Rothamsted Repository Download

A - Papers appearing in refereed journals

Cudjoe, D., Okyere, F., Virlet, N., Castle, M., Buchner, P. H., Parmar, S., Sadeghi-Tehran, P., Riche, A. B., Sohail, Q., Mhada, M., Ghanem, M., Waine, T. W., Mohareb, F. and Hawkesford, M. J. 2023. Using proximal sensing parameters linked to the photosynthetic capacity to assess the nutritional status and yield potential in quinoa. *Acta Horticulturae.* 1360, pp. 373-379. https://doi.org/10.17660/ActaHortic.2023.1360.45

Using proximal sensing parameters linked to the photosynthetic capacity to assess the nutritional status and yield potential in quinoa

D.K. Cudjoe[1,2], F.G. Okyere[1,2], N. Virlet[1], M. Castle[1], P. Buchner[1], S. Parmar[1], P. Sadeghi-Tehran[1], A. Riche[1], Q. Sohail[3], M. Mhada[3], M. Ghanem[3], T.W. Waine[2], F. Mohareb[2] and M.J. Hawkesford[1,a]

[1]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, United Kingdom; [2]School of Water, Energy and Environment, Cranfield University, Cranfield, Bedfordshire, MK43 0AL, United Kingdom; [3]AgroBiosciences Department, Mohammed VI Polytechnic University (UM6P), Lot 660, Hay My Rachid, 43150, Benguérir, Morocco.

Abstract

Proximal sensing has been used extensively for decades to assess crop nitrogen (N) status using either a hand-held chlorophyll meter or vegetation indices such as the normalized difference vegetation index (NDVI) for various crops. However, little has been done on quinoa (*Chenopodium quinoa* Willd.). In this study, we investigated how the SPAD chlorophyll meter values and NDVI could be used as indicators for N status and how they can be linked to quinoa performance in terms of photosynthesis and yield. The objectives of this study were to: 1) evaluate SPAD values and NDVI as indicators of N status, 2) assess their relevance over the crop cycle, and 3) investigate their link to the performance in terms of net CO_2 assimilation and grain yield at harvest. A pot experiment based on varying nitrogen and phosphorus (P) input conditions was conducted in the glasshouse at Cranfield University, UK. The results showed that both SPAD and NDVI correlated similarly with the leaf N content (%) (R^2=0.76, R^2=0.82, p<0.001, respectively). High correlations between SPAD and NDVI were also observed at 58 DAS (R^2=0.67) and across the entire crop cycle (R^2=0.84), validating the utility of both parameters for N status monitoring. Furthermore, significant differences between treatments were observed at different growth stages when SPAD and NDVI were measured across the crop cycle. Strong significant correlations between SPAD and NDVI with the net CO_2 assimilation (A_{net}) (R^2=0.86, R^2=0.81, p<0.001, respectively) were recorded. SPAD values and NDVI significantly correlated with grain yield at harvest (R^2=0.68, R^2=0.80, p<0.001, respectively). While SPAD and NDVI are potentially useful tools to improve N fertilizer management and develop in-season yield predictions in quinoa at relatively low-cost, alternative non-saturating spectral indices need to be explored to improve accuracy.

Keywords: *Chenopodium quinoa*, SPAD, NDVI, N status, net CO_2 assimilation, photosynthesis

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a unique pseudocereal originating from the Andean region of South America. Quinoa has attracted global attention as an important food source having exceptional nutritional qualities, health benefits, and resilience to various abiotic stresses (Bazile et al., 2016; Hinojosa et al., 2018; Dakhili et al., 2019). To meet the ever-increasing demand for quinoa, farmers and breeders need improved agronomic practices combined with the breeding of more nutrient-efficient crops, especially in low-productivity regions. Therefore, adjusting N requirements based on the prediction of potential yield is a crucial part of precision agriculture for making in-season management decisions and increasing profitability.

