Yara N-tester chlorophyll meter calibration: a prequel

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Abstract

The chemical analysis of plant tissues is a widespread tool used for identification and prevention of nutritional (e.g. nitrogen, N) deficiencies in crops. However, this analysis is time consuming and costly. Therefore, there is interest in the development of fast and simple complementary tools for determining the nutritional status of crops. One such tool is the Yara N-Tester™ (Yara International ASA, Oslo, Norway) chlorophyll meter, which has been successfully used in Europe for rapid in-field N diagnosis, and fertiliser recommendations for cereals crops. However, there is no information on the suitability of the Yara N-Tester for New Zealand crops. Furthermore, considering the different agro-ecological conditions, there is the need to calibrate and validate the Yara N-Tester on crops under variable N rates. Two controlled experiments were carried out at Lincoln University in the 2018–2019 growing season, investigating the effects of different N fertiliser rates on chlorophyll content and readings from the Yara N-tester on three wheat genotypes. A close relationship between the measured total chlorophyll content and Yara N-Tester readings for the youngest, fully expanded wheat leaves, supported the need for a universal calibration independent of source plant material. Furthermore, the close relationship between leaf lamina N concentrations at different growth stages and Yara N-Tester readings indicated the apparatus' ability to detect N status in a target crop, suggesting its readings for chlorophyll absorption can be valid predictors of the crop N fertiliser requirements. However, meaningful interpretation of results requires a consistent sampling method, because chlorophyll meter readings differ with leaf age and part of the leaf measured.

Additional keywords: chemical analysis, fertiliser requirements, nitrogen, nutritional status

Introduction

The Yara N-Tester™ (Yara International ASA, Oslo, Norway), is a chlorophyll meter based on a similar meter called the SPAD 502 (Minolta Corporation, Ltd, Osaka, Japan) (Neukirchen & Lammel, 2002; Olfs *et al.*, 2005; Ortuzar-Iragorri *et al.*, 2005). The principle of measurements for the two devices are the same, but output values are presented on different scales. The Yara N-Tester provides rapid in-field diagnostics and an inbuilt function of the tool gives immediate results for plant chlorophyll concentrations and the corresponding N fertiliser rates (kg/ha) (Olfs *et al.*, 2005). The Yara N-Tester reading (0-700 scale (dimensionless -https://www.yara.co.nz/crop-nutrition/farmers-toolbox/n-tester) indicates the N supplies from fertiliser application and N mineralisation up to the time of measurement and, therefore, allows to conclude on the remaining N demand of the crop. Yara N-Tester reading above 700 units

suggests the crop has sufficient N, a reading below 650 suggests the crop is likely to be deficient. Yara N-Tester is suited to support split-N-application strategies. In contrast, the SPAD 502 meter gives relative values (Peltonen *et al.*, 1995), ranging from 0 to 80, which are proportional to the amount of chlorophyll present in the leaf. The chlorophyll content of a plant is a good qualitative indicator for leaf N concentration (N%) (Olfs *et al.*, 2005), and for several crops it has been demonstrated that leaf N and chlorophyll concentration are strongly correlated. Nitrogen fertiliser recommendations based on leaf N% have been established for many crops over the years (Shaahan *et al.*, 1999) and the use of chlorophyll measurements to derive information on the N status of plants instead of laboratory N analyses has been reported (Wood *et al.*, 1992).

There are a number of publications relating to the use of SPAD-meters (Follett *et al.*, 1992; Blackmer & Schepers, 1995); however, there are limited published data available for the Yara N- Tester, and the results are mainly for European crops (Neukirchen & Lammel, 2002; Olfs *et al.*, 2005; Ortuzar-Iragorri *et al.*, 2005; Ortuzar-Iragorri *et al.*, 2018), and not for New Zealand conditions.

The objective of this study was to determine the accuracy of the Yara N-Tester chlorophyll meter for predicting tissue chlorophyll content for three milling wheat (*Triticum aestivum* L.) cultivars grown in New Zealand. This was meant to determine whether the Yara N-Tester could be used to improve the accuracy of rate and timing of additional N fertiliser application.

