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ORIGINAL ARTICLE

Identification of nutrient deficiency in plants by artificial intelligence

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Abstract
The deficiency of macro (N, P, S, Ca, Mg and K) and micro (Zn, Cu, B, Mo, Cl, Mn and Fe) minerals has a major effect on plant development. The lack of some nutrient minerals especially of nitrogen, potassium, calcium, phosphorus and iron is a huge problem for agriculture and early warning and prevention of the problem will be very useful for agro-industry. Currently, the methods used to determine nutritional deficiency in plants are soil analysis, plant tissue analysis or combined methods between the two aforementioned ones; however, these methods are time-consuming and costly. This study proposes a new method for determining nutrient deficiency in plants based on the rapid fluorescence of chlorophyll *a*. In the process of this research bean plants are grown on a complete nutrient solution (control plants) compared with those grown on a nutrient medium, which lacks one of these elements—N, P, K, Ca or Fe. In this article, the mineral deficiency in nutrient solution is evaluated by the stress response of the plants estimated by leaves photosynthetic activity. The photosynthetic activity is estimated by analysis of the chlorophyll fluorescence using JIP test approach that reflects functional activity of Photosystems I and II and physiological state of the photosynthetic apparatus as a whole. Furthermore, the fluorescence transients recorded from plants grown in nutrient solution with deficiency of N, P, K, Ca or Fe, are used as input data in Artificial Neural Network (ANN). This ANN was trained to recognize deficiency of N, P, K, Ca or Fe in the bean plants. The ANN is presented as a potential tool for identifying/predicting nutrient deficiencies in bean plants, using records of the fast fluorescence of chlorophyll *a*.

Keywords Plant nutrient deficiency · Chlorophyll fluorescence · OJIP induction curves · JIP parameters · Artificial neural network · Prompt chlorophyll fluorescence

Introduction

To complete their life cycle and physiological functions, plants need chemical elements such as N, P, K, Ca, Mg, S, Fe, Mn, Zn, Cu, Cl, B and Mo. The elements (N, P, K, Ca, Mg, S) are required in large quantities (> 1000 mg/kg dry matter) and are called macronutrients. On the other hand, Fe, Mn, Zn, Cu, Cl, B and Mo are required in extremely small amounts (< 100 mg/kg dry matter) and are called micronutrients (Osman 2013). Soil pH levels also affect the absorption of nutrients by plants. All of these minerals are available for plants in the range of pH 5.5–6.5 (Lucas and Davis 1961).

This article will examine the ways in which the deficiency of N, P, K, Ca and Fe in *Pharsalus vulgaris* can be determined by an artificial intelligence algorithm.

Nitrogen is the most important mineral for plants and its deficiency is crucial for plant vitality. The nitrogen is involved in the building of the amino and nucleic acids, it is important for biochemistry of coenzymes, for the photosynthetic pigments and for the polyamines (Maathuis 2009). The chloroplast proteins contain almost 75% of the nitrogen that exists in the leaves of the plants and about 27% of them are utilized in Rubisco (Cetner et al. 2017). In the chloroplasts, the nitrogen is associated with the light harvesting apparatus, photosystem I (PSI), photosystem II (PSII), electron transport chain, peripheral proteins and ATF synthase.

Nitrogen deficiency leads to reduction of plant size, due to the breakdown Rubisco capacity for CO_2 fixation which leads to a decrease in the photosynthesis rate and inhibits the plant growth (Wei et al. 2015). In plants with nitrogen starvation, also decrease in chlorophyll content is observed

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(Duli et al. 2003). Furthermore, PSII activity in plants with nitrogen deficiency is interrupted at each level. Subsequently, this leads to a decrease in the electron transport rate through the electron transport chain in the thylakoid membrane (Cetner et al. 2017).

Phosphorus is another crucial microelement for plant growth. It is involved in the composition of ATP, DNA and RNA; in the phospholipids constituting the cell membranes; in the sugar–phosphate intermediates; and lastly, in photosynthesis and breathing. This element is included in almost all metabolic processes. It plays an important role in the assimilation of carbon and nitrogen, in energy processes and lipid metabolism.

Phosphorus deficiency in plants leads to limited growth and low shoot/root dry matter ratio. Also P deficiency affects the development of reproductive organs and decreases the number of flowers as well as the formation of fruits and seeds (Sarker and Karmoker 1970).

The deficiency of P affects the carbon metabolism in the plants, because orthophosphate (Pi) is a major regulator of this type of metabolic processes. Whenever the levels of phosphorus are low, they reduce the CO_2 assimilation and this leads to a reduction of photosynthetic electron transport rate (Carstensen, et al. 2018).

Potassium (K+) is a very important macronutrient for plants and it is involved in plant development and overall productivity. The potassium ion is important for photosynthesis, osmoregulation, enzyme activation, protein synthesis, and ion homeostasis. The early visual symptom of potassium deficiency is chlorosis, which then develops into necrosis. The potassium ions are not involved directly in photosynthetic metabolism; however, K-deficiency strongly affects photosynthesis as a whole due to lack of potassium leading to a decrease in the ATP synthesis and reduction of the CO_2 assimilation (Hafsi et al. 2014). Additionally, K-deficiency leads to a reduction of the photosynthetic process as a result of the low chlorophyll content (Duli et al. 2001). In addition, potassium plays a crucial role in the resistance of plants to pests and diseases (Amtmann et al. 2008).