Proximal sensing (PS) technologies offer quick, non-destructive, and accurate assessments of crop N status, which is crucial for optimized fertilizer application and precision crop management (Chawade et al., 2019; Alvar-Beltrán et al., 2020). Spectroscopy

[a]E-mail: malcolm.hawkesford@rothamsted.ac.uk

technologies (single point or imager) offer a wide range of metrics including computed spectral reflectance indices (SRIs) and have been used to assess the nutrient status of crops, diagnose nutrient deficiency, monitor growth, and predict crop yields (Padilla et al., 2018). Chlorophyll meters such as the SPAD-502 and SRIs such as NDVI (normalized difference vegetation index) are reliable indicators for assessing the N status of crop plants (Kizilgeci et al., 2019). The NDVI is a numerical indicator using a normalized ratio of the difference between the near-infrared (NIR) and the red reflectance bands. For instance, Rehman et al. (2019) demonstrated the ability of NDVI to assess N status in rice and predict grain yield at harvest. The NDVI at panicle initiation was most closely related to crop N uptake and positively correlated (R^2=0.58) with grain yield at harvest. On the other hand, the SPAD-502 measures the relative leaf chlorophyll levels at light absorbances of 650 nm (red) and 940 nm NIR (Li et al., 2019). The SPAD has been successfully used as a selection criterion for nitrogen use efficiency and improved grain yield in durum wheat (Kizilgeci et al., 2019). In another study, Chetan and Potdar (2016) showed that yield potential in corn could be accurately predicted in-season with NDVI and SPAD. A strong correlation (r=0.98) was achieved between NDVI, SPAD, and grain yield at the tasselling stage.

Previously, most studies employing SRIs have focused largely on cereals. However, the use of PS parameters to assess the nutritional status and crop performance in quinoa has not been thoroughly studied. Recently, Alvar-Beltrán et al. (2020) tested proximal optical sensing tools to monitor quinoa growth in field conditions with various N inputs. The authors showed that SPAD-502 and GreenSeeker were effective at making in-season predictions of crop biomass at harvest (R^2=0.68 and 0.82, respectively).

As the amount of chlorophyll in the leaves provides valuable information on the physiological status and is directly linked to the photosynthetic capacity and therefore primary production (Li et al., 2019), we decided to focus the present study on these three components. The main objectives of this study were to: 1) evaluate the SPAD and leaf-level NDVI as indicators of N status in quinoa, 2) monitor N status across the season using SPAD and NDVI, and 3) assess how both reflect the crop performance in terms of net CO_2 assimilation and grain yield at harvest.

MATERIALS AND METHODS

Plant material, growth conditions, and crop establishment

A pot experiment with quinoa (*Chenopodium quinoa* Willd var. *temuco*) was conducted in the glasshouse at the Plant Growth Facility at Cranfield University, UK, from September 2020 to January 2021. The conditions were set as: day/night temperature 24/21±2°C, relative humidity 60%, a photoperiod of 14 h with a light intensity of 400-500 µmol m^{-2} s^{-1}. Before sowing, quinoa seeds were stratified at 4°C for 3 days and sown in wet vermiculite compost on a mini pot tray and incubated in the dark. After 3 days, germinated seeds were illuminated to prevent etiolation. Seedlings of similar size (5 cm) were transplanted into pots. At the two-leaf stage, the seedlings were thinned to one plant per pot. Quinoa plants were grown to maturity on a reconstituted Levington F1, low-nutrient compost, as detailed in the following section.

Experimental design, compost preparation, and application of nutrient treatments

The experiment was structured in a randomized complete block design (RCBD) with five replications. The compost used was Levington F1, low-nutrient compost (ICL, Everris, UK). Compost was washed to remove soluble nutrients, by flooding one part of the compost with five-part deionized water, mixing, breaking up aggregates, and draining through a double 0.8-mm sieve (adapted from Masters-Clark et al., 2020). The washing process was repeated five times and the washed compost was oven-dried at 80°C. Nutrients were reconstituted in the washed compost with macro- and micronutrients in a modified Letcombe nutrient solution (Masters-Clark et al., 2020). The N and P inputs were applied in four nutritional levels designated (HN-HP, HN-LP, LN-HP, and LN-LP, with H and L for high and low levels, respectively). The concentrations for HN and LN were 49.12 and 14.59 mM and for HP and LP were, 13.38 and 3.33 mM, respectively. Each pot (21 cm tall by 19 cm diameter) was filled with 360 g of washed compost and mixed with 58 g of silver sand and 790 mL of nutrient solution. Pots were replenished with 790 mL of nutrient solution at 23, 44, 65, and 79 DAS based on the designated treatments. Plants were irrigated with deionized water.