Materials and Methods

Experiments and experimental designs

This work comprised two separate controlled experiments at Lincoln University (43°38'37.3"S 172°28'03.4"E), Canterbury, New Zealand in the 2018-2019 growing season. Two separate experiments are described, one of which was carried out in a Conviron BDW 120 plant growth room (Andrews *et al.*, 2019; Beechey-Gradwell *et al.*, 2018) (Experiment 1) and the other in a glasshouse (Experiment 2). The treatments for both experiments were factorials of different N fertiliser rates by three milling wheat genotypes [‘KWM31’ (marketed as ‘Discovery’), ‘Duchess’ and ‘Reliance’]. Eight seeds were sown per pot (Experiment 1) or tube (Experiment 2) to 20 mm depth, and thinned to five seedlings one week after emergence, which translated to 280 plants per m^2.

Experiment 1 was a randomised, complete block design, consisting of 18 treatments (three genotypes, six N rates), replicated three times. The crops were grown in 2-L pots filled with Dalton’s™ washed sand. These pots were watered every second day with 110 mL of basal nutrient solutions (Andrews *et al.*, 1992) containing appropriate N treatments. The N treatments were: 1, 2, 4, 6, 8 or 10 mM concentration of nitrate-N, supplied as potassium nitrate (KNO_3^-). Before the experiment, the potting mix was analysed for fertility, and results showed: pH 7.3, and ≤ 1 mg/L for all the other nutrients [nitrate-N, ammonium-N, Olsen phosphorus (P) < 1 mg/L, sulphate-S (S), potassium (K), magnesium (Mg), calcium (Ca) and sodium (Na)]. The K concentration was made equal in all treatments by the addition of potassium sulphate (K_2SO_4) as required, but SO_4 was not balanced. Experiment 1 was sown on 9

August 2018 and harvested on 20 September 2018.

Experiment 2 was carried out in a glasshouse and was a randomised complete block design, replicated six times. The treatments were three milling wheat genotypes and two rates of N fertiliser: low (85 kg N/ha) and optimum (285 kg N/ha). The genotypes were grown in a 60% sieved composted bark (4 mm diameter) and 40% pumice (3 mm diameter) mix, in 80-cm long PVC tubes, with an inner diameter of 15 cm (total surface area = 176.8 cm^2). The base of each tube was covered with perforated 1-L pots, pushed in upside down, to allow for free drainage. The PVC tubes were positioned on solid 4-L pail containers to capture all the mineral solution draining through the bottom. The tubes were filled with 14.1 kg of the potting mix. As the crops were watered on alternate days, the mineral solution collected in the 4-L pails was returned to the respective tubes between watering events. Experiment 2 was sown on 8 September 2018 and harvested at grain maturity on 21 January 2019.

The potting mix was analysed for fertility, and results showed: pH 6.1, nitrate-N 1 mg/L, ammonium-N <1 mg/L, Olsen phosphorus (P) 6 mg/L, sulphate-S <1 mg/L, potassium (K) 39 mg/L, magnesium (Mg) 2 mg/L, calcium (Ca) 3 mg/L and sodium (Na) 8 mg/L potting mix. Base fertiliser was added during potting mix preparation at the following application rates (Nguyen *et al.*, 2017): 0.02 g/L Osmocote® (38-0-0), 0.30 g/L superphosphate (0-9-0-11-0-20), 0.30 g/L horticultural lime (primarily calcium carbonate), and 0.30 g/L Osmocote (0-0-37), 0.30 g/L Micromax® (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo and 1% Zn) and 1.0 g/L Hydraflo® (wetting agent). An additional 0.03 g/L (equivalent to 50 kg N/ha) was applied to all tubes at three weeks after emergence. The remaining 200 kg N/ha for the high N fertiliser treatments was applied at rates equivalent to 100 kg N/ha (1.04 g urea per tube) at the start of tiller formation (Growth Stage (GS) 21) (Zadoks *et al.*, 1974), and 50 kg N/ha each, at appearance of the second node (GS32) and flag leaf (GS39), as urea (46% N) to the respective tubes. The urea was dissolved in 8 L of water, and 110 mL of solution was applied per tube, followed by light irrigation.

