A non-invasive method for redox potential measurement
(Neinvazivní metoda měření redoxních potenciálů u rostlin)

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Souhrn

Redoxní potenciál (RP) byl dosud převážně měřen vpichem platinové elektrody do pletiva rostlin. RP je možno měřit v některých případech i neinvazivní metodou plíškovou platinovou elektrodou a srovnávací nasycenou kalometovou elektrodou umístěnými volně vedle rostlinných částí ponořených do vody. Umístěním pletiv do vody se navodí prostředí se sníženým obsahem kyslíku, dýchání buněk je podstatně omezeno a RP klesá až ke spodní hodnotě dýchacího řetězce buňky. Práce uvádí návrh neinvazní metody měření redoxního potenciálu pro pokusy a předběžné výsledky s její aplikací na základní fyziologické pochody rostlin. Z měření vyplývá, že přenašeče elektronů činné při dýchání jsou rozpustné ve vodě a difundují snadno z buněk do vnějšího prostředí. Nemůže se tedy jednat o ubichinon, který je rozpustný v tucích a je vázaný na stěnu buněčnou. Přenašeče elektronů nejsou oxidovány přímo vzdušným kyslíkem, ale jen prostřednictvím dýchacích enzymů. Kyslík produkovaný fotosyntézou může zčásti zabránit poklesu RP pletiv v hypoxii, stejně jako dusičnanový ion v exudátu kořenů. RP i ve vnějším vodním prostředí odráží vnitřní stav pletiv rostlin a lze jej použít pro vysvětlení celé řady základních fyziologických procesů. Dýchací procesy i změny RP probíhají relativně velmi rychle, např. pokles z +180 mV na -500 mV proběhne v některých případech i za 15 minut.

Klíčová slova: dýchání, kyslík, ubichinon, fotosyntéza, hypoxie, pšenice, ječmen, brambory, exudát

Summary

Until 2007, redox potential (RP) was measured generally by incision into plant tissue with a platinum (Pt) electrode. In some cases it is possible to measure RP by the non-invasive method using a Pt sheet electrode and comparative saturated calomel one. Both electrodes were put beside the plant parts submerged in water. Environment with decreased content of oxygen is induced by putting tissues into water, the cell respiration is substantially reducing and the RP value is sinking to the bottom value of respiration chain of cells. In this work, I present the proposal of a non-invasive method for RP measurement and some preliminary results with its application in basic physiological processes of plants. Based on the measurement it may be deduced that the electron carriers are soluble in water and that they are diffusing easilly from cells to the outer environment. It is not the ubichinon, which is soluble in fats and it is fixed to the cell wall. The electron carriers are not oxidated directly by the air oxygen but by the respiration enzymes only. Oxygen produced by photosynthesis can partly prevent the tissues RP sinking in hypoxia as well as nitrate ion in root exudates. Even in outer water environment RP reflects the inner plant tissue situation and it can explain many basic physiological processes. The respiration actions go on relatively very quickly, e.g. the sink from +180 mV to -500 mV can pass in some cases even in 15 minutes.

Key words: respiration, oxygen, ubichinon, photosynthesis hypoxia, wheat, barley, potatoes, exudate

Introduction

The RP research was primarily initiated for the elucidation of changes in host resistance against obligate parasites. In further experiments, it was discovered that RP could substantially contribute to explanation of plant integrity concept (especially for determination of auxin transport direction) and a number of further functions, for example cell respiration, nutrition and others. In contrast to current RP conception in physical chemistry, when constant electric values are looked for for redox pair combinations, RP of plant tissues depends on respiration and changes all the time.

In the last forty years (see the review of publications *Benada* 2008), RP has been examined using Pt electrode incision into the roll of leaves of cereals or other plants or into the tough tissue (for example into the potato tuber).

