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Influence of reducing and oxidating compounds on iron accumulation and chlorophyll content in oat leaves

EDWARD ŚLESAK

Department of Experimental Botany, Institute of Botany, Wrocław University

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Abstract

The influence of reducing-oxidating potentials, via the introduction of reducing or oxidating substances into the medium, on iron accumulation and chlorophyll content in oat leaves was investigated. Lowering of the redox potential increases iron accumulation and chlorophyll content in leaves and vice versa. Experiments demonstrated that the redox potential is one of the factors determining the availability and physiological activity of iron in plants.

INTRODUCTION

The availability of iron and its uptake by plants are determined by a number of factors. The most frequently studied ones are: hydrogen ion concentration (pH) (De Kock, 1955), phosphate concentration (Rediske, Bidulph, 1953; De Kock et al., 1969; Bentley et al., 1970), heavy metal ion concentration (De Kock, 1956; O'Sullivan, 1969), hydrogen carbonate (HCO_3^-) (Brown, 1959; Wallinhan, 1959; Gumiński et al., 1965b). Among the better known factors the redox potential of the nutrient solution should also be included. This is supported by the results of a number of investigators dealing with iron metabolism in plants. Thus, the results of Jeffrey (1960) indicate that the redox soil potential plays a role in establishing equilibrium between Fe^{2+} and Fe^{3+}, which, according to Arndt (1969), determines the growth of roots in depth. The studies of Gumiński (1950, 1952, 1953) call attention to the relation between the redox conditions and the effect of humic compounds. This is particularly interesting in view of the fact that the experiments of Gumiński et al. (1965a) and of Czerwiński 1967, 1968) point to the role of humate as iron chelate. It was observed that the presence of hydrogen sulphide may prevent chlorosis (Gumiński et al., 1965b, Yasu et al., 1968), and that it lowers the redox potential of the nutrient solution.

The works of Machold (1966, 1967, 1968a) on chlorophyll tomato mutants demonstrated that a depression of the redox potential enhances chlorophyll synthesis in the leaves of the mutants. The close relation between iron accumulation and chlorophyll synthesis is stressed by Agarvala and Sharma (1961), De Kock (1960), O'Sulivan (1969) and Machold (1968).

In view of the results reported in the above mentioned papers and the suggestions concerning the importance of the "redox" factor in iron metabolism of plants, finding its expression in chlorophyll synthesis, studies were undertaken on the iron and chlorophyll content in the leaves of oats growing on a medium with addition of substances reducing or increasing its potential.

MATERIAL AND METHODS

The experiments were performed with the Późny Antoniński oat variety, on Hoagland's medium ($Ca(NO_3)_2$ — 5 mM, KNO_3 — 5 mM, KH_2PO_4 — 1 mM, $MgSO_4$ — 1 mM) with addition of microelements after O'Sullivan (1969) (in mg/l. of medium: Mn — 0.55, Cu — 0.064, Zn — 0.13, B — 0.54) for 21 days, with illumination of 3000 lux — 15 h daily, that is somewhat higher than the minimum indispensable for flowering of cereals (Grodzinskii and Grodzinskii, 1973) at 24°C in 5 replications. The experiments were carried out in 4 groups under the same conditions but at various dates. The pH of the medium was 6.0 (Lastuvka and Minar, 1967). The medium was not aerated, but delicately stirred with a magnetic stirrer to prevent sedimentation of insoluble iron compounds (Gumiński et al., 1965; Czerwiński, 1967, 1968). Iron was added to the medium in $FeCl_3$ form or FeEDTA in the amount of 0.5 mg/l. The leaves were numbered in succession of their development. The reducing and oxidating compounds were introduced into the medium in amounts giving the desired potential level.

The redox potential was measured with a pH-meter MV-11 (Clamann u. Grachnert) with the use of a platinum electrode and a saturated calomel electrode. The platinum electrode was washed with alcohol before the measurements and then with aqua regia followed by distilled water, and electrolysis was started in 1 N KOH. First the negative pole was connected to the electrode for 1 min, and then the positive pole for 2 min. After washing with distilled water the electrode was placed in 0.1 per cent methyl blue for 15 min (Spruit, 1952; Deibner, 1956). The electrode thus purified was calibrated in Michaelis buffer (+406 mV, 20°C). The medium potential was calculated from the formula

$$E_h = E_{cal} + E_{exp}$$

where E_h — value of redox potential of medium in relation to hydrogen electrode

E_{cal} — value of calomel electrode potential

E_{exp} — value of medium potential obtained in experiment.

