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Potential indicators based on leaf flavonoids content for the evaluation of potato crop nitrogen status

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Introduction and objective

The evaluation of in-season crop nitrogen status

improve crop nitrogen management

and nitrogen fertilizer efficiency

Why?

1. For all the known consequences of Nitrogen (N) fertilization on:
   - Tuber yield and quality (dry matter content, tuber size, …)
   - Risks to the environment (N losses)
   - Economic loss for producers (fertilizer's prices)

2. The establishment of provisional field-specific N recommendation can never be accurate !
   (influenced by several unpredictable factors)

Evaluation of in-season crop N status

How?

Potato N monitoring implies the use of plant-based indicators:

The Nitrogen Nutrition Index (NNI): likely the best indicator of crop N status

•requires destructive sampling ! **But** •long delay to get result !

Can be used as a reference to calibrate other easier, quicker and non-destructive methods

Evaluation of in-season crop N status

How ?

These methods are currently based on optical measurements of :

1. **Chlorophyll content**: highly correlated to N status

- Leaf transmittance: hand held chlorophyll-meters

  But: Chlorophyll-meters can detect only deficiency situations for N !

- Crop light reflectance: Ground-based radiometers

  But: Interferences such as soil reflectance should be taken into account !

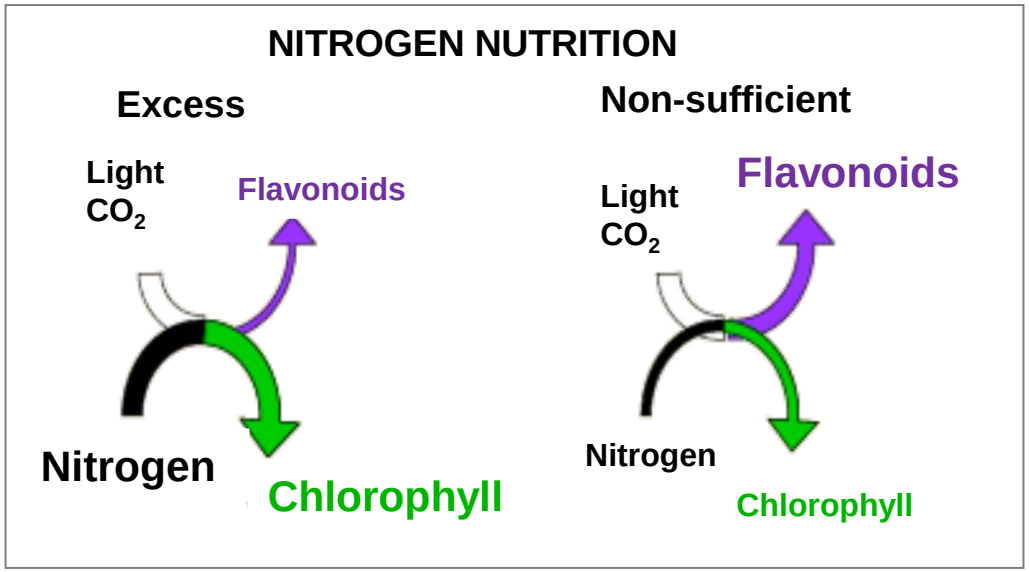
2. **Flavonoids content:** potential indicators of N status

- Leaf fluorescence: fluorometers

Evaluation of in-season crop N status

How?

3. Chlorophyll and Flavonoids content



The combined ratios should improve the discrimination between N treatments due to the **opposite dependance** of chlorophyll and flavonoids with N status

The carbon-nutrient balance
(Coley and al.,1985)

Global objective

The objective of the current study was to evaluate the potentialities of leaf flavonoids content considered alone or combined to leaf chlorophyll content for the evaluation of potato crop N status.

3 criteria were evaluated:

1) The sensitivity of the studied index,
2) The earliness of the diagnosis,
3) The stability of the index.

Material and Methods

Experimental design

- The 2010 experiment was conducted in Gembloux (Belgium)
- The experiment included two potato cultivars: Bintje and Charlotte
- 5 increasing N rates were applied at planting:

Bintje: T_1 : 0,T_2 : 115,T_3 : 165, T_4 : 251 and T_5 : 248 kg N ha^{-1}
Charlotte: T_1 : 0, T_2 : 100, T_3 : 140, T_4 : 180 and T_5 : 210 kg N ha^{-1}

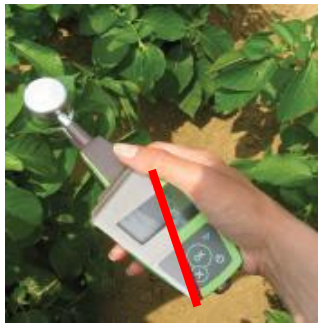
- A completely randomized block design with 3 replications was used