Calcium deficiency is associated with poor plant growth, leaf necrosis and deformation. Calcium is vital for the metabolism of plants and regulates the structure of plants. Calcium ion plays a crucial role in membrane structure and its functionality, especially for membrane permeability (Hepler 2005). Ca ions are involved in the regulation of enzyme synthesis (protein kinases or phosphatases) in the synthesis of new cell walls, which demonstrates that Ca-deficiency is be extremely harmful to plants. Calcium deficiency disrupts plant photosynthesis because Ca is a part of Mn_4CaO_5 cluster.

Iron deficiency causes chlorosis due to the reduced amount of chlorophyll, and leaves without Fe are smaller than normal (Osman 2013). The Photosynthesis is a sensitive process for the lack of Iron. This element is important for chlorophyll synthesis and as it participates in Fe–S proteins, in the ferredoxin and in the cytochrome in the photosynthetic electron transport chain. Moreover, Fe is represented in the cytochrome b559, as non-heme Fe in the PS II acceptor side and in the stromal part of core proteins between quinones Q_A and Q_B (Yruela 2013).

Plants emit several kinds of light: prompt fluorescence (PF), delayed fluorescence (DF), thermoluminescence and phosphorescence. For the aims of this research are used prompt fluorescence signals emitted by plants and its signals were used as an input data in the Neural Network. The choice of fluorescence of chlorophyll *a*, is specifically due to the fact that the fluorescence signals are sensitive to the nutritional stress of the plants (Kalaji et al. 2012). The chlorophyll fluorescence is a rapid, non-destructive method for a diagnostic of the plant stress conditions. Overall, two methods are used for the measurement of the prompt chlorophyll fluorescence a PF signal produced following a pulse-amplitude-modulated excitation and a PF signal emitted during a strong continuous actinic excitation (Kalaji et al. 2012). In the process of the experiments, the latter method was used to measure PF signals. The fluorescence rise during the first second of illumination from the initial (Fo) to the maximal (Fm) fluorescence value. The nomenclature of the kinetic induction curves of the fast (up to 1–2 s) Chl a fluorescence transient is OJIPS. The analysis of these curves is called "JIP test ", which is based on the theory of energy fluxes in biomembranes (Strasser et al. 2004). The different parameters from PF signals and the induction curves are developed which are linked with the different steps and phases of the PF transient and the redox states of PSII. This makes the OJIPS curves and the JIP parameters as possible tools to study the nutritional content of plants (Strasser et al. 2004). There are some articles that shows that there is a good correlation between fast Chl a fluorescence and nutrient deficiency in plants. Put differently, fluorescence of the chlorophyll *a* is a good indicator of the nutritional status of plants (Aleksandrov et al. 2014; Kalaji, et al. 2014; Cetner et al. 2017). Due to the difference in induction curves emitted by plants with different nutritional status, it is permissible to use the OJIPS curves and JIP parameters as Artificial Neural Network (ANN) input data. Artificial neural network is one of the most important tool in modern science and this paper provides evidence for the uses of ANN in recognition of nutrient deficiency of plants. In this study is used ANN with backpropagation of errors (Svozil et al. 1997).

The purpose of the study is to investigate how the kinetic induction curves and JIP test parameters are changed in nutrient deficiency in plants and to use these parameters and curves as input data for ANN to determine the nutritional status of the plants.

Materials and methods

Plant material

Bean plants (*Phaseolus vulgaris* cv. Cheren Starozagorski) was grown in 1 dm^3 dark glass pots filled with a modified Hoagland nutrient solution (see Tables 1, 2 and 3 for the components of solution). Solutions were supplied with oxygen by electrical pumps and replaced every 2 days. The pH of the nutrient mediums was about 5.0 for all modified solutions. The average temperature for day/night was 26/18 °C, respectively, relative humidity was 50–60%, and the photoperiod for the day/night cycle was 16/8 h. The maximum photosynthetically active radiation was about 4000 µmol (photons) m^{-2} s^{-1}. After a week of growth in full Hoagland solution, the plants were moved to stressed nutrient mediums. 14 days after the stress application (21 days after emergence) prompt chlorophyll *a* fluorescence (PF) measurements were done on 9 fully developed leaves for each treatment.

Chlorophyll fluorescence measurement

Induction kinetics of PF were measured with a Multifunctional Plant Efficiency Analyzer, MPEA (Hansatech Instrument Ltd., King's Lynn, Norfolk, PE30 4NE, UK) (Strasser et al. 2010). Prior to taking measurements as part of the experiment, each plant was kept in a dark area at least for 30 min. Measurements were made on the abaxial surface of fully developed leaves on the middle part of the chosen leaf. Measured signals were analyzed by M-PEA-data analyser version 5.4 software (this software is laboratory designed in the Dept. of Biophysics and Radiobiology, Sofia University by Petko Chernev, PhD).

