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Responses of Corn (*Zea mays* L.) Nitrogen Status Indicators to Nitrogen Rates and Soil Moisture

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Abstract: Plants usually experience fluctuating water supply during their life cycle due to continuous changes in climatic factors. Soil water content (SWC) is one of the most critical factors affecting nitrogen (N) availability, movement, and uptake by crops. Consequently, SWC levels may confound the assessment of crop N status. The present study compared the sensitivity of tissue N concentration, SPAD readings, Dualex readings, and SPAD/Dualex ratios for assessing corn (*Zea mays* L.) N status under different water supply conditions. A greenhouse trial was conducted with four N fertilizer application rates (0, 50, 50+75, and 200 kg ha⁻¹) and three watering levels (drought, drought followed by rewatering, and fully-watered). Tissue N concentration, SPAD, Dualex, and SPAD/Dualex values were influenced significantly by N rates and by SWC. Tissue N concentration, SPAD, and SPAD/Dualex increased with N rates, whereas Dualex decreased. In the first phase of reaction to drought, tissue N concentration, SPAD and SPAD/Dualex decreased rapidly but Dualex increased; however, the opposite pattern of response was observed in the long term. Under rewatering, tissue N concentration, Dualex and SPAD/Dualex gradually recovered, whereas SPAD values did not change significantly as they did in the drought treatment. There were highly significant relationships between SPAD ($r = 0.92$), Dualex ($r = -0.86$), or SPAD/Dualex ($r = 0.63$) and tissue N concentration. However, SPAD and Dualex were better predictors of tissue N concentration under drought conditions (SPAD: $r = 0.90$; Dualex: $r = -0.83$) than under fully-watered conditions (SPAD: $r = 0.39$; Dualex: $r = -0.44$) at the end of the trial. Among the indicators, Dualex is better able to discriminate N treatments, with consistent results across SWC levels.

Keywords: Indicators; N status; drought; water recovery

不同水氮处理对玉米氮素诊断指标的影响

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摘要: SPAD-502 叶绿素仪与 Dualex-3 多酚仪均可诊断作物的氮素营养状况, 为了探讨不同水分条件下该指标对作物施氮量的响应, 以玉米品种 Pioneer 38B84 为研究对象, 在温室盆栽条件下, 研究 3 个土壤水分水平(干旱、干旱后复水和水分良好)和 4 个施氮水平(0、50、50+75 和 200 kg hm⁻²), 即 12 个处理对植物含氮量、SPAD 值、Dualex 值以及 SPAD/Dualex 比值的影响。结果表明, 植物含氮量、SPAD 值和 SPAD/Dualex 比值均随施氮量的增加而增加, Dualex 值随施氮量的增加而减少。干旱胁迫初期, 植物含氮量、SPAD 值和 SPAD/Dualex 比值迅速下降, Dualex 值迅速增加; 随着干旱胁迫时间的推进, 各指标逐渐呈相反趋势变化。干旱复水后, 植物含氮量、Dualex 值和 SPAD/Dualex 比值逐渐恢复, 然而 SPAD 值恢复较小。SPAD 值($r = 0.92$)、Dualex 值($r = -0.86$)及 SPAD/Dualex 值($r = 0.63$)与植物含氮量呈极显著的相关性, 但试验后期, 由于生长阶段的不同, SPAD 值和 Dualex 值与植物含氮量在干旱条件下(SPAD: $r = 0.90$; Dualex: $r = -0.83$)的相关性高于在水分良好条件下(SPAD: $r = 0.39$; Dualex: $r = -0.44$)。通过比较不同水分条件下, SPAD 值、Dualex 值和 SPAD/Dualex 值对不同施氮量的响应, 发现 Dualex 值在不同水分条件下均能较好地反应施氮水平。

关键词: 诊断指标; 氮素; 干旱; 复水

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The SPAD-502 Chlorophyll Meter (Soil Plant Analysis Development, Minolta Camera Co., Ltd., Japan) and the Dualex (contraction of *Dual* + *excitation*, Force-A, Paris, France) are leaf-clip instruments that can be used to assess crop nitrogen (N) status quickly and non-destructively. The SPAD instrument is based on the measurement of leaf chlorophyll (Chl) content, whereas Dualex measures polyphenolics (Phen) concentrations.

