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A novel LIBS method for quantitative and high-throughput analysis of macro- and micronutrients in plants†

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Laser induced breakdown spectroscopy (LIBS) is an emerging technique for the analysis of elements in plant tissue. This study reports the validation of a newly developed LIBS instrument and a method for analysis of plant material. The LIBS setup consists of a press, a searing unit, and an analyser with an Nd:YAG laser with a pulse energy of 0.15 mJ operating at a central wavelength of 1064 nm in a nitrogen atmosphere. The LIBS measurements were conducted on 257 plant samples from eight different plant species. The plant samples were also analysed with inductively coupled plasma optical emission spectroscopy (ICP-OES) to obtain reference values for phosphorus (P), potassium (K), magnesium (Mg), sulphur (S), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), boron (B), and copper (Cu). Based on the reference values and the LIBS spectra, partial least squares regression was used to build prediction models for each nutrient. Mixed models and specific models for wheat and faba bean were made. Specific models for wheat and faba bean performed better than mixed species models. Prediction models for P, K, Mg, S, Ca, Zn, Fe, B and Cu from wheat were superior and were sufficiently precise and accurate to enable detection of plant nutrient deficiencies. However, for Mn the accuracy needs to be improved. The results document the usefulness of the novel LIBS setup for plant tissue analysis and for detection of plant nutrient deficiencies.

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Introduction

Plants require 17 elements to grow and complete a life cycle. Three of them are acquired from the air or water (carbon (C), oxygen (O) and hydrogen (H)) and 14 are normally obtained from the soil. The 14 nutrients are classified as either macronutrients (nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), sulphur (S), calcium (Ca)) or micronutrients (iron (Fe), zinc (Zn), manganese (Mn), boron (B), copper (Cu), nickel (N), chloride (Cl) and molybdenum (Mo)). It is common practice to carry out soil analysis before sowing or planting to get an indication of the soil nutrient status. However, soil nutrient analysis is often poorly related to plant availability of nutrients.[1] Visual diagnostics can be used to decide if a plant is nutrient deficient, but in many cases, the deficiencies are latent without distinct symptoms, and especially the early stages of nutrient deficiencies can be difficult to diagnose.[2] Plant tissue analysis is a well-established method for determining nutrient status and is often carried out with atomic spectrometry based methods such as inductively coupled plasma optical emission spectroscopy (ICP-OES) or inductively coupled plasma mass spectrometry (ICP-MS).[3] While these methods provide reproducible and reliable results, they are time-consuming and expensive, which to some degree hinders the widespread adoption of plant tissue analysis in farming. In addition to the atomic spectrometry based methods, various fast non-destructive *in situ* methods exist.[4] Chlorophyll *a* fluorescence can be measured with a handheld device and used to assess P and Mn status,[5,6] but while this represents a fast and reliable measure it has recently been shown to be less reliable under high light intensity.[7] Several studies have confirmed the ability of near infrared spectroscopy (NIRS) to predict N, P, K, S, Ca, Mg and Fe status in different crops. However, measurements of dried and homogenized leaves yield more accurate results as compared to NIRS measurements of fresh leaves.[8] Another useful technique is X-ray fluorescence spectrometry (XRF) that exists both as benchtop and portable instruments. X-ray fluorescence spectrometry can be used to quantify a range of plant nutrients including P, K, Ca, Mg, S, Fe, Zn, Mn and Cu, although Mg, Cu, Zn and Fe are often not included in studies or do not have a limit of detection (LOD) sufficiently low to be fit-for-purpose.[9,10] Despite the advantages of these fast and non-destructive methods, there is still a need for fast and reliable methods that enable multi-element analysis of plant tissue.

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Laser induced breakdown spectroscopy (LIBS) is a promising technology for performing multi-element analysis of plant tissues.[9] As compared to ICP-OES that requires a liquid sample, the LIBS plasma is generated by a high-energy laser pulse that has the potential to ionize both liquid and solid samples. This makes LIBS a very versatile technique that can be used for analysis in a broad range of industries, including agriculture where soil and crop plants can be analysed.[11,12]

For diagnosis of plant nutrient deficiencies, LIBS measurements must perform well across the entire relevant concentration span for the given nutrient and be able to deliver accurate data that allows the farmer to distinguish between nutrient deficient and healthy plants. It is challenging to establish thresholds for deficient, normal, or high concentrations of a given nutrient in plants. There is considerable variation between the optimal nutrient concentration between plant species,[9,13,14] and even between different genotypes of the same species.[15] Furthermore, tissue and sampling time affects the concentration.[5,16–18] It is beyond the scope of this paper to fully cover this rather complicated situation, but for the purpose of discussing the usefulness of the LIBS data of this study the values for critical, midrange and high concentrations of wheat in Fig. 1 will be used. Nitrogen cannot be measured with the LIBS setup used in this study, since we use nitrogen gas for optical path purging, and is consequently not included in the figure.

Despite a decade long interest in using LIBS for plant analysis, it is still not adopted as a widely used standard procedure. In addition to the lack of an automated LIBS system that would reduce the required labour, another aspect holding back the use of LIBS is the matrix effects that arise from the laser–sample interaction. This is affected by laser characteristics, but also by physical properties, the homogeneity, and the particle size distribution of the sample.[32] Due to the matrix effect, it is not possible to use calibration standards as is common practise in analytical chemistry. For many analytical procedures, including ICP-OES, a set of calibration standards consisting of a number of liquid solutions with known concentrations of the elements of interest are used to convert the output from the instrument into a dry matter concentration for an unknown sample. This approach is not applicable for LIBS analysis since the calibration standards would need to be matrix matched, which is a challenge for plant tissue analysis. Some attempts to create standards for plant analysis have been made. da Silva Gomes *et al.*[33] used acid extraction of sugarcane leaves to create a sugarcane powder with very low concentrations of Ca, Mg, K, P, Cu, Mn and Zn. This powder was used as a blank standard, and samples consisting of different ratios of blank and original sugarcane powder were used as concentration standards. This study achieved a good correlation with ICP-OES results. It was also stated that this is a suitable method for determining LOD of LIBS. However, Fe was not released by the acid extraction, deeming the method unsuitable for quantifying Fe. Another approach is to build a calibration curve based on certified reference material. This approach was applied in a study of 11 plant species, and reasonably good correlations between ICP-OES and LIBS data were established. The setup consisted of a 360 mJ pulse Nd:YAG laser operating at 1064 nm, and an Echelle spectrometer with ICCD detector. Ten spectra were collected for each sample by exposing ten different portions of the sample with 8 pulses each. Certified reference materials were used to construct calibration curves for P, K, Ca and Mg, and single peak emissions lines were used to calculate the element concentration in the dried and powdered leaves. Lines that were free from interference from other elements were chosen for analysis. The coefficients of variation were relatively high with values between 5% and 25%. The authors suggest that one of the main reasons for variance in this setup is the heterogeneous nature of the samples combined