JIP test parameters

These parameters are obtained from various characteristic points of photoinduced chlorophyll fluorescence transients and are a useful instrument for analysis of plant photosynthetic apparatus (Strasser et al. 2004; Strasser et al. 2010). The parameters used in this paper are described in Table 4.

Statistical analysis

All of the experiment data were statistically analyzed and the non-parametric Kruskal–Wallis one-way analysis of variance by ranks was applied to the study.

Artificial neural network

The artificial neural networks ("ANN" hereafter) are computer models based on ideas for multiple regression and classification analysis that consist of several elements operating in parallel. The functionality and capacity of the network

Table 1 Nonmodified Hoagland solution

Compound	Molecular weight	Concentration of stock solution	Volume of stock solution per liter of final solution	Element	Final concentration of element	
	g mol^{-1}	gL^{-1}	ml		mM	ppm
Macronutrients						
KNO_3	101.10	101.10	6.0	N	16	224
				K	6	235
$Ca(NO_3)_2 \cdot 4H_2O$	236.16	236.16	4.0	Ca	4	160
$NH_4H_2PO_4$	115.08	115.08	2.0	P	2	62
$MgSO_4 \cdot 7H_2O$	246.48	246.49	1.0	S	1	32
				Mg	1	24
Compound	Molecular weight	Concentration of stock solution	Volume of stock solution per liter of final solution	Element	Final concentration of element	
	g mol^{-1}	gL^{-1}	ml		µM	ppm
Micronutrients						
KCl	74.55	1.864	2.0	Cl	50	1.77
H_3BO_3	61.83	0.773	2.0	B	25	0.27
$MnSO_4 \cdot H_2O$	169.01	0.169	2.0	Mn	2.0	0.11
$ZnSO_4 \cdot 7H_2O$	287.54	0.288	2.0	Zn	2.0	0.13
$CuSO_4 \cdot 5H_2O$	249.68	0.062	2.0	Cu	0.5	0.03
NaFeDTPA(10% Fe)	468.20	30.0	0.3–1.0	Fe	16.1–53.7	1.00- 3.00

Table 2 Modified Hoagland solution

Hoagland solution	Full pH 5.05	(–Ca) pH 4.89	(–K) pH 4.82	(–N) pH 4.87	(–P) pH 4.94	(–Fe) pH 5.12
Ca (NO_3)·4H2O	4	–	4	–	4	4
KNO_3	6	6	-	–	6	6
$MgSO_4·7H_2O$	2	–	2	2	2	2
$NH_4H_2PO_4$	2	2	2	–	–	2
Mg $(NO_3)_2·6H_2O$	–	4	–	–	–	–
$MgCl_2·6H_2O$	–	–	–	–	–	–
Na_2SO_4	–	2	–	–	–	–
$NaNO_3$	–	–	6	–	–	–
$CaCl_2$	–	–	–	4	–	–
KCl	–	-	–	2	–	–
NaH_2PO_4	–	–	–	2	–	–
NH_4NO_3	–	–	–	–	1	-
1% Iron Citrate	1	1	1	1	1	-
Microelements (solution A)	1	1	1	–	1	
Microelements (solution B)	–	–	-	1	–	–

The composition of the various culture media in mM. The concentration of minerals was achieved by addition X cm3 of concentrated stock solution (1 mol per 1 dm^3) of corresponding component per 1 dm^3 of medium. Numbers in the brackets indicate the pH of each nutrient solution

Table 3 Salts containing micronutrients (without iron) used in modified Hoagland solution

Salts containing micronutrients	Quantity (g dm^{-3} H_2O)	
	Solution A	Solution B
H_3BO_3	2.85	2.85
$MnSO_4·4H_2O$	1.10	–
$ZnSO_4·7H_2O$	0.28	–
$CuSO_4·5H_2O$	0.10	–
$(NH_4)_6Mo_7O_{24}·4H_2O$	0.02	-
NaCl	3.12	3.12
$MnCl_2·4H_2O$	–	0.93
$ZnCl_2$	–	0.13
$CuCl_2·2H_2O$	–	0.07

depend on the links between the neurons that build it, and the way they are located. In order for an ANN to function properly, it must be trained for the work it is to perform. The topology of ANN is formed from nods (neurons) which are grouped by layers. The first layer is called the input layer. The last layer is called the output layer. In-between, there are other layers that are called the hidden layers or computational layers (Abiodun et al. 2018). Depending on the structure and way of learning, there are different types of ANNs: feed-forward ANN, recurrent ANN, Elman and Jordan ANN, long short-term memory, Bi-directional ANN (Bi-ANN), Self-Organizing Map (SOM), stochastic ANN. There exist three major types of learning: supervised learning, unsupervised learning and reinforcement learning (Krenker 2011).

In this study, the feed-forward ANN with supervised learning is used. The supervised learning is a machine learning algorithm used for training of an ANN to recognize or classify data. A set of training data is used for this purpose. The training data consist of input and desirable output data. In order to complete the training of an ANN the input and output data have to be fit. A learning algorithm called back-propagation of errors is used for the purposes of this study. This type of learning consists of two transmissions through the layers of an ANN a forward transmission and a backward transmission. In the forward transmission, an active signal is applied to the input layer and then the signal propagates through the hidden layers to the output layer. At the point when this signal reaches the output layer, it produces a signal in response to the input signal and then the output signal provides "feedback" to the input layer. The propagated output signal changes the input layer as such that the next input signal produces an output signal with close properties to the desirable output signal. This process is repeated until it reaches the desired signal (Rumelhart et al. 1986).