SPAD values have been demonstrated to be positively correlated with leaf N concentration in a wide range of investigations involving several crops. Many studies have established critical SPAD readings below which crop yield responds to N fertilization. SPAD readings above 45.4, 52.1, 55.3, and 58.0 at corn (*Zea mays* L.) stages of three to four, six to seven, and ten to eleven fully expanded leaves and at silking, respectively, have been recommended in order to obtain high grain yields^[1]. The range of 52 to 56 SPAD units is defined as critical for separating N sufficiency from N deficiency at the 1/4 milk line stage of corn^[2]. N stress may be present at SPAD readings lower than 38–40, and a value above 44 at GS-45 has been linked to excess N uptake in spring wheat (*Triticum aestivum* L.)^[3]. However, problems have been reported with the use of SPAD for N status assessment in plants. One problem relates to the saturation of the Chl level^[4] which results in an indication of excess N because Chl is only partly affected by N uptake^[5]. SPAD measurements are also affected by crop variety, water and cold stress, location, season, and insect damage^[6-7].

The Dualex can be used to measure polyphenolics (Phen) concentrations in plants based on a Chl fluorescence assessment. A highly significant relationship with leaf extracted Phen concentration was demonstrated ($R^2 = 0.94$ for Goulas et al.^[8] and $R^2 = 0.81$ for Cartelat et al.^[9]). Phen are a diverse class of plant secondary metabolites which have many functions^[9-10]. Polyphenol synthesis and accumulation in plants is generally stimulated in response to biotic and abiotic stresses^[11]. Phen are primarily composed of carbon (C), which is assumed to be the limiting resource for their production. N availability determines C fixation rates and C availability. According to the Protein Competition Model (PCM)^[12], the competitive relationship that exists between protein and Phen is due to the common precursor (the amino acid phenylalanine [PHE]). When N is a limiting factor for crops, leaf growth and C fixation increase with N fertilizer application rates. As a result, the amount of PHE allocated for protein synthesis is enhanced with increasing N supply, whereas the amount of PHE allocated for Phen synthesis declines. Therefore, the Dualex has been used successfully to evaluate N status in many crops, based on a strong negative correlation with leaf N concentration^[4,9-10].

The gradients of both Chl and Phen along wheat leaves increased from the base to the tip^[9]. In order to alleviate this variability, Cartelat et al.^[9] suggested that the simple Chl/Phen ratio would be a good indicator of N

status. The discrimination between unfertilized control and rich-fertilized treatment in corn obtained with SPAD/Dualex (Chl/Phen) ratio was much higher than for Dualex or SPAD readings alone^[10]. Many reports have confirmed that the SPAD/Dualex ratio is a good indicator of N status in other species^[5,13].

Plants usually experience fluctuating water supply during their life cycle due to continuously changing climatic factors. Water is one of the most important environmental factors regulating plant growth and secondary metabolites^[14]. It is the most critical factor influencing N availability, movement, and uptake in crops^[15]. N-uptake ability was reduced to about 20% of the well-watered control when soil water content (SWC) was decreased to 5%; however, N-uptake ability recovered after rewatering^[16]. Many studies have been done on the response of SPAD readings to water stress. Schröder et al.^[5] showed that SPAD readings decreased in corn under water stress, but Martinez et al.^[17] indicated that SPAD readings increased by almost three units when leaf relative water content (RWC) decreased by 6.5% in wheat. Contradictions related to water status have also been reported for Phen concentration in leaves. Cheruiyot et al.^[14] found that Phen concentration was reduced under drought stress in tea (*Camellia sinensis* L.), which is in agreement with Scalabrelli et al.^[18] in grapevine (*Vitis vinifera* L.). However, Estiarte et al.^[19] reported a 10% increase of Phen in wheat leaves under drought stress.

Users of N diagnosis instruments are interested in obtaining measurements that are as specific as possible for plant N status and robust against confounding factors such as water supply. Many studies have investigated the influence of N and water status on individual N indicators. Very few have actually compared the performance of several N status indicators in a context of contrasted and transient water status. The aim of this study was therefore to understand the influence of N rates and soil water status (as well as their interaction) on N concentration in leaves, SPAD readings, Dualex readings, and SPAD/Dualex ratios in order to identify the best water and N stress indicator.