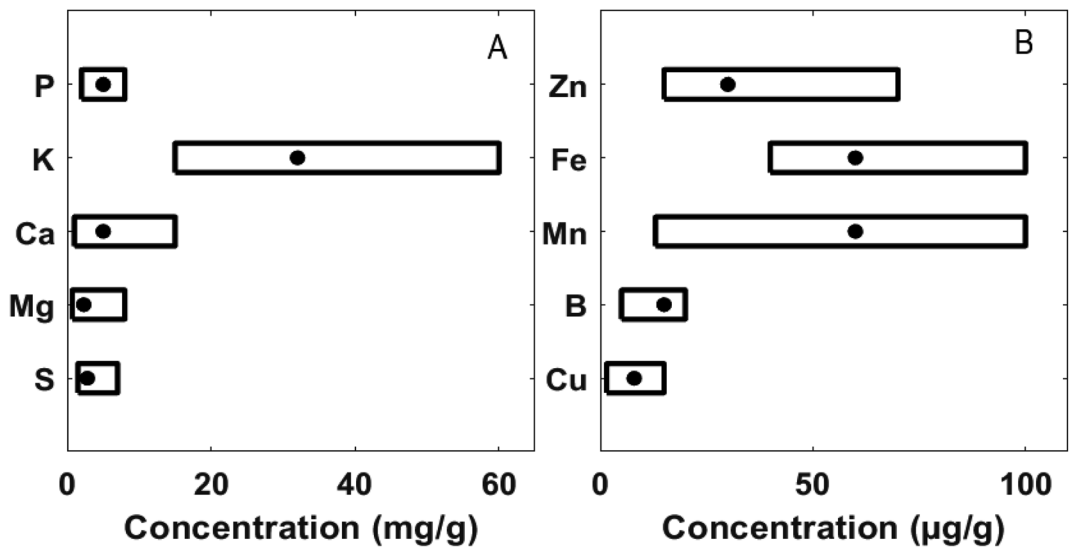

Fig. 1 Critical, midrange and high concentrations for selected macronutrients in mg g^{-1} (A) and micronutrients in µg g^{-1} (B) in wheat for youngest fully developed leaf. The left side in each box represents the deficiency level and the right side represents a high level. The black dots represent a midrange concentration. Values are based on the findings of ref. 16, 17, 19–31.

with the low number of pulses per plant pellet. Increasing the number of pulses per sample could decrease the uncertainty.[34] The same authors subsequently published a study where they used the same setup to measure the micronutrients Fe, Zn, Mn, Cu and B. While the LOD was deemed to be satisfactory for plant analysis, the correlation to ICP-OES data varied considerably. Repeatability was also a challenge with variation from 4% to 30%. Eleven different plant species were measured, and there was no clear trend in which plants species yielded a better correlation between LIBS and ICP-OES.[35] Devey *et al.*[36] studied pasture grass and quantified sodium (Na), K, Mg, Ca, P, S, Mn, Fe, Cu, Zn and B in 100 samples of dried grass. An Nd:YAG, 1064 nm, 200 mJ laser was used in combination with four charge-coupled device detector chips, with an overall spectral range of 190 to 950 nm, and a resolution of approximately 0.1 nm. No atmospheric purging was used during analysis. With a partial least squares (PLS) calibration (multivariate approach), they achieved strong correlations between LIBS and ICP-OES data for all elements except S, Cu, and Zn. Several recent studies on LIBS based plant tissue analysis are only based on a few samples, and very few quantify 10 nutrients at a time. Boron and S are rarely quantified when LIBS is adopted for analysis of plant tissue.[9,37] Sulphur quantification is often a challenge for LIBS studies since strong S emission lines are absorbed by atmospheric oxygen. In the present study, a newly developed automatic LIBS instrument and method, with a sample searing procedure and an N purged atmosphere is tested, and the ability to quantify P, K, Mg, S, Ca, Fe, Zn, Mn, B and Cu in plant samples is demonstrated. Furthermore, the usefulness of the method as a tool to diagnose nutrient deficiencies in plants is discussed. The performance goals for the novel LIBS method are:

- Rapid sample preparation (max 60 seconds per sample).
- Rapid analysis (max 60 seconds per sample).
- Quantification of macro- and micronutrients in plant tissue with a wide concentration range (from deficient to high concentrations).
- Analytical performance: relative standard deviation <5% for triplicate measurements.

Materials and methods

Plant material

Eight different plant species were chosen for analysis; faba bean (*Vicia faba*, $n = 78$), wheat (*Triticum aestivum*, $n = 64$), oat (*Avena sativa*, $n = 30$), pea (*Pisum sativum*, $n = 29$), maize (*Zea mays* $n = 18$), tomato (*Solanum lycopersicum*, $n = 12$), rapeseed (*Brassica napus*, $n = 12$) and soybean (*Glycine max*, $n = 11$). Oat, pea and 30 of the faba bean samples were field grown in Denmark. To cover the full range of relevant nutrient concentrations, plant material with normal or extreme nutrient levels were produced in a greenhouse. Wheat, faba bean, tomato, soybean, rapeseed, and maize were grown in either hydroponics or sand. Plants that were grown in hydroponics were germinated in vermiculite, and seven days after germination they were transferred to 5 litre buckets containing a nutrient solution. The nutrient solution contained 0.2 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.3 mM $MgSO_4 \cdot 7H_2O$, 0.1 mM NaCl, 0.3 mM $Mg(NO_3)_2 \cdot 6H_2O$, 0.9 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.6 mM KNO_3, 0.05 mM Fe(III)-EDTA-Na, 0.001 mM $MnCl_2 \cdot 4H2O$, 0.0007 mM $ZnCl_2$, 0.0008 mM $CuSO_4 \cdot 5H_2O$, 0.002 mM H_3BO_3 and 0.0008 mM $Na_2MoO_4 \cdot 2H_2O$. The buckets were constantly aerated, and the nutrient solution was renewed once a week. pH was adjusted to 5.5–6.0 every second day. Nutrient deficient plants received a normal dose of nutrients for 5 weeks, and after that the nutrient of interest was omitted from the nutrient solution. Plants grown in sand were germinated from seeds directly in pots containing 2 litres of sand (four plants per pot). Each pot received 800 ml of nutrient solution per week. For the first 4 weeks after germination the nutrient solution contained 0.63 mM KH_2PO_4, 0.63 mM K_2SO_4, 0.94 mM $MgSO_4 \cdot 7H_2O$, 0.32 mM NaCl, 0.94 mM $Mg(NO_3)_2 \cdot 6H_2O$, 2.8 mM $Ca(NO_3)_2 \cdot 4H_2O$, 1.9 mM KNO_3, 0.16 mM Fe(III)-EDTA-Na, 0.0032 mM $MnCl_2 \cdot 4H_2O$, 0.0022 mM $ZnCl_2$, 0.0025 mM $CuSO_4 \cdot 5H_2O$, 0.0063 mM H_3BO_3 and 0.0025 mM $Na_2MoO_4 \cdot 2H_2O$. After four weeks the concentrations were doubled. Nutrient deficiencies were induced by removing the nutrient of interest from the nutrient solution, and plants with a high concentration of a given nutrient were produced by doubling the nutrient of interest in the nutrient solution. All plants were harvested after three months, and all the leaves from the plants were sampled.

Sample preparation. To remove dust particles, leaves from field-grown plants were washed in Milli-Q water (Milli-Q Element, Millipore) containing 1% tween and subsequently rinsed with Milli-Q water. They were then freeze-dried for 48 hours. Plant samples from the greenhouse were dried for 48 hours at 60 degrees Celsius in a drying oven. The dry plant samples were milled to a fine powder in a shaker mill using zirconium balls.

ICP-OES analysis. Samples were digested and analysed by the following procedure: 100 mg of plant sample was transferred to an acid washed Teflon liner. Then 2.5 ml 70% v/v HNO_3 and 1.0 ml 15% v/v H_2O_2 were added. The samples were digested in a Milestone ultrawave single reaction chamber microwave digestion system (Milestone Srl) and afterwards diluted to 50 ml with Milli-Q water (Milli-Q Element, Millipore). The samples were analysed with ICP-OES (Agilent 5100, Agilent Technologies). The following emission lines were used: P: 213.62, K: 766.49, Ca: 318.13, Mg: 280.27, S: 180.67, Fe: 259.94, Zn: 202.55, Mn: 259.37, B: 249.68, Cu: 327.4. Limit of detection was determined as the average blank value +3 times the standard deviation of 10 blanks. Limit of quantification (LOQ) was determined as the average blank value +10 times the standard deviation, and data below LOQ were rejected. Certified reference material (CRM) (NIST 1515, apple (*Malus* spp.) leaf, National Institute of Standards and Technology) was included in every batch (22 samples), and only elements that were within ±10% of the certified values were accepted. The samples were analysed in two batches five months apart, with the field grown pea, faba bean and oat samples in the first batch and the greenhouse grown wheat, faba bean, tomato, rapeseed, soybean, and maize in the second batch.