With regard to eventual objections that RP can be changed by incision into plant tissues, it was necessary to test the RP in cases when the electrode was not incised into the tissue. In principle, this technique is acceptable because the electron carriers determining RP are soluble in water and they diffuse easily out of the cell as could be deduced from the host-parasite relationship (*Benada* 2008).

The present work demonstrates a concept of a non-invasive method for RP measurement and some preliminary results with its application to basic physiological processes of plants. The results are shown in a preliminary form because it may be a guideline for successors how to arrange the experiments.

Methods (a proposal of techniques and preliminary results)

The non-invasive method for RP measurement can be used especially under conditions of substantial decrease in air access to plant tissues by their submergence in water. In following experiments, current tap water was used. In this water no redox systems are present which could react with redox systems of the cell. It is possible also to use distilled water whose conductivity is satisfactory by dissolving diffusible substances from the cell. In tap as well as distilled water the air oxygen is dissolved that could react with a Pt electrode. The concentration of this redox system is however low. The amount of dissolved oxygen will be changed in dependence on atmospheric pressure. This must be the object of further investigation.

The value of proper RP system diffusing from the cell can be influenced by enzyme systems (by respiration). The electron carriers of cells do not react with oxygen directly, only by means of respiration enzymes.

RP was measured by a bright Pt electrode and a comparative saturated calomel electrode placed freely beside the plant parts submerged in water. The RP values are presented in a form directly obtained from measurement without conversion to value of saturated calomel electrode (+244 mV). All presented data come from experiments conducted in 2008. For individual experimental groups, only a limited number of measurements or only an experimental proposal are presented, and the results are to be regarded as preliminary.

Table 1.: RP values in water with germinated grains

Measurement No.	Crop/ variety	Start of measurement/ date and time	RP value mV	End of measurement/ date and time	RP value mV
1	Wheat Ebi	on 11.04. 7.00	+70	on 11.04. 7.45	-540
2	Wheat Ebi	on 17.04. 8.30	+160	on 17.04. 11.15	-505
3	Barley Jersey	on 10.04. 7.00	-	on 10.04. 7.30	-560
4	Barley Jersey	on 10.04. 8.00	-	on 10.04. 9.00	-570
5	Barley Jersey	on 10.04. grains without pregermination 11.00	+120	on 11.04. 7.00	-552
6	Barley Jersey	on 12.06. germinated for 3 h measured at 10.00	+54	on 13.06. 7.00	-568

1) RP of germinating seeds in hypoxia

a) cereal grains

Winter wheat and spring barley grains were germinated and submerged into water in which RP was measured (Table 1).

Partial conclusion: The RP measured corresponds with the bottom RP value of respiration chain NADP (RP of calomel electrode +240 mV–560 mV = standard RP NAD + $NADH^+$ -320 mV).

b) RP comparison of various cereal species and varieties or even other plants, especially peas with hypogeic cotyledons, and other legumes to observe changes in time

c) The influence of weight of germinated grains on the decrease rate, influence of temperature on reaction rapidity

I propose to test the temperatures of 5, 15, 20, 25 and 35 °C.

d) The influence of germination level on decrease degree of RP

e) The influence of boiling on the reaction of grains (destruction of enzymatic system by boiling)

Some preliminary results:

Germinated grains of wheat Ebi were boiled for 15 minutes. They were removed from hot bath and cooled with water 20 °C in which the RP was tested. The RP value sinked slowly within 20 hs from + values to -450 mV.

The same procedure was used for barley var. Jersey: The onset of measurement at 9.00, RP value +108 mV, at 10.00 +116 mV, at 11.00 +89 mV, during 20 hs RP sinked to -314 mV.

In the following experiment, the germinated grains of Jersey barley were submerged into boiling water and then cooled with fresh water in which the RP was measured. The RP values sank within 2 hs to -446 mV.

Partial conlusion: Both the electron carriers (probably phenolic substances) and the relevant enzyme systems are relatively resistant to the action of high temperature.