Iron was determined by the o-phenanthrolin method (Marczenko, 1959) and chlorophyll by the spectrophotometric method (MacKinney, 1941). For statistical elaboration Student's t test was applied with $t = 2.78$ at confidence level 0.95, and for dry mass and chlorophyll content values $t = 2.31$ at confidence level 0.95.

Statistical analysis was applied to all combinations in reference to "control + $FeCl_3$" (t_1) and to "control + FeEDTA" for the combination with FeEDTA (t_2).

RESULTS

Influence of substances lowering the redox potential

The medium has a certain reducing-oxidating potential determined by a number of factors such as its composition, the amount of oxygen present etc. Changes in this potential may be accomplished by introducing various chemical compounds as it is commonly done in microbiology (Hevitt, 1950; Rodina, 1968). From the many substances reducing the potential of the medium noteworthy were considered those with relatively low toxicity or which, being components of redox systems in the plant, are "endogenous reductors". The following substances capable of reducing the redox potential of the medium were used: cysteine, ascorbic acid, hydroquinone, pyrocatechin, sodium sulphite and sodium sulphide. The potential of the medium without chemical compounds added was about +490 ±20 mV. The amount of the particular substances was so adjusted as to give a potential of about +390 ±15 mV.

All leaves on the plant were subjected to analysis (1—4). The most characteristic differences appeared in young leaves (3rd and 4th), therefore in the present paper the results for these leaves will be given as illustration.

The influence exerted by the lowered redox potential on the increase in dry weight of the plants (Table 1) is due to the action of the particular potential-depressing substances. Thus, in the case of hydroquinone, pyrocatechin, and sodium sulphite a certain decrease in dry weight is observed or it remains within the limits of the control values. A specially strong inhibitory action on growth was noted in the case of sodium sulphide.

Table 1

Influence of redox potential-depressing substances on dry weight of plants

No. of expt	Combination		Dry weight of 1 plant mg	t_1	t_2
1	Control	$+FeCl_3$	42	×	
2	Control	+FeEDTA	49	2.45	×
3	Hydroquinone	$+FeCl_3$	38	2.01	
4	Hydroquinone	+FeEDTA	44	1.80	2.38
5	Cysteine	$+FeCl_3$	77	4.85	
6	Cysteine	+FeEDTA	74	3.86	4.05
1	Control	$+FeCl_3$	43	×	
2	Control	+FeEDTA	52	2.45	×
3	Pyrokatechin	$+FeCl_3$	47	2.25	
4	Pyrokatechin	+FeEDTA	50	2.39	1.29
5	Ascorbic acid	$+FeCl_3$	69	3.42	
6	Ascorbic acid	+FeEDTA	72	4.82	3.09
7	Sodium sulphite	$+FeCl_3$	35	2.29	
8	Sodium sulphite	+FeEDTA	38	1.52	2.41
1	Control	$+FeCl_3$	51	×	
2	Control	+FeEDTA	57	2.41	×
3	Sodium sulphide	$+FeCl_3$	24	4.12	
4	Sodium sulphide	+FeEDTA	27	4.35	3.82

On the other hand, cysteine and ascorbic acid produce a significant increase in dry weight. In the presence of polyphenols, sodium sulphite and sulphide there are differences in the increase in dry weight in the combination with various iron forms ($FeCl_3$ and FeEDTA), but not when cysteine or ascorbic acid are applied. In Table 2 the resunlts of analysis of iron and chlorophyll content are shown under conditions of lowered potential. The leaves of plants growing in the presence of potential--depressing compounds exhibit an enhanced iron accumulation. This increase depends on the form of iron applied (Fe_3Cl, FeEDTA), and for polyphenols sodium sulphite and sulphide the differences are wide, while in the presence of cysteine or ascorbic acid they are slight. Cysteine and ascorbic acid exert the strongest influence on iron accumulation. Analysis of chlorophyll content showed that a lowered redox potential of the medium causes an increase in the content of this pigment. It should be mentioned that the fourth leaf growing on control medium with iron chloride was chlorotic, while no such leaves were observed in the remaining combinations. Cysteine, ascorbic acid and pyrocatechin had the strongest influence not only on iron accumulation, but on the increase of chlorophyll content, notwithstanding the form of iron applied.