The code for the ANN is written on Python (a coding software widely used for the purposes of experiments in AI). A free-forward neural network was created for this paper with a hyperbolic tangent sigmoid transfer function in the hidden layers and a linear transfer function in the output layer (Hanrahan 2017; Haykin 2004).

Table 4 Definition of terms and formulas for calculation of the JIP test parameters from the Chl a fluorescence transient OJIP emitted by dark-adapted leaves

Fluorescence parameters	Description
F_0	Minimal fluorescence, when all PS II RCs are open (at $t=0$)
F_M	Maximal fluorescence, when all PS II RCs are closed
$V_J=\frac{F_J-F_0}{F_M-F_0}$	Relative variable fluorescence at the J-step
$\varphi_{Po}=1-\frac{F_0}{F_M}$	Maximum quantum yield of primary photochemistry (at $t=0$)
$\varphi_{Eo}=\left(1-\frac{F_0}{F_M}\right)(1-V_J)$	Quantum yield of electron transport (at $t=0$)
$\varphi_{Ro}=\left(1-\frac{F_0}{F_M}\right)(1-V_I)$	Quantum yield for reduction of end electron acceptors at the PSI acceptor side (RE)
$\psi_{Eo}=1-V_J$	Probability (at $t=0$) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
δ_{Ro}	Efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE)
$\gamma_{Rc}=\frac{Chl_{RC}}{Chl_{total}}$	Probability that a PSII Chl molecule ·functions as RC
k_n is proportional to $\frac{1}{F_M}$	Non-photochemical de-excitation constant
$PI_{ABS}=\frac{\gamma_{RC}}{1-\gamma_{RC}}\cdot\frac{\phi_{Po}}{1-\phi_{Po}}\cdot\frac{\psi_{Eo}}{1-\psi_{Eo}}$	Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors
$PI_{total}=PI_{ABS}\frac{\delta_{Ro}}{1-\delta_{Ro}}$	Performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors
$ABS/RC=\frac{1-\gamma_{RC}}{\gamma_{RC}}$	Absorption flux (of antenna Chls) per RC
M_0	Approximated initial slope (in ms^{-1}) of the fluorescence transient $V=f(t)$
$TR_o/RC=M_0\left(\frac{1}{V_J}\right)$	Trapping flux (leading to Q_A reduction) per RC
ET_o/RC	Electron transport flux (further than Q_A^-) per RC
RE_o/RC	Electron flux reducing end electron acceptors at the PSI acceptor side, per RC
$RC/CS_o=\varphi_{Po}F_0\left(\frac{V_J}{M_0}\right)$	Density of RCs (Q_A^- reducing PSII reaction centers)

The input signals in the ANNs are PF induction curves and JIP test parameters recorded and calculated, respectively, from leguminous plants grown hydroponically in nutrient mediums under various nutrient deficiencies described hereafter. The number of the input induction curves used is 150 for each plant grown in deficiency solution and for the control plants. The hidden neurons are 8 and input parameters are 2 induction curves for control plants and induction curves for plants with deficiency of some nutrient elements. The ANNs are trained with 600, 800 and 1000 repeats of the learning algorithm. The ¾ of all data sets were used for the training of the ANN and ¼ of the data sets were used for tests of the ANN.

Results

Chlorophyll a fluorescence and JIP test

Prompt chlorophyll fluorescence is considered to be sensitive indicator for nutrient deficiency in plants (Aleksandrov et al. 2014; Kalaji et al. 2014; Cetner et al. 2017). The PF is measured from all the leaves and the displayed OJIPS transient curves then plotted on a logarithmic time scale. The OJIPS curves in health plants have two points between O and P points. J is displayed about 2 ms and I is 30 ms after the beginning of fluorescence emitted by the chlorophyll *a*. The OJ phase depends on light and contains information on antennae size and connectivity between the PSII reaction centers (Stirbet, et al. 2014). The rise of transient from J to P is called thermal phase and depends on the reduction of the rest of the electron transport chain (Schansker et al. 1706).

In this study, it is observed that the curves of induction kinetics for different deficits were changed.

Figure 1 shows the fluorescence curves measured in *Phaseolus vulgaris* L. (Cheren Starozagorski), grown as a water culture in Hoagland complete solution (control) or in modified Hoagland solution for nutrient deficiency. The induction curves of chlorophyll fluorescence are presented in a logarithmic scale of time. In all induction curves, the characteristic J and I phases are clearly observable.

On Table 5, the values of JIP test parameters for plants grown in deficiency solutions are presented. Only parameters with statistically significant differences in the values between control and experimental plants are discussed.