2) The influence of various nitrogen forms in nutrient substance on RP change in hypoxia

The nitrate ion under experimental hypoxic conditions in root exudate prevented the RP sink in experiments with cereal plants grown in sand culture and having one leaf in presented results (*Benada* 1995).

In new experiments, plants grown on rolled filter paper and employed for determination of seed health state (strips of filter paper 10 x 50 cm, grains were laid out in the distance of 2 cm from the top, then the paper was rolled and erected in a tray with a low water level (*Benada* 1995)) were used. During the germination, the aerobic environment for the roots was provided by capillary action in the paper. RP was high, but it decreased promptly in hypoxia.

a) RP change in the barley roots eluate grown on rolled filter paper by hypoxia

Barley variety Bodega grains were laid out on rolled filter paper by 50 pieces and at the time when the leaves were approximately 10 cm long, RP was measured in the area of roots. The values obtained: +197 mV, +193 mV, +198 mV, +193 mV.

Then the rolled paper with plants was placed into pots with a volume of 200 ml and submerged into water (Table 2).

Table 2. : RP of young barley plants submerge in water on March 28 (hypoxia)

Sample No.	Time	RP mV	Time	RP mV
1	8.10	+93	8.30	-612
2	8.30	+90	9.12	-561
3	9.13	+70	9.44	-544

Partial conclusion: The rolled filter paper used for health state estimation of grains fits for the study of RP decrease.

b) RP change in the roots eluate of cereals grown on rolled filter paper by hypoxia and influence of different forms of nitrogen salts

At the beginning of the experiments, the leaf length was aproximatelly 40 mm. The rolled paper was put into pots with 200 ml volume and submerged into water. The salts with content of nitrogen were added into pots at the amount of ca. 0.2 g (Table 3).

Partial conclusion: For prevention of RP decrease, the nitrate salt must be added to roots from plants having first leaf submerged in water. The plants must not be grown under conditions with saturation of nitrogen nutrition. Amonium salt as well as urea do not prevent the RP sink under similar conditions.

Table 3. The influence of various nitrogen forms on RP in root eluate of plants with first leaf under hypoxic conditions

Variant	Exposition time (hs)	RP mV
Wheat Ebi – control	24	-340
Wheat Ebi – nitrate	24	+185
Barley Jersey – control	24	-518
Barley Jersey – nitrate	48	+100
Barley Jersey – urea	48	-549
Barley Jersey – ammonium sulphate	48	-570

c) Influence of nitrate salt on RP in eluate of germinating grains in hypoxia

– wheat Ebi: the addition of calcium nitrate solution to the pots where the germinating grains were put in. RP sank to -540 mV within 30 minutes,

– another experiment: germinating grains of wheat var. Ebi, the addition of potassium nitrate, RP decreased to -372 mV within 30 minutes.

Partial conclusion: The nitrate anion does not prevent the RP decrease in germinating grains of wheat.

d) It is necessary to explain why the nitrate prevents RP decrease in root eluate only in plants with formed first leaf? What is the reaction of plants with more leaves?

e) Will RP in eluate of isolated roots from "rolled paper" plants decrease after addition of nitrate in hypoxia?

Table 4. RP of various variants of plants with leaves and roots in hypoxia

Variant	Time (h)	RP mV
1) Barley Jersey leaves + roots – control	20	-570
2) Barley Jersey leaves + roots – nitrate	20	-370
3) Barley Jersey roots only – control	20	–495
4) Barley Jersey roots – nitrate	20	-191
5) Wheat Ebi leaves + roots – control	22	-528
6) Wheat Ebi roots	20	-520
7) Wheat Ebi roots – nitrate	20	-120

Partial conclusion: For RP decrease the roots are suffucient in hypoxia. The nitrate stops the RP decrease in the experiments with roots only, but the plants from rolled paper should be older, i.e. having longer leaves by several days of cultivation. In the presented experiments, the nitrate addition prevented RP decrease at different rates.