1 Materials and methods

1.1 Experimental design

The greenhouse experiment was carried out at Agriculture and Agri-Food Canada's Horticulture Research and Development Centre in St-Jean-sur-Richelieu (45°18'N, 73°15'W, elevation 47 m), Quebec, Canada, using the corn cultivar Pioneer 38B84. Soil used in the greenhouse trial was obtained from the L'Acadie experimental farm (0–30 cm layer), Quebec, Canada in 2009; it was kept at room temperature in sealed barrels and sieved to pass a 1 cm mesh. The soil was a clay loam (Aquolls, Humaquepts). A pre-sowing soil test gave the following mean values: soil pH (water) 6.6; organic matter 4.0%;

NO₃-N 35.5 mg kg⁻¹; available P (Mehlich 3) 86.8 mg kg⁻¹; and available K (Mehlich 3) 138.9 mg kg⁻¹. Corn was sown on 11 January, 2010. Seeds were sown in plastic pots (outside diameter: 20.3 cm, height: 15.9 cm) containing 2.85 kg of soil. A previous experiment showed that this soil type tends to dry fast and get very hard in pots. A rockwool block (length: 10 cm, width: 10 cm, height: 5 cm) was put in each container to provide a water reserve buffer within the soil volume. Before starting the measurements, the smallest and largest plants were removed; seven plants were kept in each pot to ensure uniform plant. Phosphate and potash fertilization was applied using 2.6 g of triple super-phosphate (0-46-0 N-P-K) and 5.8 g of potassium-magnesium sulphate (0-0-22-11 N-P-K-S) per pot. P, K and N (except the top-dressing N) fertilizers were applied in the surface (0–10 cm layer) layer of soil and mixed thoroughly with the soil before sowing.

A completely randomized block design was used with seven replications. Nitrogen was provided in the form of calcium ammonium nitrate (CAN). Four N treatments were provided at sowing (0, 0.64, 0.64, and 2.55 g of N pot⁻¹), and one N treatment at 21 DAS referred to as “50+75” (V2-V3; topdressing stage) (0, 0, 0.96, and 0 g of N pot⁻¹, respectively) (Table 1). The N rates tested for the greenhouse experiment were therefore estimated as a “field equivalent” of 0, 50, 50+75, and 200 kg N ha⁻¹. Three watering treatments were established: 1) drought (D, 40–45% of field moisture capacity); 2) drought followed by rewatering (D-R, 40–45% of field moisture capacity before 47 DAS and 80–90% of field moisture capacity thereafter); 3) fully-watered (W, 80–90% of field moisture capacity). The soil water regime in all pots was maintained at a fully-watered level for all treatments until N topdressing (21 DAS). Soil water content (SWC) in the pots was tested thereafter using a type HH₂ soil

moisture meter (Delta-T Devices Ltd. Cambridge, UK). SWC (0–10 cm layer) was measured every two days prior to rewatering and every day thereafter, based on five sampling points in each pot. When measurements reached the lower limit of the field capacity established for each treatment, SWC was increased to the upper limit of the established range.

1.2 Sampling

Plant samples were taken seven times (at 20 [V2], 32 [V3-V4], 39 [V4-V5], 46, 53, 60, and 67 DAS, respectively). Due to treatment effects on plant growth, different growth stages occurred at different watering levels from 46 DAS onwards. They were V4-V5, V5-V6, V6-V7, and V7-V8 for drought condition; V6-V7, V7-V8, V11-V12, and VT-R1 for fully-watered condition; V4-V5, V6, V7-V8, and V11-V12 for drought followed by rewatering condition. At each sampling time, one plant was randomly selected from each pot for the SPAD, Dualex, aboveground biomass, and tissue N concentration measurements.

1.3 Plant measurements

Readings were taken with a Minolta SPAD-502 (Soil Plant Analysis Development, Minolta Camera Co., Ltd., Japan) and a Dualex-3 (Force-A, Orsay, France) on the uppermost fully expanded leaves of the plants. The Dualex-3 is a portable instrument for the evaluation of leaf flavonoids concentration from the measurement of UV (375 nm) absorbance of the leaf epidermis by double excitation of Chl fluorescence [8]. The instrument makes use of a feedback loop that equalizes the fluorescence level induced by a reference red light to the UV-light-induced fluorescence level [7]. All measurements were made on the lamina, avoiding midribs, except measurements at V2 and V3-V4 because the leaves were not wide enough. Since either leaf side can be used to assess Phen status [7], only adaxial readings were made for SPAD and

Table 1 Description of soil water content (SWC) and nitrogen (N) treatments (g pot⁻¹)

SWC treatment	N treatment	N at sowing	N at topdressing	Total N
Drought (D, 40–45% of field capacity)	N0	0	0	0
	N50	0.64	0	0.64
	N50+75	0.64	0.96	1.60
	N200	2.55	0	2.55
Drought followed by rewatering from 46 DAS (D-R, 40–45% of field capacity first; then 80–90% of field capacity)	N0	0	0	0
	N50	0.64	0	0.64
	N50+75	0.64	0.96	1.60
	N200	2.55	0	2.55
Fully watered (W, 80–90% of field capacity)	N0	0	0	0
	N50	0.64	0	0.64
	N50+75	0.64	0.96	1.60
	N200	2.55	0	2.55

Dualex in order to reduce sampling time. No specific time of day was set for taking the measurements. A minimum of 20 readings were taken at different places on each leaf sampled, and the averaged data were used for statistical calculations.