LIBS instrumentation and analysis. The LIBS system (Micral, FOSS Analytics) consists of three units; an automatic press, a searing unit, and an analyser (Fig. 2). The automatic press can pelletize three samples simultaneously with a dwell time of 30 seconds. The searing unit, which can handle three samples at

a time, sears the sample surface of the pellets. The duration of the searing process is 15–20 seconds for all three samples. The analyser is equipped with a sample cassette that can hold 60 sample containers at a time, and it spends ~one minute for the analysis of one sample. The analyser houses a microchip neodymium-doped yttrium aluminium garnet (Nd:YAG) laser with a pulse energy of 0.15 mJ, 1.5 ns pulse length, and a repetition rate of 200 Hz, operating at 1064 nm. It uses an ultraviolet (UV) diode array spectrometer and a silica fiber optic. Integration time for the spectrometer is set to 5.7 ms, and the integration starts simultaneously as every other laser pulse is triggered. Thus, two full plasma lifetimes are covered by each sub-spectrum. The spectrometer covers the wavelength range from 174 nm to 430 nm, with a pixel resolution of 0.125 nm resulting in 2048 measured data points. Each sample is hit with 6000 laser pulses and from each sample 3000 spectra are collected (one LIBS sub-spectrum covers 2 plasma life cycles). To avoid hitting the same spot twice the sample is moved in a spiral movement. The full optical path atmosphere, *i.e.*, from the sample surface to the spectrometer detector, is purged using nitrogen gas. This ensures a good LIBS signal for wavelengths shorter than 190 nm, where the strong sulphur 181 nm emission lines are present.

For each sample measurement, 1.5 ml of powdered plant sample was transferred to a 14 mm diameter pellet container and pelletized with the press at 2000 kg cm^{-2}. After pelletization, the sample surface was seared in the searing unit. Next, the pellet was pressed again to ensure a flat surface. Total preparation time for 3 samples is ~150 seconds. Fig. 2 shows a schematic overview of the steps for the sample preparation. One out of seven samples were analysed in triplicates and the rest were analysed as single samples. In every run (60 samples) triplicates of an internal standard sample consisting of a mix of oat, pea and faba bean were included (referred to as internal standard).

Partial least squares regression models. All data analysis was carried out in MatLab R2022a (MathWorks, Inc., Natick, MA, USA) and PLS Toolbox 8.9 software.

PLS-regression (PLSR) is a multivariate data analysis method that is useful for relating two data matrices. It can be used to predict Y from X and is able to handle data with many noisy variables. For each model, the optimal number of latent variables (LVs) was selected to minimize the risk of overfitting. The selection of optimal LVs was determined by inspecting the root mean square error (RMSE) *versus* the number of latent variables, then selecting the number of latent variables where the minimum RMSE was achieved without increasing the distance between the RMSE for the calibration (RMSEC) and RMSE for the cross-validation (RMSECV). PLS-regression is thus well suited for finding correlation between LIBS derived spectra and reference data obtained with ICP-OES or other similar methods.[38,39]

PLS-regression was applied with ICP-OES reference data as the Y matrix and the pre-processed LIBS spectra as the X matrix. For each nutrient three types of models were built. A mixed model based on all the sample types ($n = 257$), one based on the faba bean samples ($n = 78$), and one based on the wheat samples ($n = 64$). The rest of the plant species did not consist of enough samples to build individual models.

Prior to prediction modelling, all of the LIBS spectra were corrected for dark signal and wavelength calibrated. The wavelength calibration was based on the spectrum from a calcium

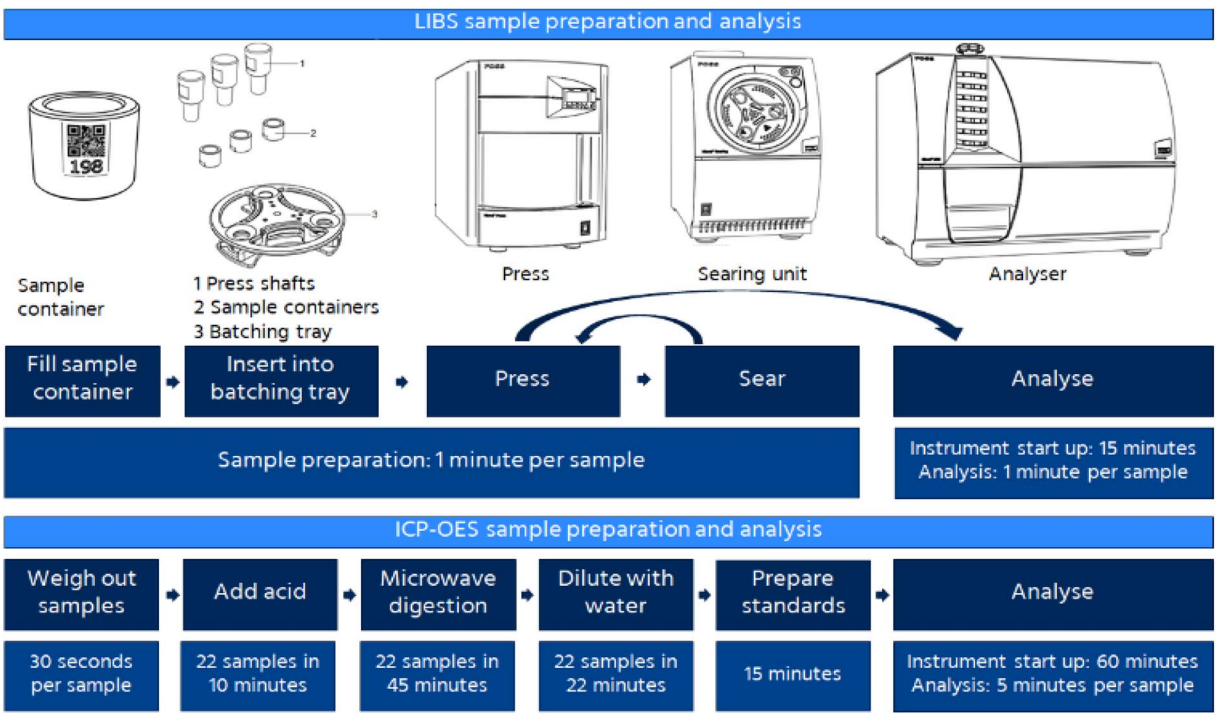

Fig. 2 Schematic overview of sample preparation and analysis with the LIBS and ICP-OES setup used in the present study (illustrations not to scale).

lactate ($C_6H_{10}CaO_6$) sample that is rich in Ca and C. This allows for a wavelength calibration for the full wavelength range covered by the spectrometer. The final LIBS spectra were the mean of the 3000 sub-spectra from each sample.

For most of the models, mean-centering (MC) of the data, as a pre-processing method, resulted in the most accurate models. The only exceptions were the mixed S model and all Fe models where multivariate scatter correction (MSC) and MC resulted in the best models, and the P models where standard normal variate (SNV) and MC resulted in the best models (see ESI Table 1†). Outliers were removed based on the Hotelling T^2 *versus* residual Q plot. Random subsets were used as cross validation and the dataset was split in 5 with 20 iterations. To avoid overfitting, root mean square error of calibration (RMSEC), and root mean square error of cross-validation (RMSECV), were used to estimate the most appropriate number of latent variables (LVs). Typically, the number of LVs where RMSECV was no longer improving was chosen. For the mixed models the data was split into a calibration and a validation set (Kennard-Stone, 66% calibration set and 34% validation set). The root mean square error of prediction (RMSEP) was used to evaluate prediction model accuracy. The faba bean and wheat sample sets did not contain enough samples to be split into a calibration and a validation set.