Measurement parameters

1. Weekly measurements.

From 23 DAS, the chlorophyll index and spectral data were measured weekly using a SPAD-502 chlorophyll meter (Soil Plant Analysis Development, Minolta Camera Co., Ltd., Japan) and a PolyPen instrument (PolyPen, Photon Systems Instruments, Czech Republic). NDVI was extracted from the PolyPen data using the 780 and 630 nm wavelengths. At 58 DAS, NDVI was calculated by taking an average of the 51 and 65 DAS because the 58 DAS data were missing due to an instrument failure. Measurements were realized on fully expanded leaves at the top of the plants. Three readings were made and then averaged.

2. Gas-exchange measurement.

The net CO_2 assimilation (A_{net}) was measured at 46 DAS in a fully expanded leaf from the top of each plant, employing a gas-exchange system (Li-6400XT, Li-COR Inc., Lincoln, NE, USA). The photosynthesis measurements were done between 10 am and 2 pm Additional SPAD data were collected on the same leaves.

3. Sampling for nitrogen content and yield determination.

At 60 DAS, leaves were sampled for nitrogen content analysis. Total nitrogen (N) content (%) was determined by the Leco combustion method. At maturity, manual harvesting was done to separate matured seed heads from the vegetative parts (i.e., panicles and stems). Harvested seed heads were dried at 40°C for 48 h in a forced-air oven and threshed manually. Chaffs were removed to retain cleaned grains. The grain yield per pot (g pot^{-1}) was further determined based on 13% moisture content.

Statistical analysis

Data were analysed using linear regression to investigate the relationship between variables. Analysis of variance (ANOVA) using R software was employed to assess differences between treatments throughout the crop cycle. All results were evaluated at a 5% level of significance.

RESULTS

Evaluation of N status during the reproductive/inflorescence growth stage

Table 1 shows the summary statistics of leaf N content determined at 60 DAS, SPAD, and NDVI at 58 DAS for each treatment. An increase in the mean values for each variable was observed with the higher nutrient supply treatments, except for SPAD and NDVI for the LN-HP treatment, for which values were lower than the LN-LP. The linear regression of the leaf N content with SPAD and NDVI showed high correlations (R^2=0.76, R^2=0.82, respectively; Figure 1a, b). The relationship between the N predictors (SPAD and NDVI) was also high (R^2=0.67; Figure 1c).

Time course of proximal sensing parameters (SPAD and NDVI) and their relationship throughout the crop cycle

Figure 2 (a, b) displays the time course of SPAD and NDVI measured from 23 to 93 DAS. A significant difference between treatments was observed from 37 DAS for SPAD (Figure 2a) and 30 DAS for NDVI. High statistical differences between treatments ($p<0.001$) were observed constantly from 37 DAS for SPAD and 44 DAS for NDVI. For both variables, higher values were obtained for the HN-HP treatment. The lowest SPAD values were observed for the LN-HP except at 44 DAS. Similarly, for NDVI, the lowest values were observed for the LN-HP treatment from 65 DAS. The relationship between SPAD and NDVI across the crop cycle is shown in Figure 2c. Nonlinear regression was fitted to the data displaying a high R^2 (0.84). Higher data variation was seen for the lower values of SPAD and NDVI, reflecting the observations on the time course for the LN-LP and LN-HP treatments.

Table 1. Descriptive statistics of leaf N content (%) at 60 DAS, and SPAD and NDVI at 58 DAS. Abbreviations used are minimum (Min); maximum (Max); standard deviation (SD); coefficient of variation (CV). Each treatment represents five replicates.