Sampling and chlorophyll extraction procedure

Yara N-Tester chlorophyll meter readings were taken mid-way along the length of the uppermost/youngest fully expanded leaf (repeated 30 times), in the three (Experiment 1) or six (Experiment 2) replicates, at GS21 and GS32 for both experiments, and an additional measurement at GS39 in Experiment 2. For each plant, an outline on the leaf where the Yara N-Tester™ measurement was taken was marked, and the length and width determined using callipers (to calculate area harvested). These were cut out and immediately placed in a pre-labelled 15 mL Falcon tube containing 5 mL undiluted *N, N*-dimethylformamide **(**DMF) (Inskeep & Bloom, 1985). The caps were immediately screwed tight and tubes stored in a box lined with tinfoil, to prevent photo-degradation of chlorophyll in DMF. The samples were stored overnight, in a dark cool room at ~5°C.

Chlorophyll content extraction was completed by agitating in 4 mL of DMF in darkness, followed by colorimetric determination on a Shimadzu UV1800 UV/Vis Spectrophotometer at 664.5 and 647 nm. The cuvettes were rinsed with 2 mL undiluted DMF to wash out the last sample. The chlorophyll concentration was calculated according to an established

equation (Inskeep & Bloom, 1985), and adjusted to chlorophyll content (mg/m^2) per unit leaf area.

For Experiment 2, leaf lamina biomass and N% were determined at flag leaf (GS39), 50% anthesis (GS65) and harvest maturity (GS92) by destructive harvest.

Statistical analyses

For both experiments, responses were analysed using a mixed model approach, fitted with REML as implemented in Genstat (Genstat 18th edition). Assumptions were checked via standard residual plots. Fixed effect in the models were N rate, genotype and its interaction. Random effects accounted for the position (row+column) within the experiment. In Experiment 1, for Chlorophyll A, only row was included in the random effect as the denominator degree of freedom (d.f.) could not be calculated with both row and column present. For both experiments, each variable was analysed separately. Order was considered and the largest *P*-value reported.

Results

In Experiment 1 (Figure 1), there were no significant differences (P=0.10) among the wheat genotypes when relating total chlorophyll (*a*+*b*) measurements and Yara N-Tester reading with overall averages of 433±16.8 and 616±21.1 units, respectively. However, the Yara N-Tester reading increased significantly (P<0.001) with N supply, from an average of 453±30 units for the 1 mM treatments to 666 and 731 units for the 6 mM and 10 mM treatments, respectively.