Table 2

Influence of substance lowering redox potential of medium on iron and chlorophyll content in oat leaves

No. of expt	Combination	Iron content in dry weight mg/g.						Chlorophyll content in fresh weight					
		leaf no. 3			leaf no. 4			leaf no. 3			leaf no. 4		
		$\bar{x}$	t_1	t_2	$\bar{x}$	t_1	t_2	$\bar{x}$	t_1	t_2	$\bar{x}$	t_1	t_2
1	Control + $FeCl_3$	220	×		133	×		0.52	×		0.29	×	
2	Control + FeEDTA	265	2.81	×	241	2.98	×	0.70	3.21	×	0.82	3.25	×
3	Hydroquinone + $FeCl_3$	249	2.80		212	2.82		0.45	3.61		0.79	3.41	
4	Hydroquinone + FeEDTA	310	2.95	2.86	249	2.43	2.79	0.89	3.45	3.25	0.92	3.59	2.89
5	Cysteine + $FeCl_3$	421	4.03		325	3.49		1.14	4.11		1.28	5.03	
6	Cysteine + FeEDTA	433	3.85	3.34	318	3.72	3.04	1.16	4.05	3.18	1.30	4.29	3.21

Influence of substances raising the redox potential

As potential-raising substances hydrogen peroxide, potassium permanganate (Gumiński, 1950; Geller, 1950) and potassium dichromate were used (Leman, 1966). As in the case of potential-depressing compounds such amounts were used which gave as effect a redox potential of +590 ±20 mV. These substances raising the redox potential of the medium decreased the dry weight increment of the plants (Table 3). Both

Table 3

Influence of substances raising redox potential in medium on dry weight of plants

No. of expt	Combination	Dry weight of plant mg	t_1	t_2
1	Control + $FeCl_3$	48	×	
2	Control + FeEDTA	55	2.41	×
4	Hydrogen peroxide + $FeCl_3$	25	3.28	
4	Hydrogen peroxide + FeEDTA	28	3.18	3.21
5 6	Potassium permanganate + $FeCl_3$	37	2.82	
6	Potassium permanganate + FeEDTA	45	1.82	2.77
7	Potassium dichromate + $FeCl_3$	39	2.39	
8	Potassium dichromate + FeEDTA	41	2.41	2.80

in combination with iron in the form of chloride and in complex with EDTA a decrease of the iron content values in the leaves of plants growing in conditions of raised potential was noted (Table 4). In the combination where iron was applied in the form of chloride this decrease was more pronounced than in the case of iron in complex. The strongest effect in this respect was exerted by potassium dichromate and permanganate. Chlorosis of the 4th leaf was observed in all the combinations with raised potential, and in the combination with potassium compounds chlorosis begins to appear in the 3rd leaf. This disturbance finds confirmation in the lowered chlorophyll content in the leaves, the decrease in chlorophyll being smaller in the presence of complexed iron than of iron in the form of chloride.

Table 4

Influence of substances raising redox potential in medium on iron and chlorophyll content in oat leaves

No. of expt.	Combination		Iron content in dry weight mg/g						Chlorophyll content in fresh weight mg/g					
			leaf no. 3			leaf no. 4			leaf no. 3			leaf no. 4		
			x	t_1	t_2	x	t_1	t_2	x	t_1	t_2	x	t_1	t_2
1	Control	$+FeCl_3$	214	×		145	×		0.57	×		0.36	×	
2	Control	+FeEDTA	245	2.81	×	221	3.11	×	0.88	2.82	×	0.68	3.25	×
3	Hydrogen peroxide	$+FeCl_3$	212	0.69		121	2.82		0.65	2.41		0.31	1.82	
4	Hydrogen peroxide	+FeEDTA	240	2.67	0.32	205	2.91	0.98	0.72	2.74	2.29	0.52	2.13	2.62
5	Potassium permanganate	$+FeCl_3$	180	2.79		108	3.02		0.32	3.49		0.31	2.32	
6	Potassium permanganate	+FeEDTA	205	1.95	2.90	193	2.80	2.71	0.58	1.15	3.31	0.40	0.52	3.21
7	Potassium dichromate	$+FeCl_3$	183	2.84		95	3.23		0.40	3.18		0.25	2.51	
8	Potassium dichromate	+FeEDTA	224	2.39	2.69	171	2.12	2.92	0.72	2.51	2.81	0.49	2.29	2.49

DISCUSSION

The investigations indicate that the effect of the redox potential in the nutrient solution should be considered as direct and indirect influence. The direct effect is conected with the state of availability of iron to the roots in the nutrient solution, whereas the indirect influence is associated with the physiological activity of iron in plant tissue.