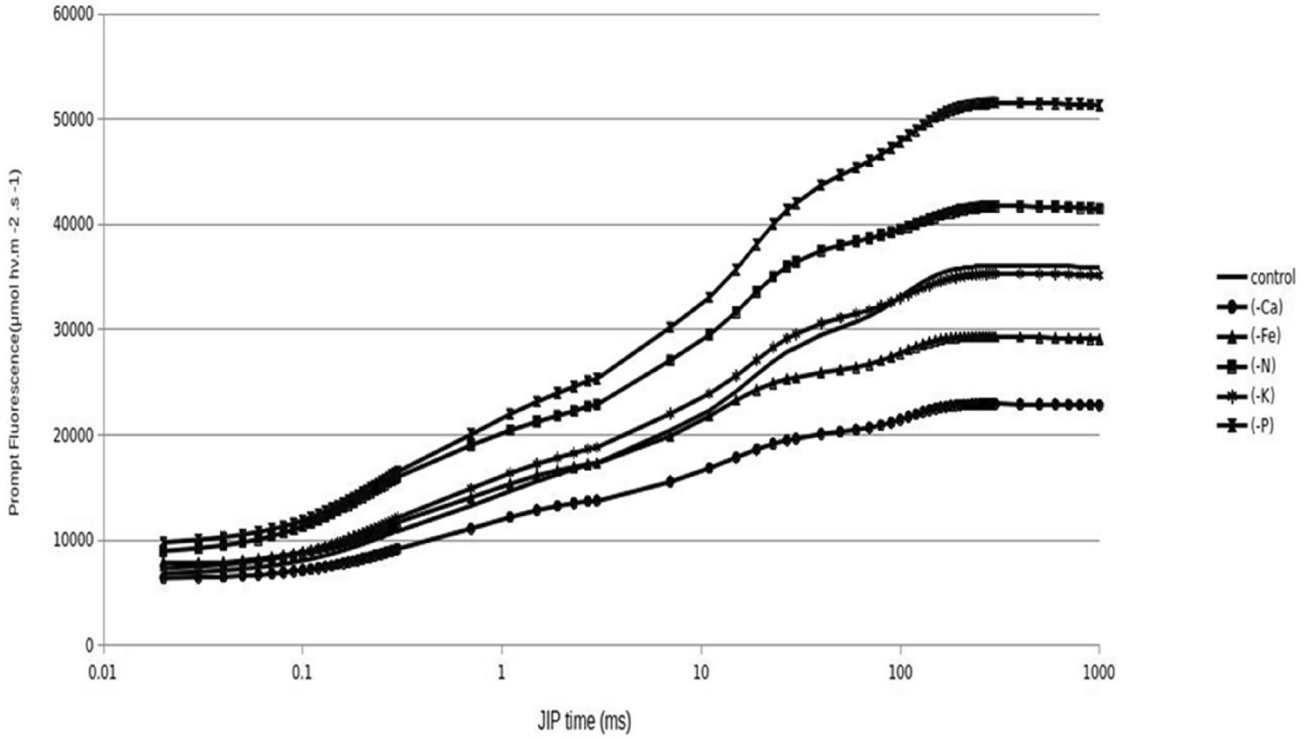

Fig. 1 Induction curves of PF, measured in Phaseolus vulgaris leaf, control and grown in Ca, N, K, P and Fe nutrient deficiencies. Fluorescence was measured by illumination of the plant with red light with an intensity of 4000 µmol hv m^{-2} s^{-1}

Artificial neural network

The JIP analyses made for plants grown in the absence of a nutrient element indicate that the ICs of the PF and the JIP test parameters are different for each missing element. Based on these differences, it is designed an ANN to identify the missing nutrient in plants. To construct an ANN, as input data, is used the induction curves measured in the *Phaseolus vulgaris* leaf as well as some JIP test parameters. The plants were grown in modified Hoagland solutions at different nutrient deficiency. In this case the deficits presented in the network were: (–Fe), (–K), (–N), (–P), (–Ca) and (Con) -the control plants grown in a complete nutrient medium. The 6-component output vector of the type [1, 0, 0, 0, 0, 0, 0], and 1 in this vector matches the data for the first deficit delivered at the entrance of ANN. Once the data for the first deficit are submitted, the data for the second are given and the output vector in this case has the form [0, 1, 0, 0, 0, 0, 0], etc. The network with Bayesian Regularization method was trained and for this purpose was used ¾ of the data. The training from 600 to1000 times was repeated. The first task was to measure the PF fluorescence signals of bean plants and then to use them as input network data. The second task was to check whether these signals can be used to detect nutrient deficiency in plants. The data obtained during the training of the network is presented in Table 6. By using all the induction curves (Fe–Fe, (–K), (–N), (–P), (–Ca) and Control Plants), optimal network operation for 600, 800 and 1000 replicates are reached at 8 hidden neurons. Increasing the number of hidden neurons does not increase the accuracy of the network. On the other hand, increasing the repeatability of the training (epochs) after 800 repetitions in practice does not increase the accuracy of the network and for this reason it is accepted that the optimal number of repetitions is 800.

Results presented in Table 7 are obtained when the network is trained to detect only one nutrient deficiency. As an input data are used two data sets—the data for the deficit of one macroelement and the data for control plants.