1.4 Laboratory analyses

Shoots were cut at ground level and oven-dried at 70°C for 7 d, after which the dried biomass was weighed. Samples were ground through a 1-mm screen in a Wiley mill, and stored at room temperature before laboratory analyses. Samples of 0.5 g of dried biomass were mineralized using a mixture of sulphuric and selenious acids, as described by Isaac and Johnson^[20]. The tissue N concentration was measured on a QuikChem 8000 Lachat autoanalyzer (Lachat Instruments, Milwaukee, WI) using Lachat method 15-501-3^[21].

1.5 Statistical analysis

The database was subjected to correlation analysis by the SAS^[22] PROC CORR procedure and analysis of variance (ANOVA) by the SAS PROC MIXED procedure followed by orthogonal contrast analyses of linear, quadratic, and other similar effects for quantitative treatments^[23]. When both linear and quadratic analyses were significant, the trend was referred to as curvilinear^[24]. Time-repeated measures analysis was used to determine the influence of N rates, SWC, and growth stages on SPAD, Dualex, and SPAD/Dualex values by the SAS PROC MIXED procedure^[25].

2 Results

2.1 Biomass

Shoot biomass data are presented here to show the effect of treatments on crop growth, and as a reference for the response of N status indicators in the same context. Overall, the effects of treatments on shoot biomass followed the expected pattern. Shoot biomass increased with DAS but the degree of increase interacted with both SWC and N treatments (Fig. 1-A). N rates and improvements of SWC increased shoot biomass at each sampling date. Nitrogen was the most important limiting factor for growth, as evidenced by the N0 treatment, which resulted in minimal growth, irrespective of the SWC treatments.

2.2 Response of N status indicators to different levels of N and SWC

2.2.1 Tissue N concentration Tissue N concentration decreased gradually with DAS and the degree of reduction was influenced mainly by the SWC treatment (Fig. 1-B). Tissue N concentration was lower in the drought (D) treatment after 32 DAS. N treatment effects were significant. The N0 treatment stood apart from the others with very low tissue N levels.

2.2.2 SPAD, Dualex, and SPAD/Dualex SPAD readings (Fig. 1-C) and SPAD/Dualex ratio (Fig. 1-E) decreased gradually with DAS, while Dualex readings increased (Fig. 1-D). Already from the first sampling date

(20 DAS), the N0 treatment stood out for all indicators, irrespective of SWC treatment. SPAD levels for the other treatments decreased with DAS without a clear separation among treatments. Dualex measurements of the N0 treatment increased steeply at 32 DAS. They levelled off after that point for the fully-watered (W) treatment, but decreased for the drought (D) treatment. Dualex readings were clearly segregated according to N and water treatments from the rewatering stage onwards (47 DAS). Improvement of water supply conditions clearly increased Dualex levels. The SPAD/Dualex ratio was characterized by a wide range of levels, attributable mainly to SWC treatments before rewatering (47 DAS). The levels tended to decrease with DAS. The N0 treatment always had the lowest SPAD/Dualex values. The fully watered treatment produced higher SPAD/Dualex values than the drought treatment before the rewatering stage (47 DAS) but lower values thereafter. The rewatering treatment (D-R) tended to produce slightly lower SPAD/Dualex values than the drought treatment. At each date, increases in N supply were positively related to SPAD/Dualex levels.

2.3 Relationships between N status indicators and N tissue concentration

There were strong linear correlations between SPAD readings, Dualex readings, and SPAD/Dualex ratio and tissue N concentration at each sampling date and all SWC levels (Table 2). Positive correlations were observed for SPAD and SPAD/Dualex ratio, whereas negative correlations were found for the Dualex readings, as expected. Correlations with tissue N concentration were on average strongest for SPAD, followed by Dualex and then SPAD/Dualex. The strength of the relationships tended to decline for the fully-watered treatment as of 46 DAS.