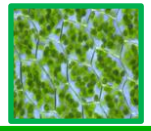
Experimental field
(Gembloux, CRA-W, 2010)

Optical data collection

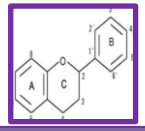
Optical measurements were carried weekly during the potato growth from mid of June to end of July

8 measurements dates (expressed as Day After Emergence: DAE)

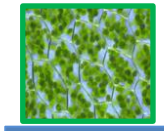
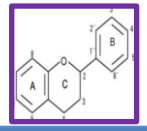
Dualex (Force-A, Orsay, Paris, France)	Multiplex fluorometer (Force-A, Orsay, Paris, France)	Chlorophyll-meter (Yara, Oslo, Norway)	Cropscan radiometer (Cropscan Inc, Rochester, USA)	Chlorophyll-meter & Dualex or & Multiplex
				
CHL index transmittance	**SFR-R index fluorescence**	**SPAD index transmittance**	**R index reflectance**	**SPAD/FLV index**
FLV index fluorescence	**FLAV index fluorescence**		**NIR index reflectance**	**SPAD/FLAV index**
NBI index	**NBI-R index fluorescence**			



Chlorophyll indicators



Flavonoids indicators

Chlorophyll & Flavonoids indicators

Samples collection and analysis

Plant samples were collected periodically during the growing cycle allowing the dertermination of:

- Total biomass
- Tuber yield

Plant tissue sub-samples were subjected to laboratory analyses allowing the determination of:

- Total N for different parts of the plant
- Total N-uptake
- NNI

Statistical analysis

The studied indices were subjected to analysis of variance (ANOVA) and to polynomial contrasts

Results and discussion

Comparison of studied indices among the criteria

Sensitivity

Statistical analysis	Observed effect
Effect of nitrogen rate among the whole sampling dates	N* or N**or N*** : significant N responses for all the studied indices
Effect of nitrogen rate for each sampling date	FLAV (Bintje) and NBI (Charlotte) was able to reveal significant N responses respectively for eight and six different sampling dates
Trends of indices	Mainly curvilinear indicating a low difference between the medium and high N rates

N*, N**, and N***: statistical significance nitrogen effect respectively at P ≤0.05, P ≤ 0.01, and P ≤ 0.001.

FLAV : related to flavonoids content (Multiplex) , **NBI** : related chlorophyll and flavonoids content (Dualex),

Comparison of studied indices among the criteria

Sensitivity

The Sensitivity index SI corresponds to the ratio between changes in the response of the measuring index divided by the corresponding change of N concentration in aerial parts:

$$SI = \frac{(Index_{T_5} - Index_{T_1})/(Index_{T_1})}{(N_{T_5} - N_{T_1})/(N_{T_1})}$$

(Adapted from the definition of Bockstaller et al., 2008 and Jolicoeur, 2002)

$Index_{T5}$ and $Index_{T1}$: Studied index corresponding respectively to T_5 and T_1 treatments.
N_{T5} and N_{T0}: Nitrogen concentration in aerial parts corresponding respectively to T_5 and T_1 treatments (% of DM)

a) SI ranges from 0 to 1 for indices having the same dependance on N status
b) SI ranges from -1 to 0 for indices having inverse dependance on N status

➡ |SI|(average absolute value of SI) classified **the combined chlorophyll to flavonoids** indicators as the **highest sensitive** indices

Bintje: NBI=0.85, SPAD/FLV= 0.8
Charlotte: NBI-R=0.88, NBI and SPAD/FLV= 0.76

Comparison of studied indices among the criteria

Earliness of diagnosis

Responses of studied indices to applied N dose at the first date of sampling

			Index										
			CHL	FLV	NBI	SPAD	SFR-R	FLAV	NBI-R	R	NIR	SPAD/FLV	SPAD/FLAV
N effect	17 DAE	Bintje	NS	*	*	*	NS	*	NS	NS	NS	**	**
	5 DAE	Charlotte	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS

N: Nitrogen effect. DAE: Days After Emergence. *, **, and ***: statistical significance respectively at P ≤0.05, P ≤ 0.01, and P ≤ 0.001.
NS : no significance at P>0.05.

Comparison of studied indices among the criteria

Stability

The stability was assessed through the absence of interaction between N rate and date of measurements expressed as DAE N.DAE ^{NS}

Responses of the studied indexes to N*DAE interaction

		Index										
	Variety	CHL	FLV	NBI	SPAD	SFR-R	FLAV	NBI-R	R	NIR	SPAD/FLV	SPAD/FLAV
N*DAE	Charlotte	**NS**	**	**NS**	*	**NS**	*	**	*	**NS**	**NS**	*
	Bintje	**NS**	***	**	**NS**	*	***	***	**NS**	**NS**	**NS**	**

N: Nitrogen effect. DAE: Days After Emergence. *, **, and ***: statistical significance respectively at P ≤0.05, P ≤ 0.01, and P ≤ 0.001.
NS : no significance at P>0.05.