Treatment	Leaf N content (%)					SPAD					NDVI				
	Mean	Min	Max	SD	CV (%)	Mean	Min	Max	SD	CV (%)	Mean	Min	Max	SD	CV (%)
HN-HP	5.1	5.0	5.3	0.12	2.35	49.2	45.5	53.3	2.83	5.75	0.55	0.55	0.56	0.00	0.00
HN-LP	3.2	3.0	3.3	0.14	4.38	40.3	38.8	41.5	1.01	2.51	0.52	0.51	0.54	0.01	1.92
LN-HP	1.6	1.4	1.9	0.23	14.38	33.8	32.1	35.0	1.13	3.34	0.49	0.47	0.5	0.01	2.04
LN-LP	1.5	1.3	1.8	0.20	13.33	38.9	35.6	41.6	2.36	6.07	0.50	0.47	0.51	0.02	4.00

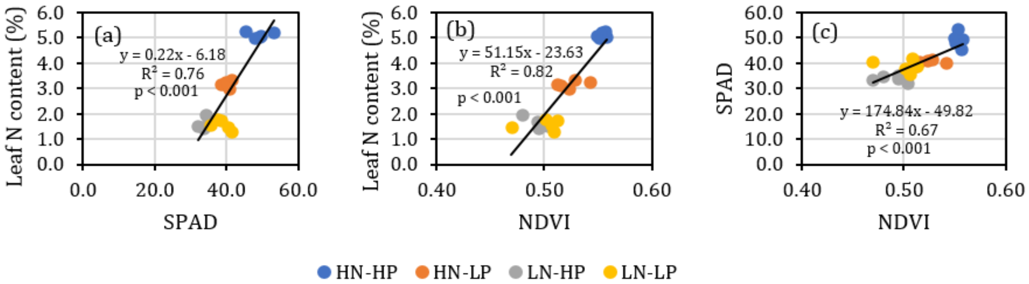

Figure 1. Linear regression between leaf N content at 60 DAS and SPAD (a), NDVI (b) at 58 DAS, and between NDVI and SPAD at 58 DAS (c). Each treatment represents five replicates. Significant level is ***p<0.001.

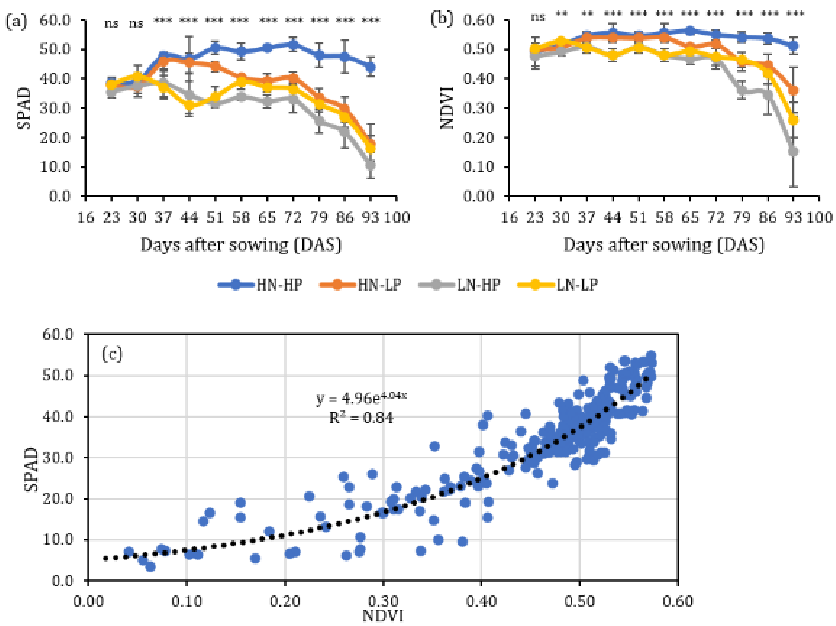

Figure 2. Time course of SPAD (a) and NDVI (b) from 23 to 93 DAS. Relationship between NDVI and SPAD over the same period (c). Error bars represent mean ± SD (*n*=5). Asterisks indicate significant difference between treatments (**p<0.01, ***p<0.001) using student's *t*-tests. Non-significant is denoted as ns (p>0.05).

Assessment of how well the proximal sensing parameters reflected crop performance

Table 2 highlights the summary statistics of the crop performance indicators under different nutritional treatments. An increase in the mean values for each variable was observed with the increase in nutrient supply. To assess how the N status predictors (SPAD and NDVI) reflected crop performance in terms of photosynthesis (net CO_2 assimilation) and grain yield at harvest, correlation analyses were performed (Figure 3). The results showed a strong significant correlation between A_{net} and leaf N content, SPAD, and NDVI (R^2=0.68, R^2=0.86, and R^2=0.81, respectively). In parallel, the N status predictors were significantly correlated with grain yield (R^2=0.86, R^2=0.68, R^2=0.80, respectively).