It was found that the value of the redox potential determines the degree of iron oxidation in the medium, both when it is present in ion form or in complex. The two forms of iron used in the experiments differ widely by the degree of their solubility in the nutrient solution. Iron chloride introduced at pH 6.0 is precipitated as iron hydroxide and phosphate and in this form it is little available to plants. On the other hand FeEDTA is well soluble under the same conditions giving an assimilable form of iron.

The rise of redox potential of the medium due to oxidating substances gave as effect he presence in the solution of only trivalent iron. Lowering of the redox potential of the medium by introduction of reducing substances led to a reduction of trivalent iron, so that an increase in bivalent iron occurred. The iron oxidation level is closely bound with its solubility. This may be best illustrated by Granick's results (1957) who demonstrated that at pH 7.0 of the solution the concentration of soluble Fe^{2+} is 10^{-4} M and of trivalent iron it is as low as 10^{-17} M. Thus, at constant pH value of the medium, the amount of soluble Fe^{2+} is determined by the value of the redox potential. This finds confirmation in the iron accumulation in plants, which is most intensive in those growing under conditions of lowered potential. These results agree with the suggestions of those investigators who consider that iron is taken up in bivalent form (Kliman, 1937; Erkama, 1950; Sommers and Shive, 1942; Thorne and Wallace, 1944; Granick, 1957).

Although there are wide differences in ionic iron solubility at various oxidation levels, complexed iron, both Fe^{2+}-EDTA and F^{3+}-EDTA, is readily soluble.

Iron was introduced into the medium in the form of Fe^{3+}-EDTA, but under conditions of lowered potential this iron undergoes rather readily reduction (Garvon, 1964). Similarly as in the case of ionic iron, an enhanced accumulation was noted when the potential was lowered, but this cannot, as already mentioned, be explained by the better solubility of the ferrous complex. According to the hypothesis of Hill-Cottingham (1955, 1957, 1965), and the results of Chaney, Brown and Tiffin (1972), the first step in trivalent complexed iron assimilation is its reduction. It is possible that under conditions of depressed potential this first step of iron assimilation occurs in the medium, owing to which it is more readily assimilated.

Chlorophyll accumulation in the leaves is a manifestation of physiological activity of iron in the plant. In the present experiments accumulation of chlorophyll in the leaves was associated with iron accumulation in them. Both the ionic form of iron and the complex were found to increase the chlorophyll content when the potential was lowered in the medium. This proves that the increase in soluble bivalent iron content stimulates chlorophyll accumulation. Bivalent iron activates synthesis of chlorophyll precursors, δ-aminolevulinic acid (Marsh et al., 1963) and coproporphyrinogen III (Lascelles, 1961; Stephan, and Machold, 1969). A decrease in the amount of bivalent iron in conditions of raised potential causes a reduction of chlorophyll content in the leaves. According to Thorn et al. (1950), oxidation of bivalent iron inhibits chlorophyll synthesis, and this seems to decide on the content of this pigment in the leaves. Physiological activity of bivalent iron is stressed by Machold et al. (1968) who found that chlorotic leaves do not contain this form, in contrast to green ones. In spite of the differences in the action of the particular reducing or oxidating substances, manifested in their different effect on plant growth, beside some specific influence, all these compounds enhanced iron and chlorophyll accumulation, while all oxidating substances had a reverse effect.

When the results of investigations on the relation between the redox potential value of the medium and iron accumulation and chlorophyll content in leaves are analysed in the light of the quoted suggestion of Machold (1968) the causative relation between this factor and physiological processes may be represened as follows:

Redox potential of the medium

↓

Fe^{3+} : Fe^{2+} ratio

↓

metabolism (chlorophyll synthesis).

CONCLUSIONS

1. The redox potential of the nutrient medium is one of the factors determining the availability and physiological activity of iron in plants.

2. Lowering of the redox potential of the medium stimulates iron accumulation in leaves, whereas a rise of this potential has an inhibitory effect.

3. Changes in redox potential of the medium influence chlorophyll accumulation in leaves. This is manifested by an increase of chlorophyll content at lowered potential and a decrease under conditions or raised potential.

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