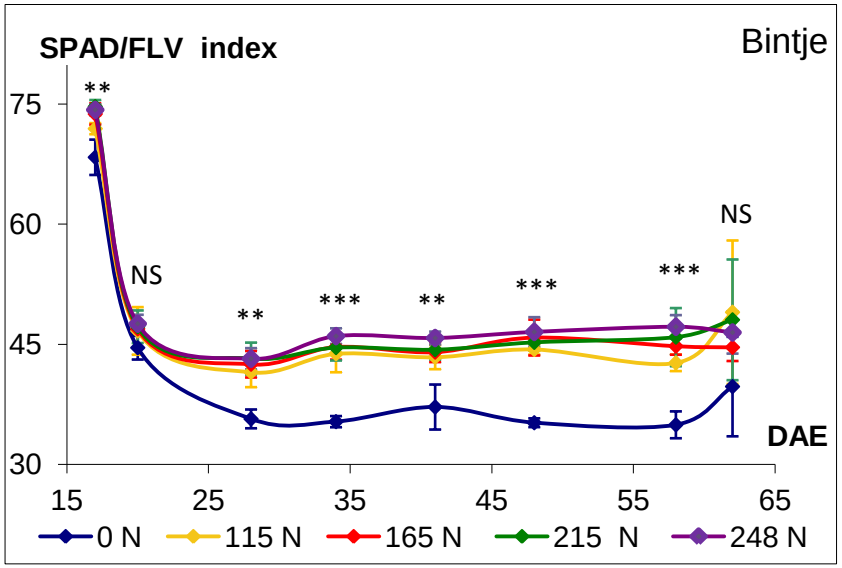
Identification of valuable indices

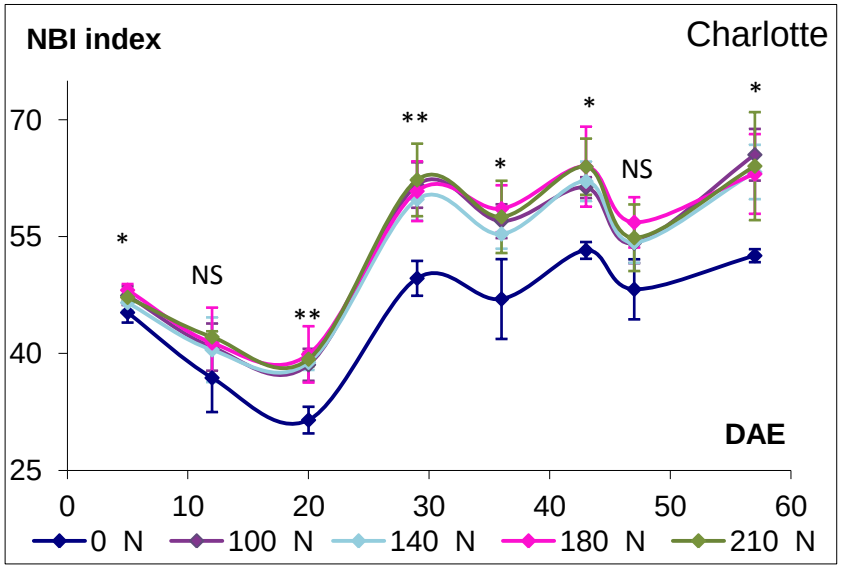
Combining the three criteria

SPAD/FLV index for Bintje and **NBI** index for Charlotte were valuable in this study

Optical devices		**Chlorophyll-meter & Dualex**	**Dualex**
Indices		**SPAD/FLV**	**NBI**
Sensitivity	N effect for the whole sampling dates	N***	N***
	N effect for each sampling dates	Significant N effect at 6 sampling dates	Significant N effect at 6 sampling dates
	Sensitivity index	0.8	0.76
Earliness of diagnosis		N** at 17 DAE	N* at 5 DAE
Stability		N.DAE ^{NS}	N.DAE ^{NS}

Identification of valuable indices





Time course of SPAD/FLV and NBI indices for increasing nitrogen rates (N) (kg ha^{-1})

N: Nitrogen effect. DAE: Days After Emergence. *, **, and *** : statistical significance respectively at P ≤0.05, P ≤ 0.01, and P ≤ 0.001. NS : no significance at P>0.05

Conclusion and prospects

❑The combination of leaf flavonoids content to leaf chlorophyll content as ratios (SPAD/FLV and NBI) suggests that these indicators could be used as valuable tools to assess potato crop N status.

❑The identification of valuable indices in this study on the basis of the analyzed criteria should not eliminate considerations of others in different contexts and have to be studied further.

❑The specificity of the selected index should also be investigated for further experimental years.