3) RP in eluate of cereal leaves and ears in hypoxia

a) Hypoxia in cereal green leaves

– The leaves of wheat variety Briliant were rolled and submerged into water in the beaker with on May 26 at 8.30: RP was +172 mV. On May 27 at 8.00: RP obtained was +198mV. Additional RP measurement under aerobic conditions; the rolled leaves were incised with a Pt electrode, on May 26 at 8.30: RP obtained was +78 mV with lower turn point +62 mV.

– The leaves of spring barley var. Sebastian were rolled and submerge into water in the beaker. On May 23 at 9.45 RP was +172, at 11.00 it was +187 mV.

Additional RP measurement under aerobic conditions on May 28 at 7.00: RP +225, after 5 days with leaves continuously emerged in water RP was -595 mV.

Partial conclusion: In green cereal leaves, RP does not sink in hypoxia. The oxygen evidently released by photosynthesis is used for respiration.

b) Hypoxia in cereal green ears

On June 9, two green ears of flowering wheat were put into a beaker, the ears were rolled and submerged into water. At 10.00 RP was +29 mV, at this value RP stopped at least for 30 minutes. The next day on June 6 at 6.30 h, RP was -559 mV.

Partial conclusion: It may be concluded that the photosynthesis and oxygen production in green ears proceeds more weakly than in leaves.

c) Hypoxia in green leaves of other plants

– Poppy leaves were rolled, put into a beaker and submerged into water. On June 6 at 10.00, RP was +26 mV, at 11.00 -42 mV, on June 11 at 7.00 -307 mV, on June 12 at 6.00 -541 mV.

Partial conclusion: **In poppy leaves the assimilation and RP decrease goes on a low level.**

– Grapevine leaves. The measurement onset on June 17 at 7.15, RP was -93 mV, at 8.00 +7.00 mV, at 8.30 +30 mV.

– Potato leaves: at 8.00 RP was -6 mV, at 9.00 -37 mV, at 10.30 +9 mV.

Partial conclusion: **The influence of assimilation in grapevine and potato on RP in hypoxia is to be tested in further experiments.**

4) Influence of hypoxia on RP of organs where photo-synthesis does not proceed (other than cereal roots)

– Potato tuber slices were put into a beaker, submerged into water. The onset of measurement on May 27 at 11.00, RP was +90 mV, on May 28 at 8.00 -471 mV, at 8.30 -507 mV.

Partial conclusion: In potato tuber the photosythesis and production of oxygen do not proceed, RP sinks to the bottom limits of respiration chain.

5) Investigation of influence of temperature and light intensity on RP of eluate from tissues in hypoxia (Proposal)

Partial conclusions from trials with the influence of hypoxia on green parts of plants

The oxygen produced in photosynthesis prevents the RP sink in hypoxia. The experiments were carried out in diffused illumination in the laboratory where the light and photosynthesis intensity was low. Different organs have different photosynthesis levels and thus the oxygen production. Moreover, the leaves were rolled and this decreases light access and effect of photosynthesis.

6) The testing of assumption that plant tissue eluate has RP similar to that of the tissues

The potato slices were put into a beaker filled with water.The RP measurement was performed using a Pt electrode incision into the tissue or the electrode was submerged into the eluate beside plant tissues. The onset state of RP on June 16 was as follows: at 7.00 -3 mV, at 7.30 -18 mV (Table 5).

Table 5.: The comparison of RP in potato tissues and in eluate of potato slices

Date	June 16				June 17
Time	8:30	9:00	10:00	10:30	7:00
Eluate	-128	-173	-230	-260	-569
Tissue	-139	-300	-212	-328	-575

Partial conclusion: Close RP values were found in eluate and tissues of potato slices.

7) The RP change in eluate from potato tubers after putting them away

The potato slices were submerged into water on June 18 at 7.00. At 9.00 the RP was -290 mV, at 9.30 -385 mV. Then the slices were removed from water, RP began to increase: at 9.35 to -149 mV, at 9.45 to -67 mV. On June 19 at 7.00 it decreased to -266 mV.