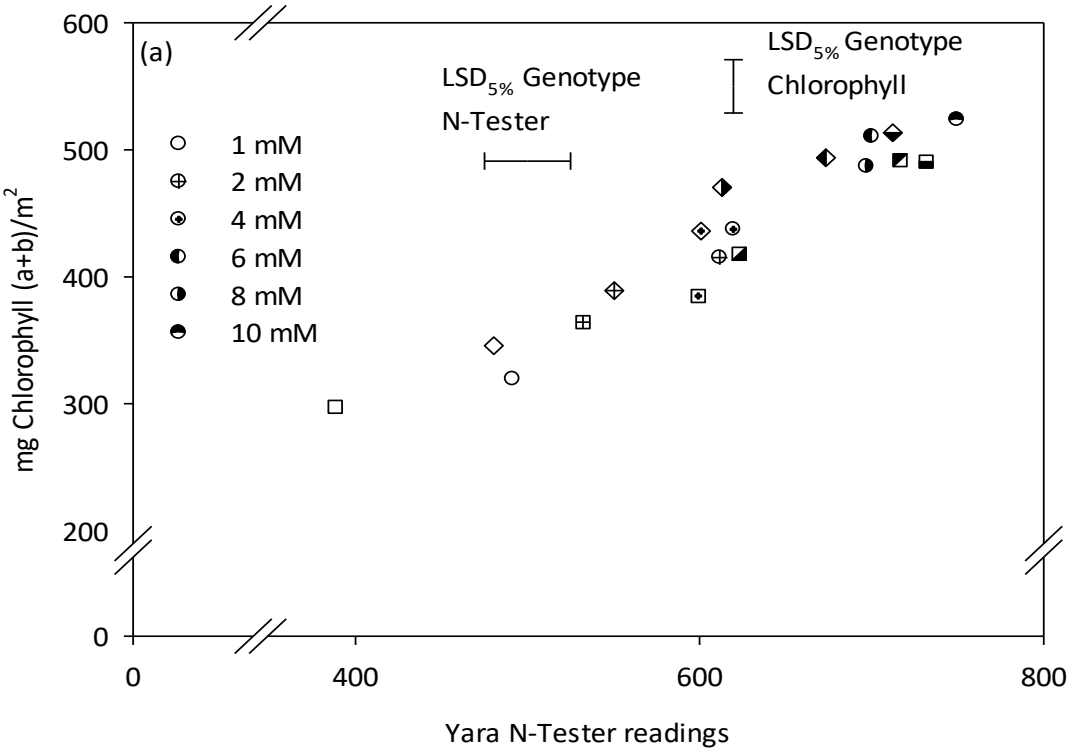

Figure 1: Total chlorophyll (a+b) against Yara N-Tester™ measurements for leaves of three wheat genotypes (● 'Discovery', ■ 'Reliance', ◆ 'Duchess') grown under six nitrogen (N, 1—10 mM) rates in a growth chamber at Lincoln University, Canterbury, in 2018 (Experiment 1). Vertical and horizontal lines are the least significant differences ($LSD_{0.05)}$ values) for the total chlorophyll and N-Tester results, respectively.

Similarly, total chlorophyll significantly increased (P<0.001) with N supply (Figure 1), from an average of 321±23.8 mg/m^2 for the 1 mM treatments to 390, 474 and 509 mg/m^2 when N was applied at 2, 6 and 10 mM, respectively. There were no differences between the 8 and 10 mM treatments. There was a very strong relationship (R^2=0.92) between Yara N-Tester readings and total chlorophyll measurements. For all genotypes, there was minimal change in chlorophyll amounts after Yara N-Tester reading of about 700 units.

In Experiment 2 (Figure 2), the responses of total chlorophyll and Yara N-Tester reading to N fertiliser rate and genotype treatments were inconsistent. The Yara N-Tester readings decreased (P=0.041) with maturation, from an average of 609±14.8 units for harvest 1 (2 November) to 583 on 9 November 2018 (harvest 2). The Yara N-Tester readings increased (P<0.001) with N supply from 510±14.8 units for the 85 kg N/ha to 681 units for the 285 kg N/ha but were unaffected (P=0.31) by genotype.

Total chlorophyll was unaffected (P=0.54) by the harvesting time (Figure 2), with an average of 519±16.9. However, total chlorophyll increased (P<0.001) with N supply from 408±16.9 units for the low N crops to 630 units when 285 kg N/ha was applied. Total chlorophyll content differed (P<0.001) among the genotypes at high N rate (Figure 2). Discovery had the higher total chlorophyll (569 mg/m^2), compared with an average of 494±20.1 mg/m^2 for 'Duchess' and 'Reliance'. The overall relationship between Yara N-Tester reading and total chlorophyll was very strong (R^2=0.90), more so at low Yara N-Tester readings representing low N supply.