The RMSECV and RMSEP (mixed models) and the coefficient of determination (R^2) was used to evaluate model performance. Considering that the evaluation of the RMSECV value is absolute, and that there is a concentration range difference between the different data sets, a new parameter was defined that allows for a performance comparison between the data sets. The relative uncertainty (rel_{RMSECV}) is here defined as the RMSECV divided by the 50th percentile value for the reference concentrations and presented in percent:

$$rel_{RMSECV} = \frac{RMSECV}{50^{th}\ percentile\ value} \times 100$$

Results

Plant material and ICP-OES data quality

Nutrient concentration range in plant samples. The plant material obtained from the greenhouse and the field proved to be suitable for building prediction models that span the biologically relevant nutrient ranges. Nutrient concentrations ranged from deficient too high for all nutrients (Table 1). However, for individual plant species, this was not always the case. As a result, the wheat and faba bean models did not always cover the entire nutrient concentration range.

ICP-OES data quality

The predefined required accuracy of a maximal relative difference of ±10% from the certified reference value was achieved for all elements of interest (Table 2). Thus, the ICP-OES data quality was acceptable, and data was further used for establishment of LIBS prediction models.

Analytical performance of the LIBS instrument

The LIBS spectra had element emission lines for all nutrients considered in this study, and Fig. 3 shows the mean spectrum for all LIBS sample measurements with the most pronounced emission lines indicated.

To evaluate the effect of pellet surface quality on spectral repeatability for the LIBS measurements, three replicates were measured for multiple samples from each plant species. Spectral repeatability was calculated as relative standard deviation (RSD) of emission line strengths (baseline subtracted), across measurements of the same sample for element emission lines C 193.0 nm, Zn 202.6 nm, Ca 317.9 nm, and K 404.5 nm. Pooled RSD for the different emission lines is shown in Table 3. The maize samples were the only samples with a poor pellet quality. This was due to a large particle size that led to an uneven pellet surface. The maize sample measured in triplicate had an RSD of 3.7% for the C emission line, which was considerably higher than the corresponding RSD for the other plant species. For Zn, Ca and K the maize sample had RSD values that were comparable to the other plant species. This suggests that the quality of the pellet surface affects the analysis of different nutrients differently.

To test if the spectral repeatability was affected by the concentration of the nutrient of interest, the RSD was calculated for nine wheat samples that were measured in triplicates. Calcium, K and Mn emission lines were chosen because these nutrients represented the widest concentration range in the available samples that were measured in triplicates. There was

Table 1 Nutrient range for the eight different plant species as determined with ICP-OES

Nutrient	All	Maize	Faba bean	Wheat	Rapeseed	Oat	Tomato	Soybean	Pea
P (mg g^{-1})	0.6–11	0.7–7.0	0.9–5.9	1.7–7.7	0.6–11	3.1–4.6	0.8–6.2	1.1–7.1	4.2–5.5
K (mg g^{-1})	2.2–62	6.9–43	12–53	12–52	3.4–62	24–34	6.4–34	6.0–34	18–28
Ca (mg g^{-1})	0.1–45	0.1–6.6	4.3–23	2.0–14	10–45	5.1–10	8.0–25	9.0–31	6.8–13
Mg (mg g^{-1})	0.3–11	0.3–5.6	1.5–11	2.3–7.7	0.4–11	1.1–2.0	0.5–7.3	0.8–11	1.6–2.4
S (mg g^{-1})	0.4–22	0.4–4.3	1.1–5.9	2.9–6.7	1.2–22	4.0–7.8	0.7–10	1.4–3.1	2.2–3.0
Zn (μg g^{-1})	5.5–170	12–86	15–91	14–67	5.5–70	21–62	12–54	7.1–170	31–50
Fe (μg g^{-1})	23–942	28–105	67–942	38–99	23–144	82–125	78–432	35–480	74–104
Mn (μg g^{-1})	2.0–278	2.0–81	17–130	9.7–229	3.6–118	30–187	5.8–59	11–183	22–44
B (μg g^{-1})	1.7–80	5.2–21	15–73	7.6–64	1.7–70	6.9–22	10–64	7.1–80	12–18
Cu (μg g^{-1})	0.8–33	2.9–13	3.0–17	4.7–13	0.8–28	5.4–8.6	4.8–18	1.5–33	5.6–12

Table 2 ICP-OES analysis of CRM NIST 1515 ($n = 17$). Column 1: the nutrient, column 2: the certified value ± STDEV for the CRM, column 3: the mean concentrations ± STDEV based on ICP-OES analysis, column 4: the relative standard deviation (RSD), column 5: the accuracy calculated as the average deviation from CRM values in percentage, and column 6: the root mean square error (RMSE) calculated as $\sqrt{\sum (Y_{pred} - Y_{ref})^2/n}$ where Y_{pred} is the concentration from the ICP-OES analysis and Y_{ref} is the certified concentration

Nutrient	CRM (μg g^{-1})	Mean (μg g^{-1})	RSD%	Accuracy%	RMSE (μg g^{-1})
P	1590 ± 0.110	1577 ± 0.044	2.8	102	42
K	16 100 ± 0.200	16 145 ± 0.658	4.1	100	620
Ca	15 260 ± 0.150	15 513 ± 0.414	2.7	98	460
Mg	2710 ± 0.080	2652 ± 0.081	3.1	102	95
S	1800	1844 ± 0.065	3.5	98	75
Zn	12.5 ± 0.3	12 ± 0.9	7.6	103	3.2
Fe	83 ± 5	77 ± 4.3	5.6	107	7.0
Mn	54 ± 3	53 ± 2.2	4.1	101	2.2
B	27 ± 2	29 ± 0.9	3.1	94	1.7
Cu	5.6 ± 0.24	5.1 ± 0.4	8.4	109	0.63

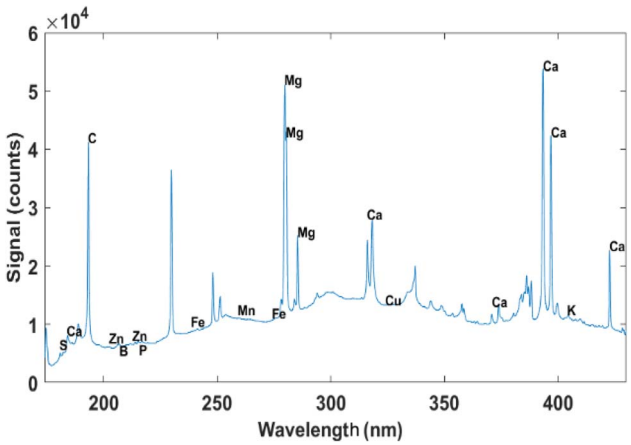

Fig. 3 Mean LIBS spectrum from 557 spectra from plant samples (257 individual samples) as observed in the 174 to 430 nm wavelength range. Relevant emission lines are marked with the corresponding element.

no clear correlation between concentration and RSD (ESI Fig. 1†).

Model parameters and model performance

Parameters included in models. For each nutrient one or more specific emission lines were chosen. The choice of emission lines to include in the models was based on known LIBS emission lines (NIST LIBS database, National Institute of Standards and Technology). The spectrometer used in this setup covers the wavelength range from 174 nm to 430 nm. For Ca, Mg, S, P, Fe, Cu, Mn, B and Zn there is an abundance of strong emission lines in this range. However, for K this is not the case and instead the weaker emission line at 404.5 nm was chosen. For some nutrients there are overlapping spectral emission lines. This is especially the case for Fe, where several emission lines had to be excluded from the models because they overlapped with emission lines from other nutrients. Due to this, only 3 emission lines could be included, despite the existence of numerous Fe emission lines. For P and S, the best models were achieved by including a broader range of wavelengths. The wavelength ranges included in the PLS models are shown in the ESI Table 1,† together with the pre-processing method.