Discussion

In this study, it is measured chlorophyll *a* fluorescence transient to analyze the changes in light phase of photosynthesis in nutrient-deficient bean plants. The plants were grown hydroponically to determine possible effects of macronutrients (N, P, K and Ca) and micronutrient (Fe) deficiency on the Electron Transport Chain in the chloroplasts. Nutrient deficiency induced changes in chlorophyll *a* fluorescence induction curves as well as in JIP parameters. Due to these changes in the fluorescence induction curves and in the

Table 5 Calculated JIP parameters in relative units for N, P, K, Ca and Fe deficiency *Phaseolus vulgaris* plants, normalized to respective parameter values, calculated for the control plants. The significance values of difference as compared to control samples based on Dunnett's Method are presented

JIP parameters	V_J	F_O	$\delta(R_0)$	$\varphi(P_0)$	$\varphi(E_0)$	$\psi(E_0)$	γ(RC)
Control	1.00	6817	1.00	1.00	1.00	1.00	1.00
(–N)	1.23 ± 0.005	8939	0.69 ± 0.004	0.98 ± 0.08*	0.85 ± 0.005	0.86 ± 0.005	0.84 ± 0.005
JIP parameters	ABS/RC	TR_0/RC	F_M	RC/ABS	PI (ABS)	PI (total)	ET_0/RC	RE_0/RC
Control	1.00	1.00	36,071	1.00	1.00	1.00	1.00	1.00
(–N)	1.32 ± 0.003	1.36 ± 0.003	48,145	0.75 ± 0.005	0.53 ± 0.002	0.29 ± 0.003	1.16 ± 0.04	0.81 ± 0.005
JIP parameters	V_J	F_O	$\delta(R_0)$	$\varphi(P_0)$	$\varphi(E_0)$	$\psi(E_0)$	γ(RC)
Control	1.00	6817	1.00	1.00	1.00	1.00	1.00
(–P)	1.06 ± 0.05	9771	0.87 ± 0.004	1.02 ± 0.08*	0.98 ± 0.08*	0.97 ± 0.07*	0.90 ± 0.05
JIP parameters	ABS/RC	TR_0/RC	F_M	RC/ABS	PI (ABS)	PI (total)	ET_0/RC	RE_0/RC
Control	1.00	1.00	36,071	1.00	1.00	1.00	1.00	1.00
(–P)	1.19 ± 0.04	1.17 ± 0.04	51,543	0.84 ± 0.006	0.82 ± 0.006	0.66 ± 0.005	1.13 ± 0.04	0.99 ± 0.09*
JIP parameters	V_J	F_O	$\delta(R_0)$	$\varphi(P_0)$	$\varphi(E_0)$	$\psi(E_0)$	γ(RC)
Control	1.00	6445	1.00	1.00	1.00	1.00	1.00
(–K)	1.17 ± 0.005	6416	0.83 ± 0.005	0.99 ± 0.09*	0.90 ± 0.05	0.90 ± 0.05	0.93 ± 0.05
JIP parameters	ABS/RC	TR_0/RC	F_M	RC/ABS	PI (ABS)	PI (total)	ET_0/RC	RE_0/RC
Control	1.00	1.00	36,084	1.00	1.00	1.00	1.00	1.00
(–K)	1.13 ± 0.03	1.14 ± 0.03	35,336	0.88 ± 0.007	0.65 ± 0.005	0.52 ± 0.002	1.03 ± 0.07*	0.85 ± 0.005
JIP parameters	V_J	F_O	$\delta(R_0)$	$\varphi(P_0)$	$\varphi(E_0)$	$\psi(E_0)$	γ(RC)
Control	1.00	6445	1.00	1.00	1.00	1.00	1.00
(–Ca)	1.57 ± 0.005	5158	0.93 ± 0.05	0.74 ± 0.005	0.58 ± 0.003	0.67 ± 0.005	1.15 ± 0.05
JIP parameters	ABS/RC	TR_0/RC	F_M	RC/ABS	PI (ABS)	PI (total)	ET_0/RC	RE_0/RC
Control	1.00	1.00	36,085	1.00	1.00	1.00	1.00	1.00
(–Ca)	0.81 ± 0.007	1.31 ± 0.007	20,836	1.40 ± 0.006	0.40 ± 0.005	0.36 ± 0.008	0.75 ± 0.007	0.71 ± 0.007
JIP parameters	V_J	F_O	δ(Ro)	$\varphi(P_0)$	$\varphi(E_0)$	$\psi(E_0)$	γ(RC)
Control	1.00	6445	1.00	1.00	1.00	1.00	1.00
(–Fe)	0.75 ± 0.006	7554	1.42 ± 0.009	1.16 ± 0.002	0.69 ± 0.005	0.75 ± 0.008	0.68 ± 0.005
JIP parameters	ABS/RC	TR_0/RC	F_M	RC/ABS	PI (ABS)	PI (total)	ET_0/RC	RE_0/RC
Control	1.00	1.00	36,085	1.00	1.00	1.00	1.00	1.00
(–Fe)	0.76 ± 0.006	0.80 ± 0.006	29,394	0.73 ± 0.006	0.41 ± 0.008	0.30 ± 0.009	0.69 ± 0.005	0.55 ± 0.003

*Non-significant differences

JIP parameters, they could be used for nutrient deficiency recognition.