3 Discussion

In this study, as expected, significant correlations between SPAD, Dualex, or SPAD/Dualex and tissue N concentration were found (Table 2). SPAD values and SPAD/Dualex ratios increased with N rates, whereas Dualex values decreased. The selected treatments produced the expected effects on shoot biomass accumulation (Fig. 1-A). The degree of variation of SPAD, Dualex, and SPAD/Dualex ratio in relation to the N treatments was consistent with previous studies^[4,10].

The Dualex quantifies the UV light that crosses the leaf epidermis and results in excitation of Chl fluorescence^[8]. The UV filtering capacity of the leaf epidermis is activated primarily by UV rays from the sun, but also by stress factors such as N deficiency and drought. The effect of drought stress, in conjunction with N deficiency, has not been quantified up to now. In the greenhouse set-up, the greenhouse glass likely reduced the UV levels reaching corn leaves. Nonetheless, Dualex readings were on average 65% higher in the N0 as compared to the fer-

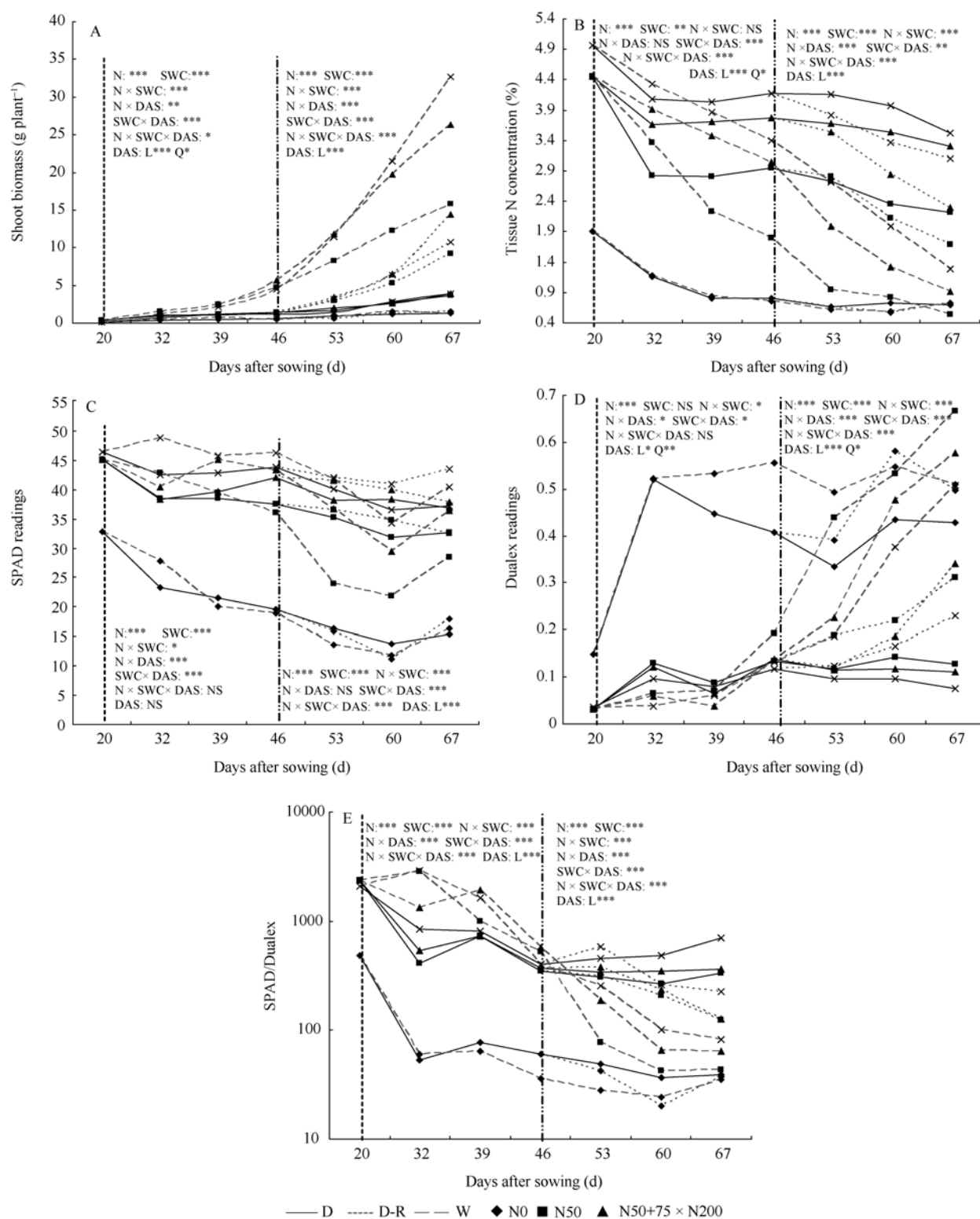


Fig. 1 Responses of (A) shoot biomass (g d m plant⁻¹), (B) tissue N concentration, (C) SPAD, (D) Dualox, and (E) SPAD/Dualox to N rates and SWC at different days after sowing