Model performance

For all nutrients the plant specific models had better performance, although the degree of improvement differed between nutrients. In Fig. 4 an example of how the sample matrix affects prediction model accuracy can be seen. The faba bean samples, when included in a model for all sample types (Fig. 4A), tend to gain an offset such that the concentration is underestimated for

Table 3 Baseline subtracted RSD values for selected emission lines for different plant species and the internal standard measured in triplicates and presented as the pooled RSD ± STDEV for C, Zn, Ca and K. The number of triplicate measurements for each plant species is indicated with *n*

Plant	RSD C 193.0 nm (%)	RSD Zn 202.6 nm (%)	RSD Ca 317.9 nm (%)	RSD K 404.5 nm (%)
Maize ($n = 1$)	3.7	2.2	2.0	1.7
Faba bean ($n = 13$)	0.9 ± 0.5	2.3 ± 1.2	1.7 ± 0.8	2.9 ± 1.1
Wheat ($n = 9$)	0.8 ± 0.4	1.5 ± 1.0	1.7 ± 0.9	2.8 ± 1.5
Rapeseed ($n = 1$)	1.3	2.3	1.9	3.1
Oat ($n = 17$)	0.9 ± 0.3	4.0 ± 2.2	1.9 ± 1.2	2.7 ± 1.5
Tomato ($n = 3$)	0.6 ± 0.4	0.9 ± 0.6	1.5 ± 0.6	4.3 ± 1.5
Soybean ($n = 1$)	0.4	0.2	0.9	1.7
Pea ($n = 7$)	0.8 ± 0.5	3.0 ± 1.7	2.1 ± 1.0	2.8 ± 2.0
Internal standard ($n = 8$)	0.6 ± 0.3	3.0 ± 1.5	1.9 ± 0.8	3.1 ± 1.2

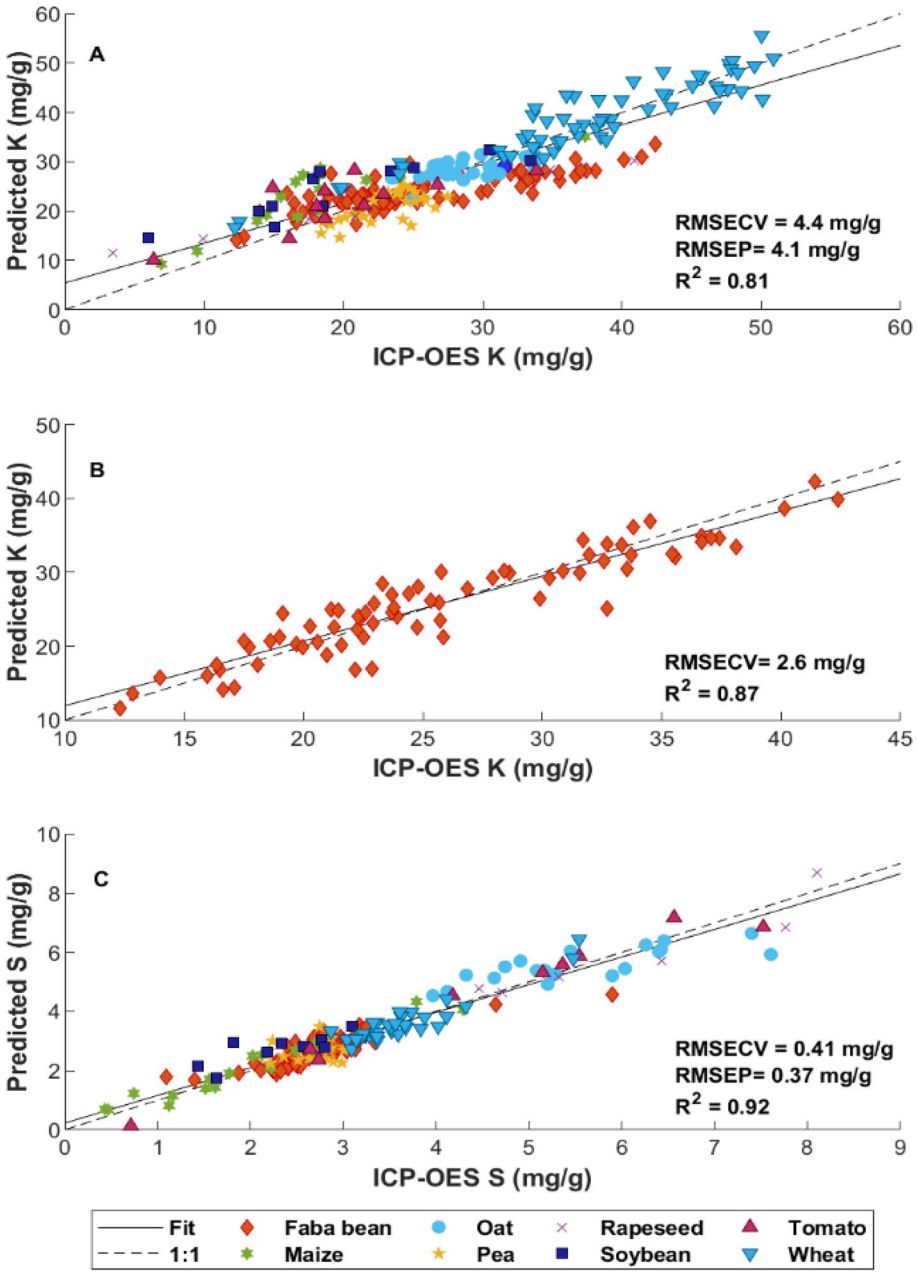

Fig. 4 PLS-regression calibrations models for the relationship between ICP-OES reference values and LIBS predictions for the K mixed model (A), K faba bean model (B), and S mixed model (C). Different plant species are indicated, and the dotted line represents the 1 : 1 fit between ICP-OES references and LIBS predictions. The solid line represents the actual best fit between ICP-OES references and LIBS predictions.

K. When a specific faba bean model was built, the accuracy improved significantly (Fig. 4B). The mixed S model (Fig. 4C) was not affected by the matrix effect to the same extent as the mixed K model. However, a small improvement for RMSECV was observed when plant specific models were explored (Table 4).

Relative standard deviation for predicted values

The internal standard sample was measured 24 times in eight triplicate measurements. For these measurements, concentrations for all 10 elements considered in this study were predicted using the PLS models based on all samples (mixed models). Table 5 shows the mean concentration with the standard deviation, the pooled standard deviation for triplicate measurements and finally the pooled RSD for triplicate measurements. The Fe and B models had the highest RSD for predicted concentrations, and except for Mn all the micronutrients had higher RSD as compared to the macronutrients.