Nitrogen deficiency

The value of Fo for plants with nitrogen deficiency is higher than the value of Fo for control plants. The higher initial fluorescence value measured in nitrogen deficiency plant is proof of the lower efficiency of transmitting of the excitation energy from the Light Harvesting Complex (LHCII) to the reaction centers of PSII (Havaux et al. 1991). On the other hand, plants subject to nitrogen deficiency have a higher value for Fm compared to the control plants. The V_j values of the control plants are less than those of plants with nitrogen deficiency. This means that PSI oxidizes stronger the plastoquinone pool in plants that develop in the absence of nitrogen. The lower value of $\psi(E_o)$ for nitrogen-deficient plants indicates that electron transport after primary quinone is limited. The lack of nitrogen in plants leads to decrease in transport of electrons through the ETC, which is reflected by the parameter $\varphi(E_o)$. The value of parameter γ(RC) for stressed

Table 6 Input and output data of ANN trained to detect nutritional deficiencies in *Phaseolus vulgaris*

Input data and repetitions	Input parameters	Hidden Neurons	Wrong answers (%)		
			Training	Test	Total
Phaseolus vulgaris PF (600)	6	2	43.6	43.8	43.7
Phaseolus vulgaris PF (800)	6	2	43.2	43.6	43.5
Phaseolus vulgaris PF (1000)	6	2	43.3	43.6	43.4
Phaseolus vulgaris PF (600)	6	4	25.7	35.8	28.2
Phaseolus vulgaris PF (800)	6	4	25.5	33.9	27.6
Phaseolus vulgaris PF (1000)	6	4	25.3	32.8	27.0
Phaseolus vulgaris PF (600)	6	8	25.9	29.6	26.8
Phaseolus vulgaris PF (800)	6	8	25.2	28.4	26.0
Phaseolus vulgaris PF (1000)	6	8	25.3	28.6	26.5
Phaseolus vulgaris PF (600)	6	10	25.5	30.2	26.7
Phaseolus vulgaris PF (800)	6	10	25.2	29.8	26.3
Phaseolus vulgaris PF (1000)	6	10	24.9	29.6	26.2

The number of trains of the network (epochs) varies from 600 to 1000. The number of hidden neurons varies from 2 to 10 and the input parameters are 6, which corresponds to measurements of the fluorescence signals of PF for plants grown in environments with different nutrient deficiencies (5 variants) + signal from control plants

Table 7 Input and output data of ANN trained to detect nutritional deficiencies in *Phaseolus vulgaris*

Input data and repetitions	Input parameters	Hidden Neurons	Wrong answers (%)		
			Training	Test	Total
Phaseolus vulgaris (800) (–Fe) controls	2	8	3.1	4.0	3.5
Phaseolus vulgaris (800) (–K) controls	2	8	0.2	1.8	0.6
Phaseolus vulgaris (800) (–N) controls	2	8	2.3	5.3	3.0
Phaseolus vulgaris (800) (–P) controls	2	8	0	0	0
Phaseolus vulgaris (800) (–Ca) controls	2	8	1.1	5.2	2.4
Phaseolus vulgaris (800) ALL	6	8	51.6	52.9	51.9

For the input parameters, the data obtained by measuring the fluorescence signals of PF are used simultaneously. The number of network exercises (epochs) is 800 iterations. The number of hidden neurons is 8 and the input parameters are 2, which corresponds to a signal measured from a plant grown in a nutrient mineral deficiency + signal from control plants

plants is lower than the value of this parameter for control plants. This means that the relative amount of chlorophyll molecules acting as RCs in plants grown under nitrogen deficiency is less than in control plants. The fact that the value of REo/RC is lower in plants grown under nitrogen deficiency compared to control plants indicates that much less electrons manage to reduce the last acceptors of PSI. The two performance indices PI (ABS) and PI (total) have very low values for stressed plants compared to unstressed. This shows that, in general, the lack of nitrogen has a strong negative effect on the photosynthetic apparatus. The higher value of the TRo/RC parameter for nitrogen deficiency plants shows that they capture more energy in the RC than the control plants. The value of the *N* parameter for the plants with nitrogen deficiency is lower than the value of this parameter for control plants. Therefore, fewer electrons are required for the complete recovery of acceptors after Q_A.

Phosphorus deficiency

The values of minimal fluorescence signal Fo and maximum fluorescence signal F_M in plants with phosphorus deficiency are higher than the value of Fo and Fm in control plants. The parameters δ(Ro) and γ(RC) are lower values than the values of the control plants. The first of these parameters gives information about the ability of the intermediate carriers to reduce the last acceptors of PSI. A lower γ(RC) value indicates that phosphorus deficient plants have a relatively small number of RCs compared to plants grown under normal conditions. In the case of phosphorus deficiency, the value for N is lower than in the control plants. The values of ABS/RC and TRo/RC for plants with phosphorus deficiency are higher than in unstressed plants. The ETo/RC parameter, which gives information about the flow of electrons after Q_A is greater in plants with phosphorus deficiency than in unstressed ones. This is an indication that a greater number of electrons are able to pass ETC after the primary quinone acceptor in PSII. On the other hand, the REo/RC parameter is not substantially altered, indicating that approximately the same number of electrons in both stressed and unstressed plants reach to the final acceptors of PSI. This means that for stressed plants, the losses of energy are mainly observed at intermediate carriers in ETC. One reason for this may be the water–water cycle (Weng et al. 2008).