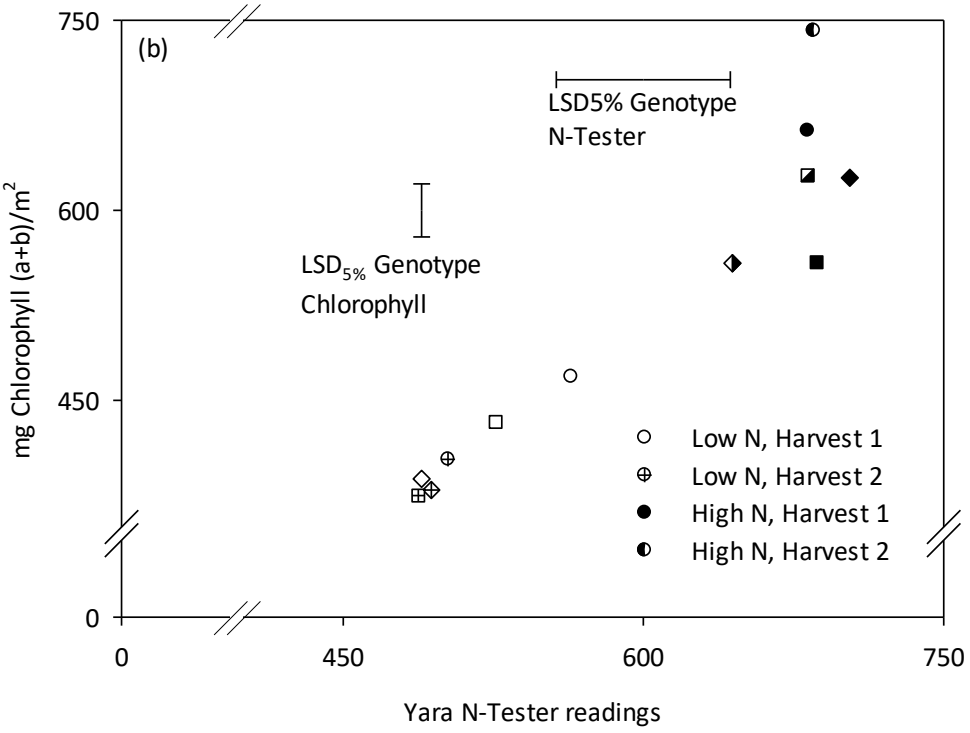

Figure 2: Total chlorophyll (a+b) against Yara N-Tester™ measurements for leaves of three wheat genotypes (● 'Discovery', ■ 'Reliance', ◆ 'Duchess') grown at low (85 kg/ha) or high (285 kg/ha) N rates in a glasshouse at Lincoln University, Canterbury, in 2018 (Experiment 2). Harvests 1 (open, and closed symbols) and harvest 2 (crossed, and half-closed symbols) and each symbol represents a mean of six replicates). Vertical and horizontal lines are the least significant differences ($LSD_{0.05)}$ values for the total chlorophyll and N-Tester results, respectively.

Crop nitrogen concentrations

In Experiment 2, there was a strong relationship ($R^2 \geq 0.72$) between leaf lamina N% and Yara N-Tester readings during vegetative and early reproductive growth stages (Figure 3a, b) and at harvest maturity (Figure 3c). Furthermore, there was a strong relationship ($R^2=0.87$) between grain yield and Yara N-Tester (Figure 3d). These relationships meant that the meter readings adequately described the lamina N status of wheat crops. In Figure 3a, leaf lamina N content of 3–4% represented Yara N-Tester readings of between 530 and 620 units. Knowledge of the critical lamina N% for the different growth stages could be used to estimate the corresponding Yara N-Tester readings, thus, the need for N fertiliser application.