Wheat model performance in relation to nutrient deficiency levels for wheat

To evaluate how the RMSECV for each prediction model affects the ability to correctly identify a plant nutrient concentration as either deficient or sufficient with the LIBS setup, a Monte-Carlo

Table 4 Summary of all PLS model results. Column 1: the nutrient, column 2: the number of samples included in the calibration set after outlier removal, column 3: the concentration range included in the model after outlier removal, column 4: the number of latent variables (LV), column 5 through 9: parameters related to the prediction model performance

Nutrient	Model	Range (mg g^{-1})	LV	R^2	RMSEC (mg g^{-1})	RMSECV (mg g^{-1})	RMSEP (mg g^{-1})	rel_{RMSECV} (%)
P	Mixed ($n = 161$)	0.64–7.7	7	0.88	0.49	0.54	0.49	13.8
	Wheat ($n = 64$)	1.7–7.7	6	0.96	0.25	0.31		5.2
	Faba ($n = 74$)	0.9–5.9	8	0.94	0.22	0.3		8.3
K	Mixed ($n = 161$)	3.4–51	3	0.81	4.3	4.4	4.1	16.7
	Wheat ($n = 63$)	12–51	5	0.92	2.0	2.5		6.5
	Faba ($n = 73$)	12–42	2	0.87	2.5	2.6		11.0
Ca	Mixed ($n = 156$)	0.47–21	10	0.95	0.91	1.1	0.71	13.8
	Wheat ($n = 61$)	2.0–13	7	0.97	0.39	0.51		8.2
	Faba ($n = 72$)	4.9–21	4	0.93	0.98	1.1		8.9
Mg	Mixed ($n = 160$)	0.26–7.8	10	0.94	0.43	0.49	0.36	14.5
	Wheat ($n = 64$)	2.3–7.7	3	0.94	0.27	0.29		6.6
	Faba ($n = 76$)	1.5–9.2	10	0.98	0.28	0.37		7.9
S	Mixed ($n = 161$)	0.43–8.1	12	0.92	0.35	0.41	0.37	13.8
	Wheat ($n = 58$)	2.9–4.4	12	0.74	0.11	0.18		5.1
	Faba ($n = 76$)	1.4–3.6	3	0.47	0.27	0.29		11.2
Nutrient	Model	Range (μg g^{-1})	LV	R^2	RMSEC (μg g^{-1})	RMSECV (μg g^{-1})	RMSEP (μg g^{-1})	rel_{RMSECV} (%)
Zn	Mixed ($n = 157$)	5.5–75	8	0.82	5.8	6.3	5.5	16.0
	Wheat ($n = 56$)	14–63	7	0.86	3.0	3.9		11.7
	Faba ($n = 71$)	15–78	7	0.89	4.1	4.8		9.3
Fe	Mixed ($n = 148$)	23–189	6	0.78	13.8	15.5	15.0	18.2
	Wheat ($n = 63$)	38–99	6	0.62	5.0	6.8		9.4
	Faba ($n = 62$)	67–159	6	0.81	7.3	11.0		9.8
Mn	Mixed ($n = 165$)	2.0–229	3	0.94	11.7	12.1	12.6	18.9
	Wheat ($n = 64$)	9.7–229	3	0.96	8.23	8.8		7.1
	Faba ($n = 70$)	23–101	4	0.77	7.69	8.5		12.1
B	Mixed ($n = 166$)	1.7–73	3	0.89	5.66	5.8	5.2	29
	Wheat ($n = 61$)	7.6–64	3	0.96	2.63	2.9		13.6
	Faba ($n = 77$)	15–73	3	0.96	3.43	3.7		9.3
Cu	Mixed ($n = 161$)	1.5–18	4	0.76	1.41	1.5	1.3	17.2
	Wheat ($n = 64$)	4.7–13	4	0.90	0.52	0.6		6.3
	Faba ($n = 77$)	3.0–17	3	0.92	0.90	0.97		10.5

Table 5 Predictions of internal standard sample based on the mixed models. Column 1: the nutrient, column 2: the mean values ± STDEV for all 24 predictions, column 3: the pooled STDEV for predictions of triplicate measurements of the internal standard sample ($n = 8$), and column 4: the pooled RSD ± STDEV for predictions of triplicate measurements of the internal standard sample

Nutrient	Mean (mg g^{-1})	Pooled STDEV (mg g^{-1})	Pooled RSD (%)
P	4 ± 0.15	0.11	2.6 ± 0.95
K	30 ± 1.1	0.64	2.2 ± 1.4
Ca	10 ± 0.5	0.23	2.2 ± 1.1
Mg	2.3 ± 0.22	0.07	3.2 ± 1.4
S	4.6 ± 0.27	0.09	1.9 ± 1.1
Nutrient	Mean (μg g^{-1})	Pooled STDEV (μg g^{-1})	Pooled RSD (%)
Zn	48 ± 4.3	2.8	5.9 ± 4.1
Fe	112 ± 16	7.4	6.6 ± 2.8
Mn	79 ± 5.6	1.9	2.4 ± 1.7
B	13 ± 2.2	0.76	6.3 ± 3.5
Cu	7.7 ± 0.71	0.42	5.4 ± 2.2

approach was adopted.[40] For each nutrient, and for a given "true" nutrient concentration that was lower than the deficiency limit, 10 000 virtual measurements from a normal distribution with a standard deviation equal to the RMSECV was drawn. The probability to detect deficiency was defined as the ratio between values lower than the deficiency limit and values above the limit. The above given procedure was repeated for a plenitude of different concentrations on both sides of the deficiency limit. To allow for comparison with ICP-OES performance, the same approach was used to construct probabilities based on the RMSE value from the ICP-OES analysis. As can be seen in Fig. 5 the range in which the diagnosis is uncertain differs considerably between nutrients and analytical method. The ICP-OES analysis generally had lower RMSE values for the macronutrients as compared to the LIBS RMSECV values for macronutrients. For micronutrients the difference was less pronounced. When considering the LIBS analysis for P, K, Mg, S, Ca, Fe, Zn and Cu predictions, the deficient plants that were misclassified as not being deficient were generally close to the actual deficiency limit, but for Mn and B the risk of misclassifying

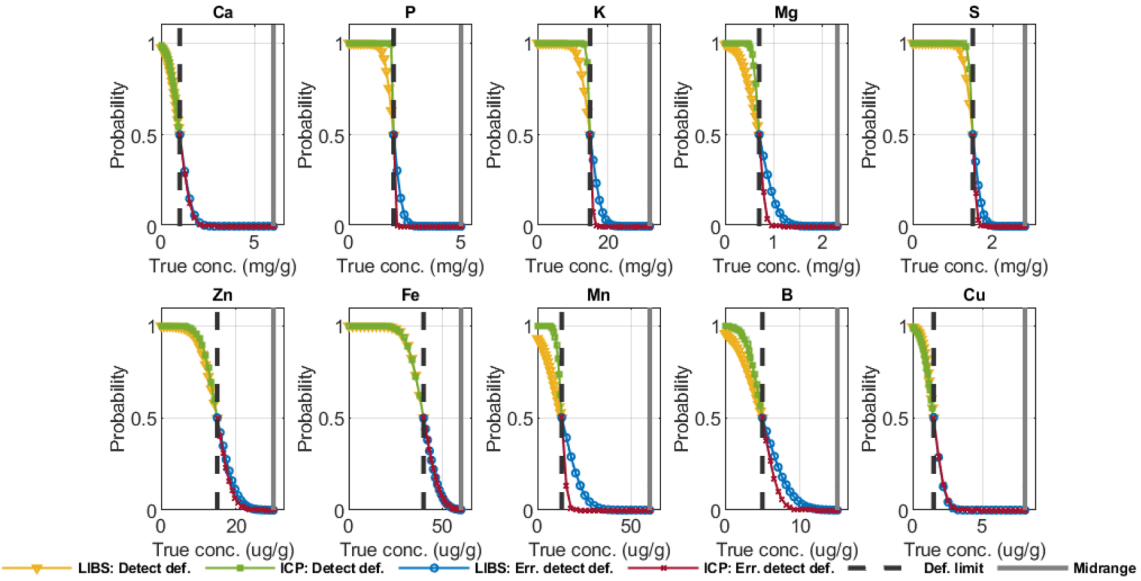

Fig. 5 Monte-Carlo distribution of probability for correctly or erroneously diagnosing deficiency at different nutrient concentrations based on RMSEC values from the wheat models or RMSE values from ICP-OES analysis of 17 CRM samples. The horizontal black dotted lines represent the respective deficiency limits, and the vertical grey lines represent midrange concentrations. Yellow triangles represent simulated samples with a concentration below the deficiency limit for LIBS predictions, and blue circles represent samples with concentrations above the deficiency level for LIBS predictions. Green squares represent simulated samples with a concentration below the deficiency limit for ICP analysis and red crosses represent samples with concentrations above the deficiency level for ICP analysis. Position on the *y*-axis is the probability of being diagnosed as a deficient plant.

a deficient plant as non-deficient was present even in plants with very low concentrations. For Mn, even if a simulated sample had no Mn present at all, the probability to detect Mn deficiency was only 93%. For the P, K, Mg, S, Ca, Zn, Mn and Cu models the uncertainties were low enough to ensure that a plant with a midrange concentration would not be erroneously classified as a deficient plant, however for Fe and B the concentrations that could be erroneously classified as deficient was close to the midrange concentration. This was also the case when ICP-OES analysis was used to quantify Fe and B concentrations. It should be noted that the above given procedure assumes that the RMSECV and RMSE are constant for all concentrations, which in reality is not the case.