N topdressing was applied at 21 DAS (V2-V3; the first vertical broken line) and rewatering was done at 47 DAS (the second vertical broken line). Time-repeated analysis was applied before and after rewatering. The *, **, and *** indicate the difference at $P \leq 0.05$, 0.01, and 0.001, respectively. L and Q indicate linear and quadratic components with DAS, respectively, based on repeated analysis.

tilized treatments, which indicates that the Dualox is able to show the effects of N treatment on corn in a greenhouse environment. Growth stages significantly affected

SPAD^[1,10] and Dualox measurements. This is likely due to growth stage-associated changes in the estimated parameters themselves, such as polyphenolics^[19] concen-

Table 2 Pearson's correlation coefficients (*r*) for the relationship between N status indicators and tissue N concentration

Indicator	SWC ¹⁾ level	20 DAS ²⁾ (-26)	32 DAS (-14)	39 DAS (-7)	46 DAS (+0 ³⁾)	53 DAS (+7)	60 DAS (+14)	67 DAS (+21)
SPAD	D	/	0.81 ^{***4)}	0.90 ^{***}	0.94 ^{***}	0.92 ^{***}	0.90 ^{***}	0.90 ^{***}
	D-R	/	/	/	/	0.90 ^{***}	0.90 ^{***}	0.79 ^{***}
	W	0.92 ^{***}	0.80 ^{***}	0.87 ^{***}	0.86 ^{***}	0.91 ^{***}	0.77 ^{***}	0.39 [*]
Duallex	D	/	-0.86 ^{***}	-0.80 ^{***}	-0.80 ^{***}	-0.78 ^{***}	-0.82 ^{***}	-0.83 ^{***}
	D-R	/	/	/	/	-0.72 ^{***}	-0.73 ^{***}	-0.63 ^{***}
	W	-0.86 ^{***}	-0.89 ^{***}	-0.68 ^{***}	-0.81 ^{***}	-0.73 ^{***}	-0.49 ^{**}	-0.44 [*]
SPAD/Duallex	D	/	0.47 ^{***}	0.56 ^{***}	0.78 ^{***}	0.84 ^{***}	0.67 ^{***}	0.61 ^{***}
	D-R	/	/	/	/	0.48 ^{**}	0.63 ^{***}	0.68 ^{***}
	W	0.63 ^{***}	0.47 ^{***}	0.50 ^{***}	0.82 ^{***}	0.79 ^{***}	0.70 ^{***}	0.56 ^{**}

¹⁾ D: 40–45% of field moisture capacity; D-R: 40–45% of field moisture capacity before 47 DAS and 80–90% of field moisture capacity thereafter; W: 80–90% of field moisture capacity. ²⁾ DAS: days after sowing. ³⁾ The number in parentheses is the days before (–) or after (+) rewating. ⁴⁾ *, **, and *** indicate significant difference at $P \leq 0.05$, 0.01, and 0.001 levels, respectively.

trations for the Duallex ^[9], as well as to the fact that the leaf selected for sampling differed among the growth stages. The SPAD/Duallex ratio has been described as a better indicator of crop N status than either SPAD or Duallex alone ^[4,9-10]. The current study supports this finding, as the range of values was greater and the difference between the unfertilized control and rich-fertilized N treatments was always larger in SPAD/Duallex ratios (89%) than that in SPAD (53%) or Duallex (70%) alone.

It has been suggested that the SPAD method is more sensitive to water than to N stress ^[26-27], since reduced crop cell turgor could influence the transmittance of NIR (the near infrared region) energy through the leaf ^[7] and this transmission of energy is the principle behind the SPAD. Under drought stress, chlorophyll content was strongly reduced in corn ^[28] and Phen content increased by 10% in wheat ^[19]. In the current trial as well, the reduction in SPAD values and the increase in Duallex values under N0 were already apparent on the first measurement date, but the effect of different SWC levels was not as apparent. There was a trend change in tissue N (Fig. 1-B) and SPAD levels (Fig. 1-C) between the D and the W treatments before and after rewating. These indicators were on average lower under drought stress ^[28] than in the fully-watered treatment before rewating but higher after. This may be explained by: 1) plant N uptake reduced by drought stress; 2) reduction of leaf growth by drought and the induction of shrinking ^[7] and; 3) maintenance of NH_4^+ and NO_3^- concentrations in the soil in the drought treatment, which then became available for uptake when water supply was restored ^[29]. As of 46 DAS, Duallex was lower in the drought stress treatment than in the fully-watered treatment (Fig. 1-D), which is inconsistent with the positive relationship reported earlier between Phen accumulation and water stress ^[30]. However, a similar observation was made in a previous study ^[9] and explained by leaf rolling, which results in reduction of the Phen content of the measured epidermis due to less sunlight accumulation. Another possibility is that