Table 2. Descriptive statistics of A_{net} measured at 46 DAS and grain yield at harvest. Abbreviations used are minimum (Min); maximum (Max); standard deviation (SD); coefficient of variation (CV). Each treatment represents five replicates, except HN-HP and HN-LP for A_{net} in which one of the replicates had wilted leaves and was removed.

Treatment	Performance indicator									
	A_{net} (μmol m^{-2} s^{-1})					Grain yield (g pot^{-1})				
	Mean	Min	Max	SD	CV (%)	Mean	Min	Max	SD	CV (%)
HN-HP	25.10	20.01	28.03	3.60	14.34	75.0	52.2	92.8	17.5	23.33
HN-LP	21.37	17.04	23.19	2.91	13.61	50.1	46.8	55.2	3.47	6.93
LN-HP	15.83	11.91	17.84	2.50	15.79	22.9	19.2	26.1	2.68	11.70
LN-LP	15.32	11.95	18.97	3.11	20.30	15.4	11.8	20.6	3.82	24.81

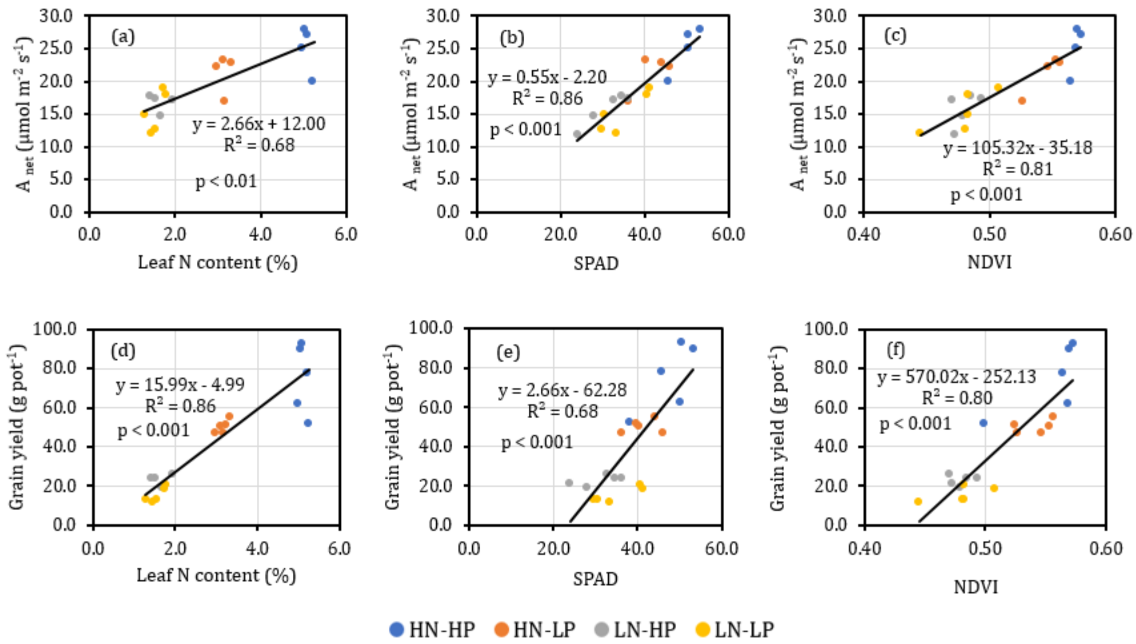

Figure 3. Correlations between leaf N content at 60 DAS and A_{net} at 46 DAS (a), SPAD at 46 DAS and A_{net} at 46 DAS (b), NDVI at 44 DAS and A_{net} at 46 DAS (c), leaf N content at 60 DAS and grain yield at harvest (d), SPAD at 46 DAS and grain yield at harvest (e), and NDVI at 44 DAS and grain yield at harvest (f). Each treatment represents five replicates, except HN-HP and HN-LP for A_{net} in which one of the replicates had wilted leaves and was removed. Significant levels are **p<0.01, ***p<0.001. SPAD and NDVI values are dimensionless.