Discussion

Sample preparation and analysis time

It is often stated that LIBS analysis requires little to no sample preparation. However, this statement is being disputed and there is now an understanding that sample preparation can significantly improve results for some sample types, including plant samples.[41,42] A study from 2018 showed that LIBS analysis of dried plant samples provides more accurate data as compared to LIBS analysis of fresh plant samples.[43] Another recent study showed that there can be a benefit from removing C from plant samples by dry ashing before LIBS analysis.[44] In our study, a novel and relatively extensive sample preparation procedure was employed to minimize sample-to-sample variation and to increase the signal. Plants were dried, homogenized, pressed, seared, and pressed again to ensure an even pellet surface. The even pellet surface is important since it ensures that the lens to sample distance variation for all 6000 laser pulses is minimized. A prerequisite to an even surface is a homogenised sample, but due to differences in fibre content, some plants species are more difficult to homogenize than others. In this study all the plant samples, except for the maize samples, were easy to homogenize to a fine powder that could be pressed into pellets with a flat surface. The maize samples contained coarser ground material, and after the second press they were more uneven than the other samples. While the maize sample had a higher RSD for the C emission line, RSD for the Zn, Ca and K line were comparable to that of the other plant species (Table 3). Although this does to some degree confirm the theoretically better performance of a flat pellet surface, the spectral repeatability was still acceptable, and the system was robust enough to handle more uneven samples. However, when possible, it is always better to use finely ground samples. This is in line with the findings from other studies, where it is seen that a smaller particle size results in a lower coefficient of variation of measurements and an increase in signal intensity.[45,46] A finely homogenized sample is also important for ICP-OES analysis, since it is a prerequisite in attaining a representative sample.[3] The first step in sample preparation is therefore similar for LIBS and ICP based analyses. However, from here the complexity and time consumption differs considerably. Fig. 1 shows the required steps and estimated time consumption for the LIBS and ICP-OES analyses conducted in this study. The required sample preparation steps before ICP analysis are considerably more time-consuming than the required steps for LIBS analysis. The three main benefits of LIBS analysis are: (i) sample

weighing is not required, (ii) it is not necessary to digest the sample into a liquid, and (iii) no hazardous chemicals are needed. Instead, the required sample portion can be measured out with a measuring spoon, and the press and searing step requires no chemicals. This results in a sample preparation time of ~60 seconds per sample for LIBS compared to ~90 minutes from powder to liquid for 22 samples for ICP. With regards to the analysis time, the LIBS analysis is also faster than ICP analysis. The LIBS instrument requires 15 minutes of purging with N gas before analysis can start. After that it can analyse one sample in 60 seconds. The ICP-OES requires the preparation of standards (~15 minutes) and a start-up procedure that takes ~60 minutes. After that it can analyse one sample in five minutes. With a combined sample preparation and analysis time of two minutes per sample the novel LIBS system used in this study is considerably faster and less labour intense, as compared to ICP-OES analysis.

Analytical performance

To judge the analytical performance of LIBS with regards to repeatability (same day triplicate measurements), the pooled RSD for measurements carried out on the same day were calculated for the internal standard. The RSD for emission line strengths from triplicate measurements for the C emission line, the Zn emission line, the Ca emission line and the K emission line were 0.6 ± 0.3%, 3.0 ± 1.5%, 1.9 ± 0.8% and 3.1 ± 1.2%, respectively. Trevizan *et al.*[35] found that when one pellet was exposed by eight laser shots in ten different locations, the ten resulting test portions had coefficients of variations between 4 and 30% for peak height intensity depending on the analyte and on the reference material that was analysed. This suggest that there was a clear benefit of collecting 3000 spectra per sample since it resulted in lower coefficient of variation. No correlation between the RSD for three replicates of wheat and the concentration of the nutrients Ca, K and Mn was observed (ESI Fig. 1†). This suggests that the repeatability is unaffected by concentration, and that the method can be applied across a wide range of concentrations.

Performance of prediction models

Reference values. Due to considerable matrix effects, it is not possible to use standards to calibrate the LIBS instrument and obtain quantitative data based on standards. Unlike ICP-OES, where standards mixed from pure chemicals can be used to translate signal into a dry matter concentration, the LIBS quantification of the element concentration must be based on a matrix matched sample set that has been analysed with ICP-OES or ICP-MS to yield known concentrations of the analytes of interest.[9] Based on the known concentrations it is possible to build PLS models that can predict the concentration of the element of interest. While this is a well proven and accepted approach,[47] it does mean that any uncertainty in the ICP analysis will be carried over to the LIBS quantification. Table 2 shows the accuracy RMSE, and corresponding RSD, for 17 CRM samples analysed with ICP-OES. Phosphorus, K, Mg, Ca, S, Mn and B had RSD values from 2.7–4.1%. However, the micronutrient Zn, Fe and Cu had relatively high RSD values (7.6%, 5.6% and 8.4%, respectively). Furthermore, Fe and Cu were close to not being within the accepted accuracy (±10% of the certified value for the CRM). The Zn and Cu concentrations in the CRM used here are low, and this could explain the relatively poor performance of the ICP analysis. It is likely that the accuracy was better for samples with higher concentrations meaning that the majority of the analysed samples in this dataset were likely to have a better accuracy than the CRM. The Fe concentration in the CRM sample, however, is close to the 50th percentile value for Fe in the plant samples used in this study, and it must therefore be expected that the ICP data for Fe were generally of a lower quality compared to the other elements.

Model performance for macro- and micronutrients

The mixed prediction models and the plant specific models both performed better when predicting the macronutrients compared to the micronutrients. The rel_{RMSECV} values were lower for the macronutrients compared to the micronutrients (mean value 10.1% and 13.3% for macronutrients and micronutrients, respectively). The pooled RSD for the predictions for the internal standard sample were lower for the macronutrients compared to the micronutrients (mean value 2.4% and 5.3% for macronutrients and micronutrients, respectively). This was expected given that the concentrations of the macronutrients are considerably higher than the concentrations of the micronutrients, and furthermore, the ICP-OES analysis also resulted in RSD values that were lower for the macronutrients compared to the micronutrients. In accordance with these results Devey *et al.*[36] also found that LIBS had a lower error for macronutrients compared to micronutrients. The mean estimated error in percentage was 9.6% for macronutrients and 30.2% for micronutrients. The explanation for the much larger difference between the error for the macronutrients compared to the micronutrients in this study, compared to what is found in our study, could be that the samples were not seared prior to analysis. The searing procedure removes volatiles on the sample surface and reduces the sample matrix complexity. This results in stronger element emission lines, and a reduction of the sample matrix effect.