Partial conclusion: RP is dependent on tissue respiration and it increases after removing the tissues from eluate. Air penetrates into eluate. The deep RP decrease on the second day (on June 19) could be interpreted by the influence of microflora expansion under hypoxic conditions.

Discussion

Even under outer water conditions RP reflects the inner state of plant tissues and it may be used for elucidation of basic physiological processes. The electron carriers operating in respiration are soluble in water and they diffuse into outer environment. That cannot be ubichinon which is soluble in fats and which is attached to cell membrane as it is given in most handbooks (e.g. *Procházka et al.* 1998, *Handbuch* 1960). Both the respiration processes and RP changes go relatively very promptly, i.e. the decrease from +180 mV to -500 mV goes within even in 15 minutes.

Hypoxia enables to uncover many significant physiological processes using RP values. Because oxidation and reduction of electron carriers are fixed to respiration enzymes and it is necessary to take into account the variability of obtained values. The RP values in the plant physiology is appreciable and dynamic factor as has been presented several times in earlier papers (*Benada* 2008). The diffusion of electron carriers acting in respiration and infiltrating the outer water environment is a substantial ingredient of new theory of recognition between the host and its parasites, and it forms the fundamentals of plant resistance against diseases (*Benada* 1991).

Conclusion

In the present work, a non-invasive method for RP measurement in plants was applied. The basis of this method is RP measurement with a sheet Pt electrode and comparative saturated calomel electrode freely placed beside the plant parts submerged in water. The work presents the methods as well as results of laboratory experiments performed in 2008 as proposals for further experiments. For individual experiments only a limited number of values or the experiment proposals are presented, and the results are to be considered as preliminary. The results are given in preliminary form because the work may be the guideline for further investigation of successors.

The following conclusions may be the basis for further experiments:

1) The electron carriers acting in respiration are soluble in water and diffuse to outer water environment.

2) The electron carrier acting in respiration is not ubichinon, because this is soluble in fats and is bound to the cell wall.

3) The oxygen formed in photosynthesis shares in respiration of green parts submerged in water and it reflects even in RP.

4) RP even in outer water environment reflects the inner state of plant tissue given by RP and it can be used for elucidation of a number of basic physiological processes.

5) The respiration processes can proceed relatively very promptly, i.e. the decrease from +180 mV to -500 mV results even in 15 minutes.

6) The RP of electron carriers is changed by respiration enzymes and it is not influenced directly by air oxygen.

7) Hypoxia enables through RP to study main physiological processes.

8) The rolls of filter paper used for estimation of grain germination are suitable material for study of influence of nitrogen form on RP sink.

Závěr:

Jako základ pro další výzkum poslouží následující závěry:

1) Nosiče elektronů činné při dýchání jsou rozpustné ve vodě a difundují do vnějšího vodného prostředí

2) Nosičem elektronů činným při dýchání není ubichinon, protože je rozpustný v tucích a je vázaný na stěnu buněčnou.

3) Kyslík produkovaný při fotosyntéze se účastní v dýchacím procesu zelených pletiv ponořených do vody a projeví se i v RP

4) RP i ve vnějším vodním prostředí odráží vnitřní stav pletiv rostlin daný RP a lze jej použít pro vysvětlení celé řady základních fyziologických procesů

5) Dýchací procesy i změny RP mohou probíhat relativně velmi rychle např. pokles z +180 mV na –500 mV i za 15 minut

6) RP nosičů elektronů je měněn dýchacími enzymy a není přímo ovlivněn vzdušným kyslíkem

7) Hypoxie umožňuje prostřednictvím RP zjistit řadu důležitých fyziologických procesů

8) Rolády z filtračního papíru používané pro hodnocení klíčení obilek jsou vhodným materiálem pro sledování vlivu formy dusíku na pokles RP.

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