DISCUSSION

This study was conducted to evaluate the usefulness of proximal sensing and indices such as SPAD and NDVI measured at the leaf level, as indicators of N status and crop performance predictors in quinoa. As there is a strong relationship between chlorophyll content and the leaf N content, leaf chlorophyll content is considered a useful indicator of the N status (Uddling et al., 2007). Here, both the SPAD and NDVI displayed similar efficiencies as indicators of N status, as they correlated strongly (R^2=0.76, R^2=0.82, respectively) with the leaf N content at 60 DAS (Figure 1a, b). As reported by Yang et al. (2010), the high correlation observed between NDVI and leaf N content at 60 DAS may be reasoned by the reflectivity of quinoa leaves as influenced by the amount of accumulated N, chlorophyll, and leaf area. The results are also consistent with Vian et al. (2018), where strong positive relationships were established between NDVI and leaf N content in wheat.

SPAD chlorophyll readings and NDVI have been established as reliable indicators to identify crop N status in many cereals (Kizilgeci et al., 2019; Rehman et al., 2019). However, as already mentioned, comparative research on their exploitation in quinoa remains scarce. Thus, the SPAD and leaf NDVI indices hold great potential to optimize N-use efficiency in quinoa. Furthermore, the strong relationship between SPAD and NDVI observed at 58 DAS during the inflorescence stage and throughout the crop cycle validates the suitability and precision of both parameters for rapid and non-destructive N status monitoring during the growing season (Figures 1c and 2c).

The time series of SPAD and NDVI showed strong significant differences between treatments at various growth stages of quinoa (Figure 2a, b). The results suggest that quinoa was very responsive to N fertilization but also phosphorus fertilization. Generally, quinoa responds well to N applications due to enhanced photosynthetic capacity and production of photoassimilates (Murphy and Matanguihan, 2015; Bascuñán-Godoy et al., 2018). This observation further demonstrates the utility of SPAD and NDVI in revealing nutritional variations during the growing season.

This study assessed how the N status predictors (SPAD and NDVI) reflected the crop performance in terms of photosynthesis and grain yield at harvest. It is well established that grain yield and net CO_2 assimilation are positively correlated to leaf or canopy N content, as both are responsive to an increase in nitrogen. Strong significant correlations were observed between A_{net} with leaf N content, SPAD, and NDVI in the present study (Figure 3). Our results indicate that SPAD and NDVI indices could reasonably and accurately assess the photosynthetic performance of quinoa when the proximal sensors are not saturated. Moreover, the linear regression with grain yield showed that grain yield is responding quite well to the increase in N and P fertilization and that SPAD and NDVI could reflect those changes. These findings demonstrate the utility of developing in-season yield predictors in quinoa based on proximal sensing.

CONCLUSIONS

This study demonstrated that SPAD and NDVI measured at the leaf level through proximal sensing are relevant as indicators of N status in quinoa. The strong relationship observed between SPAD and NDVI validates the effectiveness of both parameters for N status monitoring in quinoa during the growing season. Furthermore, the significant difference between treatments established at critical growth stages of the crop indicates the utility of both parameters in detecting nutritional variations during the growing season. Both SPAD and NDVI indices correlated strongly with net CO_2 assimilation and grain yield, indicating the utility for assessing the photosynthetic capacity and developing in-season yield predictions in quinoa. As SPAD and NDVI are potentially suitable proximal sensing parameters to improve N fertilizer management and develop in-season yield predictions in quinoa at a low cost. Alternative non-saturating spectral indices should be explored in quinoa to further improve accuracy.

ACKNOWLEDGEMENTS

This research is funded by OCP S.A. under the Mohammed VI Polytechnic University

(UM6P), Rothamsted Research, and Cranfield University program. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) through the Designing Future Wheat program [BB/P016855/1].

Literature cited

Alvar-Beltrán, J., Fabbri, C., Verdi, L., Truschi, S., Dalla Marta, A., and Orlandini, S. (2020). Testing proximal optical sensors on quinoa growth and development. Remote Sens. *12* (*12*), 1958 https://doi.org/10.3390/rs12121958.