fully-watered plants gradually experienced a relative N deficiency as they grew due to the limited N supply in the pots, a situation that would result in higher Phen levels. Crops have developed strategies for adapting to declines in SWC. Drought modifies the balance between N absorption, remobilization, and incorporation and the N cycling that occurs through the roots ^[31]. Rewatering after severe drought leads to resumption of root growth ^[16] and root vigour is stimulated with increasing N levels ^[32]. Buljovic and Engels ^[16] reported that nitrate uptake ability was restored 2 d after rewating. In the current experiment, the recovery of N-uptake ability after rewating was demonstrated by the increase in shoot biomass (Fig. 1) following rewating, and evidenced by the changes in SPAD, Duallex, and SPAD/Duallex ratio. Chl content has been found to recover as part of the transition to a normal watering regime ^[28], however, the present study showed that SPAD levels did not change significantly as they did in the drought treatment (Fig. 1). Maybe the small size of the pots resulted in a stress-induced lag in Chl recovery ^[28]. By contrast, Duallex readings were distinctly higher in the rewated treatment as compared to the drought treatment. This may be an indirect consequence of the quick resumption of growth as water supply was re-established, leading to strong demand for the limited bulk N available in the pots at this stage. After rewating, SPAD/Duallex ratios (Fig. 1) remained significantly higher in the drought treatment as compared to the D-R treatment and the W treatment.

4 Conclusion

SPAD and Duallex were better predictors of tissue N concentration under drought conditions than under fully-watered conditions as the experimental period progressed. However, Duallex measurements showed a greater sensitivity to N treatments and SWC changes resulting in better discriminative power among treatments throughout the experiment than SPAD or SPAD/Duallex ratio. Therefore, Duallex is better to evaluate N fertilizer status

than SPAD across SWC levels.