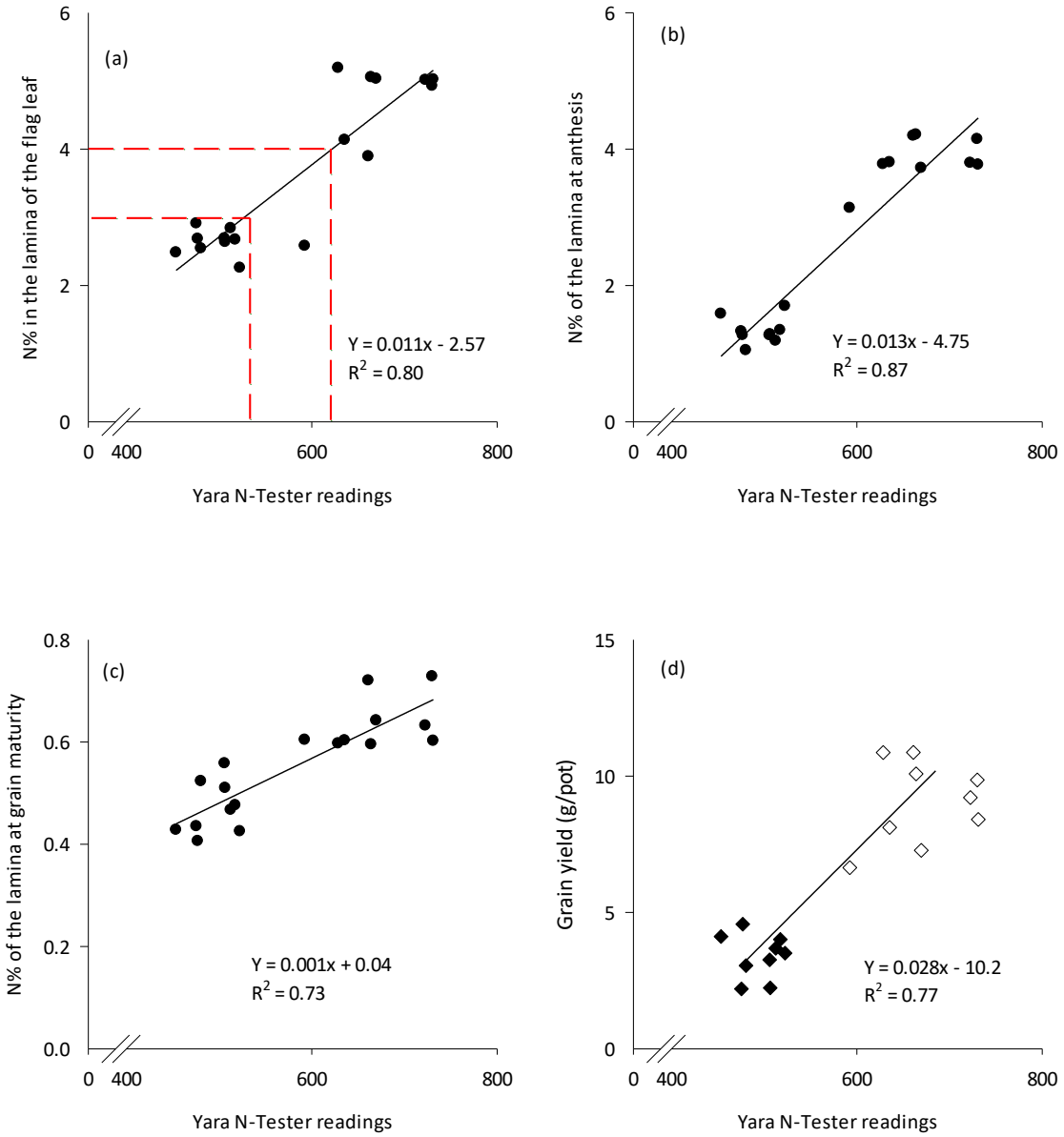

Figure 3: The relationship between lamina nitrogen (N) concentration (N%) at (a) flag leaf stage, (b) anthesis, (c) harvest maturity stages and (d) the grain yield of three wheat genotypes against the Yara N-Tester™ readings at flag leaf growth stage. In Figure 3d, open symbols represent 285 kg N/ha crops, and closed symbols are 85 kg N/ha crops.

Discussion

The objective of this study was to determine the accuracy of the Yara N-Tester chlorophyll meter for predicting tissue chlorophyll content and leaf N% for three milling cultivars grown in New Zealand. The close, linear relationship between the Yara N-Tester readings and the total chlorophyll content in the leaves (Figures 1, 2) suggests that the apparatus can be used for quantitative leaf chlorophyll determination for wheat crops. These results are consistent with previous reports for other crops such as tobacco (*Nicotiana tabacum.* L) (Neukirchen & Lammel, 2002; Brentrup, 2005) and rice (*Oryza sativa.* L.) (Takebe & Yoneyama, 1989). However, in contrast to previous reports (Uzik & Zofajova, 2000; Neukirchen & Lammel, 2002), Yara N-Tester readings did not differ among the wheat genotypes in either of the experiments. This could be because the total chlorophyll differences reported here were too small to be detected by the Yara N-Tester or the fact that 'Duchess' and 'Reliance' have common parentage (FAR, 2019; Steve Shorter, 2019, *pers.comms*) and therefore had similar total chlorophyll content in both experiments (Figure 1, 2). Results shown in Figures 1 and 2, also suggest that the Yara N-Tester saturates at about 680 units, which is consistent with the 600—700 units reported for wheat (Olfs *et al.*, 2005) and other species (Laurie-Anne *et al.*, 2015).

