relative chlorosis was 36.45% with a coefficient of variation of 67.36%. The highest percentage of relative chlorosis was 73.87%. Relative chlorosis is very significant, negative correlation between SPAD values.

Table 5 Average concentrations of Fe (μg g^{-1} DM) in the leaves (60 samples) and petioles (60 samples), the visual chlorosis rating (0-5) SPAD readings and relative chlorosis (Kl_rel %), by calculating the value of the standard deviation (SD), coefficient of variation (CV%), minimum and maximum values of the given parameters

	leaves	petioles			
	Fe (μg/g DM)	Fe (μg/g DM)	visual rating	SPAD	Kl_rel
prosjek	161.33	109.84	1.95	23.95	36.45
Sd	41.97	27.06	1.47	9.52	24.55
KV%	26.01	24.63	75.19	39.74	67.36
Min	96.11	65.36	0.00	9.72	0.00
Max	302.31	216.58	5.00	42.08	73.87

The average iron concentration (Table 5) in grapevine leaves was 161 μg g^{-1}, while the petioles average iron concentration was slightly lower (109 μg g^{-1}). According to Reuter and Robins's (1997) it is adequate concentration of iron in the leaf and stem of the vine. According to the same authors, interveinal chlorosis presents greater help in diagnosing Fe chlorosis, compared to the obtained concentrations values of iron. In many cases, however, leaves from Fe-deficient plants grown in the field have quite high leaf Fe concentrations (>80-100 μg g^{-1}DM), and there is no good correlation between leaf Fe and chlorophyll concentrations (Morales et al. 1998). In this study, the concentration of iron in the leaves and petioles of grape vines was not significantly correlated with the relative chlorosis, which can be result of physiological inactivation of iron. Gruber and Kosegarten (2002) also reported high Fe concentrations in roots and leaves on grape vines grown on calcareous soil, where plants suffered from Fe deficiency

Conclusion

Fe chlorosis appeared on locations with high pH, high carbonate content and high altitudes. Concentration of iron in the maize leaves and grape vine leaves and petioles were relatively high, but physiologically inactive leading to inhibited synthesis of chlorophyll and finally, the appearance of chlorosis. Knowing causes that lead to the Fe chlorosis it is possible to acquire preventive action to minimize or, if possible, avoid the damage caused by Fe chlorosis.

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