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References

- [1] Argenta G, Ferreira da Silva P R, Sangoi L. Leaf relative chlorophyll content as an indicator parameter to predict nitrogen fertilization in maize. *Ciência Rural*, Santa Maria, 2004, 34: 1379–1387
- [2] Piekielek W P, Fox R H, Toth J D, Macneal K E. Use of a chlorophyll meter at the early dent stages of corn to evaluate nitrogen sufficiency. *Agron J*, 1995, 87: 403–408
- [3] Vidal I, Longeri L, Hétier J M. Nitrogen uptake and chlorophyll meter measurements in spring wheat. *Nutr Cycl Agroecosyst*, 1999, 55: 1–6
- [4] Tremblay N, Fortier É, Mellgren R, Belec C, Jenni S. The Dualex—a new tool to determine nitrogen sufficiency in broccoli. *Acta Hort*, 2009, 824: 121–131
- [5] Schröder J J, Neeteson J J, Oenema O, Struik P C. Does the crop or the soil indicate how to save nitrogen in maize production? Reviewing the state of the art. *Field Crops Res*, 2000, 66: 151–164
- [6] Blackmer T M, Schepers J S. Use of a chlorophyll meter to monitor nitrogen status and schedule fertigation for corn. *J Prod Agric*, 1995, 8: 56–60
- [7] Samborski S M, Tremblay N, Fallon E. Strategies to make use of plant sensors-based diagnostic information for nitrogen recommendations. *Agron J*, 2009, 101: 800–816
- [8] Goulas Y, Cerovic Z G, Cartelat A, Moya I. Dualex: a new instrument for field measurements of epidermal ultraviolet absorbance by chlorophyll fluorescence. *Appl Opt*, 2004, 43: 4488–4496
- [9] Cartelat A, Cerovic Z G, Goulas Y, Meyer S, Lelarge C, Prioul J L, Barbottine A, Jeuffroy M H, Gate P, Agati G, Moya I. Optically assessed contents of leaf polyphenolics and chlorophyll as indicators of nitrogen deficiency in wheat (*Triticum aestivum* L.). *Field Crops Res*, 2005, 91: 35–49
- [10] Tremblay N, Wang Z, Bêlec C. Evaluation of the Dualex for the assessment of corn nitrogen status. *J Plant Nutr*, 2007, 30: 1355–1369
- [11] Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritime*. *Plant Physiol Biochem*, 2007, 45: 244–249
- [12] Jones C G, Hartley S E. A protein competition model of phenolic allocation. *OIKOS*, 1999, 86: 27–44
- [13] Meyer S, Cerovic Z G, Goulas Y, Montpied P, Demotes-Mainard S, Bidet L P R, Moya I, Dreyer E. Relationships between optically assessed polyphenols and chlorophyll contents and leaf mass per area ratio in woody plants: a signature of the carbon-nitrogen balance within leaves? *Plant Cell Environ*, 2006, 29: 1338–1348
- [14] Cheruiyot E K, Mumera L M, Ng'etich W K, Hassanali A, Wachira F. Polyphenols as potential indicators for drought tolerance in tea (*Camellia sinensis* L.). *Biosci Biotechnol Biochem*, 2007, 71: 2190–2197
- [15] Liao C F H, Bartholomew W V. Relation between nitrate absorption and water transpiration by maize. *Soil Sci Soc Am Proc*, 1974, 38: 472–479
- [16] Buljovic Z, Engels C. Nitrate uptake ability by maize roots during and after drought stress. *Plant Soil*, 2001, 229: 125–135
- [17] Martinez D E, Guamet J J. Distortion of the SPAD-502 chlorophyll meter readings by changes in irradiance and leaf water status. *Agron J*, 2004, 24: 41–46
- [18] Scalabrelli G, Saracini E, Remorini D, Massai R. Changes in leaf phenolic compounds in two grapevine varieties (*Vitis vinifera* L.) grown in different water conditions. *Acta Hort*, 2007, 754: 295–299
- [19] Estiarte M, Penuelas J, Kimball B A, Hendrix D L, Pinter P J, Wall G W, LaMorte R L, Hunsaker D J. Free-air CO₂ enrichment of wheat: leaf flavonoid concentration throughout the growth cycle. *Physiol Plant*, 1999, 105: 423–433
- [20] Isaac R A, Johnson W C. Determination of total nitrogen in plant tissue using a block digester. *J Assoc Off Anal Chem*, 1976, 59: 98–100
- [21] Lachat Instruments. 2005. Methods list for automated ion analyzers (flow injection analyses, ion chromatography) [2005-4-8] <http://www.lachatinstruments.com/applications/MethodsList.PDF>
- [22] SAS Institute. SAS for Windows. V.9.1. SAS Inst., Cary, NC, 2003
- [23] Little T M, Hills F J. Agricultural Experimentation: Design and Analysis. Paperback. Wiley, 1978
- [24] Hedeker D, Gibbons R D. Longitudinal data analysis. New Jersey: John Wiley & Sons, Inc. Hoboken, 2006
- [25] Klaus H, Oscar K. Design and Analysis of Experiments. New Jersey: John Wiley & Sons, Inc., Hoboken, 2008
- [26] Elwadie M E, Pierce F J, Qi J. Remote sensing of canopy dynamics and biophysical variables estimation of corn in Michigan. *Agron J*, 2005, 97: 99–105
- [27] Schlemmer M R, Francis D D, Shanahan J F, Schepers J S. Remotely measuring chlorophyll content in corn leaves with differing nitrogen levels and relative water content. *Agron J*, 2005, 97: 106–112
- [28] Sanchez R A, Hall A J, Trapani N, de Hunau R C. Effects of water stress on the chlorophyll content, nitrogen level and photosynthesis of leaves of two maize genotypes. *Photosynth Res*, 1983, 4: 35–47
- [29] Muh J, Franke J, Borken W. Drying–rewetting events reduce C and N losses from a Norway spruce forest floor. *Soil Biol Biochem*, 2010, 42: 1303–1312
- [30] Horner J D. Nonlinear effects of water deficits on foliar tannin concentration. *Biochem Syst Ecol*, 1990, 18: 211–213
- [31] Nicolas M, Simpson R J, Lambers H, Dalling M J. Effects of drought on partitioning of nitrogen in two wheat varieties differing in drought-tolerance. *Ann Bot*, 1985, 55: 743–754
- [32] Liu R X, Zhou Z G, Guo W Q, Chen B L, Oosterhuis D M. Effects of N fertilization on root development and activity of water-stressed cotton (*Gossypium hirsutum* L.) plants. *Agric Water Manag*, 2008, 95: 1261–1270