Performance and benefit of plant specific models

Considering that sample matrix affects the LIBS spectra, there was reason to believe that a plant specific prediction model would perform better as compared to a prediction model based on a mixture of different plant species. To test this, PLS models based on either wheat or faba bean samples were built. The models improved when a single plant species was used instead of all the eight plant species (Table 4). For the mixed models the rel_{RMSECV} was above 10% for all the models. For the wheat models the values were below 10% for P, K, Ca, Mg, S, Fe, Mn and Cu. For the faba bean models P, Ca, Mg, Zn and B were below 10% (Table 4). This clearly shows the benefit of building plant specific prediction models. This is most likely due to a remaining matrix effect caused by different physical

properties of the leaves. The difference in physical structure also resulted in slightly different powder properties, which could have affected the measurements. For all the nutrients the improvement was most pronounced for the wheat models, whereas the faba bean models generally did not perform as well. It should be noted that the faba bean plants were grown in different locations. Thirty plants were field grown and the remaining plants were greenhouse grown. It is possible that this accounts for the diminished effect of building a plant specific model. It has long been recognised that stress, *e.g.*, from wind, affects the morphology of plants, a phenomenon known as seismomorphogenesis.[48] In a study conducted on soybean leaves, strong matrix effects were observed when soybeans grown at different locations were analysed with LIBS. Plasma temperature and electron density varied considerable between samples from different sets, but only small variations were observed from the same set. The matrix effect was so pronounced that without a normalization, to adjust for the matrix effect, no meaningful connection between signal intensity and concentration could be established.[49] Considering that this type of detrimental matrix effect was not observed in the mixed model or the faba bean model, it can be concluded that the searing step included in the sample preparation step serves the purpose of decreasing the matrix effect. Combined with the multivariate approach it is possible to get meaningful correlations out of a mixed model, however, despite this it is still beneficial to make matrix matched models. Braga *et al.*[50] reached a similar conclusion stating that to avoid matrix effects it is advisable to work with plant specific calibrations. However, more research into the benefits and limitations of plant specific models is needed.

LIBS as a tool for diagnosing nutrient deficiencies in plants

This study confirms that with the demonstrated setup, LIBS analysis is faster and thus potentially cheaper than traditional ICP-OES analysis. The key question is then; is the analytical accuracy and precision good enough to make trustworthy predictions of plant nutritional status? To be a useful tool for diagnosing nutrient deficiencies, the predicted values must be accurate enough to distinguish deficient plants from normal non-deficient plants. No analytical method is perfect and there will always be an uncertainty associated with an analysis. However, this needs to be at a level where most plant samples are correctly diagnosed as either deficient or non-deficient. The RMSEP or RMSECV values are the best estimates of how well the models perform and what accuracy can be expected of future predicted concentrations. The R^2 is good for evaluating overall correlation. However, R^2 can be heavily influenced by few measurements at extreme values. Thus, in this study key results and main conclusions are not based on R^2. The mixed models generally had RMSEP values that were too high to be useful for diagnosing nutrient deficiencies. The wheat models on the other hand are considered to be sufficiently good to be useful in diagnosing deficient plants. When the RMSECV values from the wheat models are compared to the RMSE values from ICP-OES analysis it is obvious that for some nutrients ICP-OES is superior when it comes to accuracy, but for others the difference is negligible. For P, K, Mg, S and Mn the ICP-OES has a considerably better accuracy, however for Ca, Zn, Fe, B and Cu it is very similar to LIBS.

In addition to the analytical accuracy and precision, the biological variation also influences the usefulness of the data. For nutrients with a large gap between normal concentrations and deficiency levels, the acceptable uncertainty for the prediction models is larger than for the nutrients where the difference between normal concentrations and deficiency is smaller. The deficiency limits and midrange concentrations from Fig. 1 can serve as an indication of whether the prediction models are sufficiently good and can be trusted to separate deficient from normal plants. In Fig. 5 the probability of erroneously diagnosing a wheat plant as either deficient or sufficient at different nutrient levels for the nutrients considered in this study can be seen. Although, LIBS predictions of P, K, Mg and S are less accurate than ICP-OES measurements, they can still be trusted to correctly classify the vast majority of samples. For Ca, Zn, Fe, B and Cu the accuracies are very similar and predictions based on LIBS analysis are thus expected to result in nearly the same amount of correctly classified plants as ICP-OES analysis. Manganese predictions are less accurate as compared to ICP-OES analysis and for this nutrient there would be a considerable risk of misclassifying plants based on predictions from the PLS model. For future studies it would be relevant to include more samples with low concentrations, which would probably increase the likelihood of achieving sufficiently low uncertainties for the Mn model. Devey *et al.*[36] reached different conclusions when analysing pasture samples with LIBS and ICP-OES. They found that K, Mg, Ca and P measured with LIBS had accuracies similar to ICP-OES measurements. For Mn, Fe and B they found the LIBS measurements to be fit-for-purpose, although the accuracy was inferior to that of ICP-OES measurements of the same elements. However, Zn, Cu and S were not measured with sufficiently good accuracy to be considered fit-for-purpose. The difference in their findings with regards to S, can be explained by the lack of atmospheric purging in their LIBS instrumentation, which enables analysis of the S 181 nm emission line. The explanation for the finding, that the Mn model is fit-for-purpose, is likely that the concentration in the analysed pasture samples were considerably higher for this element, as compared to the samples in the present study. In a study conducted on sugarcane leaves a good agreement between predictions based on LIBS analysis and ICP-OES data was found for P, K, Ca, Mg, Mn, Fe, Zn and B. A multivariate approach yielded considerably better result than a univariate approach.[51] In agreement with findings from our study the precision was best for macronutrients. The RMSEP values obtained in the sugarcane study are generally comparable to the RMSECV values for the wheat models in our study. In accordance with our findings, Mn and Fe are the most difficult elements to obtain good prediction models for.

Although ICP-OES also suffers from the inability to measure N, it would be of great interest to work further on quantifying N with LIBS. Jull, Künnemeyer[52] used argon for atmospheric purging and achieved a reasonably good

correlation between N estimated with NIR and N measured with LIBS. This warrants further research into the quantification of N with LIBS. Another element that is currently not being analysed with LIBS is Mo.[9] This is due to its inherently low concentration in plant tissue, and not a lack of suitable emission lines. By dry-ashing the sample prior to analysis it might be possible to get a sufficiently high signal, but further studies are required to add this essential nutrient to the list of elements that can be quantified with LIBS.

Conclusion

In this study the novel LIBS set-up proved to be considerably faster than ICP-OES analysis. The combined sample preparation and analysis time was less than two minutes per sample. The nutrients P, K, Mg, S, Ca, Fe, Zn, Mn, B and Cu could all be quantified. The spectral repeatability of LIBS measurements expressed as RSD (3–4%) was comparable to the RSD for ICP-OES measurements (2.7–8.4%). Despite the reduction of matrix effects by sample searing prior to LIBS analysis, the plant species specific models performed better than the mixed models. The best performing models were for wheat where the rel_{RMSECV} was below 10% for P, K, Ca, Mg, S, Fe, Mn and Cu. Considering the limits of nutrient deficiency for wheat the accuracies for P, K, Mg, S, Ca, Zn, Fe, B, and Cu wheat models were satisfactory, whereas the uncertainty for the Mn wheat model would result in a risk of misclassifying deficient plants as non-deficient. For Mn more samples in the low concentration range are required to build more accurate models. It was also demonstrated that the combination of sample searing before analysis and purging with N gas during analysis makes it possible to quantify S, a previously challenging element in LIBS analysis, with a certainty that enables diagnosis of S deficiency in plants. In conclusion, this study demonstrates the potential of using LIBS to quantify a broad range of nutrients in plant tissue and detecting nutrient deficiencies in a highly cost-efficient way.

Data availability

The data supporting this article have been included as part of the ESI.†

Author contributions

Frederikke Neergaard Mikkelsted: methodology, investigation, validation, writing – original draft, visualization. Daniel Adén: methodology, investigation, validation, writing – original draft, review and editing, visualization, supervision. Thomas Nikolajsen: supervision, conceptualization, writing – review & editing. Kristian Holst Laursen: supervision, conceptualization, writing – review & editing.

Conflicts of interest

The first, second and third author of this publication are employed with FOSS Analytical A/S, the entity manufacturing and selling the tested LIBS system. The corresponding author has no conflict of interests.

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