Potassium deficiency

The induction curves of control plants and those with K-deficiency are distinguished slightly from each other, indicating that the lack of K does not significantly affect the photosynthetic apparatus. This may be due to partial replacement of missing potassium ions with sodium ions in processes related to photosynthesis. Significant differences exist only with two parameters: VJ and δ(Ro). The first parameter demonstrates that in plants lacking potassium there are a relatively greater number of closed reaction centers at the J level of induction curves compared to control plants. The lower values for the parameter δ(Ro) show that the probability of reduction of the last acceptor of PSI is lower for plants with potassium deficiency. The JIP parameters ABS/RC, TRo/RC, Mo, RC/ABS, PI (ABS), PI (total) and REo/RC are altered due to potassium deficiency. Low values for PI (ABS) and PI(total) indicate that lack of potassium leads to changes in ETC and lowering the photosynthetic activity of the plants.

Calcium deficiency

For plants growing in a calcium-free environment, fluorescence is much less intense than that of the control plants. This is evident from the large difference between the value of the parameter FV of the stressed and unstressed plants. Lack of calcium causes changes in almost all JIP parameters. The PI (ABS) and PI (total) parameters have very low values for stressed plants compared to unstressed ones. The JIP analysis shows that calcium deficiency has an extremely strong impact on the photosynthetic apparatus and affects almost all of its components.

Iron deficiency

The higher value for Fo indicates that the light harvesting complex of the stressed plants is less effective than the light harvesting complex of the control plants. For bean plants grown in iron deficient environments, the parameters φ(Po) and δ(Ro) have higher values than in the control plants. The first of the two parameters gives information that in the stressed plants the transfer of electrons from RC to Q_A is more likely. The second parameter indicates that the probability, with which electron reduces the last acceptors of PSI, is greater for plants grown in Iron free environments. φ(Eo) and ψ(Eo) provide information that the probability of transfer of electrons after the primary quinone acceptor is much lower for stressed plants. The reason for this is probably due to the lack of intermediate non-heme iron between Q_A and Q_B. The lack of iron in plants has a negative impact on the whole photosynthetic apparatus.

It is clear that deficiency of some nutrients lead to differences in the JIP test parameters and the fluorescence induction curves in plants. This gave reason to use artificial neural network for time efficient and accurate recognition of nutrient deficiency in bean plants. The network which used in the work was ANN with backpropagation of error.

The ¾ of all the data to train the network were used and we used Bayesian Regularization for optimisation. The network from 600 to 1000 times was trained.

As inputs the fluorescence signals of PF were used. The network with 600 repetitions was trained first, changing the number of input parameters (deficiency) and the number of hidden neurons. The number of induction curves was 648. The data obtained during the training of the network are presented in Table 6. Using all parameters (representing plants with 5 types of tested deficits: (–Fe), (–K), (–N), (–P), (–Ca) and control plants) optimal network operation for 600, 800 and 1000 reps are reached with 8 hidden neurons. Increasing the number of hidden neurons does not increase the accuracy of the network. On the other hand, increasing repetition of training (epochs) after 800 iterations does not actually increase the accuracy of the network and therefore was accepted that the optimal number of iterations is 800.

The next task was to use as an input network data only two signals—one for the signals measured by the deficiency plants and other for the signals measured by the control plants. The results are presented in Table 7. From Tables 6 and 7, it is evident that the trained network for only two parameters gives a much smaller error compared to network trained ones, to detect all deficiencies at once.

The network trained to detect Iron and Nitrogen deficiency has the biggest training error. The network trained to detect Phosphorus deficiency does not produce any erroneous results. On the other hand, when submitted the total number of data on all analyzed options: control and deficient, the error increased to 52%.

It could be suggested that the appropriate nutritional deficiency recognition strategy is to train the network to recognize each deficiency individually.

The record of OJIP transients in the experiments allowed to be quantify photosynthetic parameters that were significant for the evaluation of the photosynthetic apparatus of the investigated plants subjected to nutrient deficiency stress.

It is evident from the results that the same photosynthetic parameters calculated for plants subject to a different nutritional deficiency have different values. These results are important, as they show that some photosynthetic parameters are sensitive to nutrient deficiency and could be used as a fluorescence phenotype marker.

Applying the AI to OJIP transient data allows us to recognize which nutrients are missing in plants. This approach allows for the development of fast and strongly accurate methods for plants monitoring in vivo conditions.

Conclusion

Deficiency of all analyzed elements changed the physiological state of bean plants that was displayed in modifications of the chlorophyll fluorescence transients. The effects of the lack of these elements included the impairments in electron transport chain in both donor and acceptor sides of PSII and of PSI. The ANN with backpropagation was applied to recognize nutrient deficiency on the basis of chlorophyll fluorescence data. The results suggest that the ANN approach for early recognition of nutrient deficiency based on chlorophyll fluorescence data is a very useful and powerful tool.

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