Bascuñán-Godoy, L., Sanhueza, C., Hernández, C.E., Cifuentes, L., Pinto, K., Álvarez, R., González-Teuber, M., and Bravo, L.A. (2018). Nitrogen supply affects photosynthesis and photoprotective attributes during drought-induced senescence in quinoa. Front Plant Sci *9*, 994 https://doi.org/10.3389/fpls.2018.00994. PubMed

Bazile, D., Jacobsen, S.E., and Verniau, A. (2016). The global expansion of quinoa: trends and limits. Front Plant Sci *7*, 622 https://doi.org/10.3389/fpls.2016.00622. PubMed

Chawade, A., van Ham, J., Blomquist, H., Bagge, O., Alexandersson, E., and Ortiz, R. (2019). High-throughput field phenotyping tools for plant breeding and precision agriculture. Agronomy (Basel) *9* (*5*), 258 https://doi.org/10.3390/agronomy9050258.

Chetan, H.T., and Potdar, M.P. (2016). Yield prediction models in maize using SPAD and NDVI. Res. Environ. Life Sci. *9*, 1002–1004.

Dakhili, S., Abdolalizadeh, L., Hosseini, S.M., Shojaee-Aliabadi, S., and Mirmoghtadaie, L. (2019). Quinoa protein: Composition, structure and functional properties. Food Chem *299*, 125161 https://doi.org/10.1016/j.foodchem.2019.125161. PubMed

Hinojosa, L., González, J.A., Barrios-Masias, F.H., Fuentes, F., and Murphy, K.M. (2018). Quinoa abiotic stress responses: a review. Plants (Basel) *7* (*4*), 106 https://doi.org/10.3390/plants7040106. PubMed

Kizilgeci, F., Akinci, C., and Yildirim, M. (2019). Improving grain yield, protein ratio and nitrogen use efficiency of durum wheat (*Triticum durum* Desf.) hybrids using SPAD meter as a selection criterion. Int. J. Agric. Environ. Food Sci. *3*, 112–116 https://doi.org/10.31015/jaefs.2019.3.1.

Li, R., Chen, J., Qin, Y., and Fan, M. (2019). Possibility of using a SPAD chlorophyll meter to establish a normalized threshold index of nitrogen status in different potato cultivars. J. Plant Nutr. *42* (*8*), 834–841 https://doi.org/10.1080/01904167.2019.1584215.

Masters-Clark, E., Shone, E., Paradelo, M., Hirsch, P.R., Clark, I.M., Otten, W., Brennan, F., and Mauchline, T.H. (2020). Development of a defined compost system for the study of plant-microbe interactions. Sci Rep *10* (*1*), 7521 https://doi.org/10.1038/s41598-020-64249-0. PubMed

Murphy, K., and Matanguihan, J. (2015). Quinoa Improvement and Sustainable Production (Hoboken, New Jersey, USA: John Wiley and Sons), https://doi.org/10.1002/9781118628041.

Padilla, F.M., Gallardo, M., Peña-Fleitas, M.T., de Souza, R., and Thompson, R.B. (2018). Proximal optical sensors for nitrogen management of vegetable crops: a review. Sensors (Basel) *18* (*7*), 2083 https://doi.org/10.3390/s18072083. PubMed

Rehman, T.H., Borja Reis, A.F., Akbar, N., and Linquist, B.A. (2019). Use of normalized difference vegetation index to assess N status and predict grain yield in rice. Agron. J. *111* (*6*), 2889 https://doi.org/10.2134/agronj2019.03.0217.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., and Pleijel, H. (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. Photosynth Res *91* (*1*), 37–46 https://doi.org/10.1007/s11120-006-9077-5. PubMed

Vian, A.L., Bredemeier, C., Turra, M.A., Giordano, C.P.S., Fochesatto, E., Silva, J.A., and Drum, M.A. (2018). Nitrogen management in wheat based on the normalized difference vegetation index (NDVI). Cienc. Rural *48* (*9*), https://doi.org/10.1590/0103-8478cr20170743.

Yang, W., Nick, S., and Li, M.Z. (2010). [Nitrogen content testing and diagnosing of cucumber leaves based on multispectral imagines]. Guangpuxue Yu Guangpu Fenxi *30* (*1*), 210–213. PubMed