The close relationship between Yara N-Tester readings and leaf lamina N% at different growth stages (Figure 3 a–c) was consistent with previous reports on a number of crops (Peltonen *et al.*, 1995; de Ruiter & Davis, 1996; Shaahan *et al.*, 1999; Neukirchen & Lammel, 2002). This suggests the Yara N-Tester can be an effective surrogate indicator for plant N%, but this needs further confirmation from field experiments. As the leaf N% has been used for recommending N fertilisers for several crops (Neukirchen & Lammel, 2002), the Yara N-Tester could potentially replace the chemical leaf analysis for N. Furthermore, lamina N% at flag leaf and grain yield were closely related to the Yara N-Tester readings at flag leaf (Figure 3a, d). This shows that the Yara N-Tester is an effective indicator of grain yield and quality (Ortuzar-Iragorri *et al.*, 2005), and could be used instead of chemical analyses. However, there is the need to proceed to full field evaluation and calibration experiments to confirm these 'controlled climate' experimental results.

Yara N-Tester values indicated varying leaf N% depending on development stage (Figure 3a–c). For example, a Yara N-Tester value of 600 units corresponded to leaf N% of ~3.8% at flag leaf, but ~2.8% at anthesis and ~0.57% at harvest maturity. These differences have been reported previously (e.g. Takebe & Yoneyama, 1989; de Ruiter & Davis 1996). This, combined with leaf position (Neukirchen & Lammel, 2002), means that standard recommendations for chemical leaf analyses concerning GS or leaf position should also determine when the Yara N-Tester measurements occurred. Different chlorophyll meter readings have been reported for leaves of different ages, even for succeeding leaves on the same plant and treatments (e.g. de Ruiter & Davis, 1996; Neukirchen & Lammel, 2002). Therefore, there is need for consistency in the method of choice. Published work on wheat (e.g. de Ruiter & Davis, 1996, Peltonen *et al.*, 1995) recommend use of either the youngest, fully expanded leaf or the flag leaf and excluding the leaf mid-rib. Youngest, fully expanded leaves are likely to be more representative of the current N status of the plant, while flag leaves are considered the most appropriate as they were invariably wider and more easily measured. The higher specific leaf N for the

leaf mid-rib compared with the leaf lamina (Guillard *et al.*, 1995; Fletcher *et al.*, 2013), means that they should be excluded from measurements, as they do not represent the bulk of the N in the plant.

The close relationship between the Yara N-Tester readings and the measured total chlorophyll content in wheat leaves, supports the need for a universal calibration independent of source plant material. Furthermore, the close relationship between Yara N-Tester readings and both the leaf lamina N% at different growth stages and the grain yield, indicates the apparatus' ability to detect N status in a target crop, and therefore its readings for chlorophyll absorption are valid predictors of the crop N fertiliser requirements. However, meaningful interpretation of results requires consistent sampling methods, in relation to leaf age and part of the leaf measured (position along the leaf). Yara N-Tester readings can also be influenced by edaphic factors, such as water stress and deficiencies of other nutrients (Neukirchen & Lammel, 2002), such as P and sulphur. Thus, to derive a reliable N-fertiliser recommendation from the Yara N-Tester measurement, it is important to ensure that crops are grown under optimum conditions (Neukirchen & Lammel, 2002; Olfs *et al.*, 2005), to avoid erroneous recommendations. Furthermore, under drought stress, plants suffer from water deficiency, and the chlorophyll concentration tends to increase (Ommen *et al.*, 1999; Neukirchen & Lammel, 2002) without representing a better nutritional status. Taking into account these possible sources of variation, the use of Yara N-Tester measurement for N-fertiliser management needs, like any crop analysis, "a strict sampling protocol" (Schröder *et al.*, 2000). One of the reasons why in some studies (e.g., Gaidos, 2001) an improvement of the N-fertiliser recommendation could not be found might be that such a protocol was not followed. These results show promise for the Yara N-Tester, and now needs to proceed to full field evaluation and calibration experiments in order to confirm these 'controlled climate' experimental results.

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