Leaf nutritional indexes validity in nitrogen nutrition diagnosis and yield prognosis in organic potatoes

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Introduction
Potato (Solanum tuberosum L.) is highly responsive to N fertilizer. Therefore, adequate nitrogen supply is necessary for plant growth and economic yield. Research has reported that nitrogen supply to potato crop usually exceeds plant requirements. As a result, environmental and economic risks are common threats where potato plantation is dominant (Shreshta et al, 2010).

Attempts to optimize mineral nitrogen supply in order to reduce such risks and optimize productivity have established different nutritional indexes, i.e. SPAD index, petiole nitrate content, total chlorophyll and total nitrogen content. In addition, critical threshold of each index has been established to identify plant nutritional status whereas above this level, there is no need for additional nitrogen supply and vice versa (Rodrigues et al, 2005). Moreover, some investigations have reported the potential of employing these indexes in potato yield prognosis. In the USA, Jindong et al, (2007) have compared the efficiency of SPAD, petiole nitrate and Quickbird satellite imaginary data in determining N status of potato plants. They reported petiole nitrate content as an efficient method during early stages of plant development. However, feasibility of these indexes and their critical levels were not reported. Adriana et al, (2008) in Slovenia studied the efficiency of SPAD meter and petiole nitrate content in identifying potato nutritional status. The measured indexes were compared with plant N-tissues content and correlations with the fresh tuber yield were tested. They reported the accuracy of these indexes in monitoring plant nutrition status and fertilization decision-making. In Brazil, Coelho et al, (2010) reported 40.5 and 43.7 of SPAD index, 66.7 and 75.2 g N kg⁻¹ DM and 6.13 and 6.96 mg g⁻¹ FM of total chlorophyll as critical levels for economic yields for Agata and Astrex, respectively. Moreover, they reported positive correlations between these indexes and yield levels. In Argentina, Giletto et al, (2010) reported 40 and 36 SPAD index readings as minimum thresholds during vegetative growth and tuber bulking for economic yield in three tested varieties, respectively.

In organic potato, Shukla et al, (2007) reported highly significant linear and quadratic regressions between leaf N content, chlorophyll meter readings and tuber yield. Moreover, SPAD readings decreased as plants aged. With the exception to the previous work, no more reports are documented. With the increasing awareness towards organic fertilization and lack of precise diagnosis standards, knowledge of crop growth in relation to the slower release of organic fertilizers’ nitrogen is required for best management and optimum yields.

The current experiment aims to test the validity of some nutritional indexes, SPAD index, total chlorophyll and total nitrogen content, as affected by the GM sunhemp nitrogen doses, in organic potatoes as estimates for N nutritional status and yield prediction.

Materials and methods
A field trail was conducted in the Horta Nova experimental farm, of Universidade Federal de Viçosa, Minas Gerais state which is located at 693 m altitude, 20°45′ S and 42°51′ E, from May 27 to September 10, 2011, in a sandy clay soil (5.7pH and 2.5% OM). Prior to potato plantation, the green manure sunhemp (Crotalaria juncea L.) was planted in an adjacent area and grown until full blooming (85 DAP). Then, harvested, chopped, air dried and stored until incorporation.
incorporation, samples were collected for dry matter and total nitrogen content determination. Samples were dried until constant weight. The difference between fresh and dry weight represents dry matter content (%). Thereafter, samples were ground to pass through 2 mm sieve and subjected to total nitrogen content determination by Kjeldahl method.

The experiment consisted of five treatments, four doses (75, 150, 225 and 300 kg N ha⁻¹) from the GM sunhemp as a sole nitrogen source and the recommended mineral nitrogen (250 kg ha⁻¹) as a control. The corresponding amounts to nitrogen doses from the sunhemp air-dried biomass were 3.75, 7.5, 11.25 and 15 t ha⁻¹, respectively. Treatments were arranged in a complete randomized block design with four replicates. Fifteen days before planting, the equivalent quantities of the GM dry biomass were weighted and manually distributed then incorporated by rotary tiller. Within the control treatment, mineral nitrogen was supplied from ammonium sulphate (21% N) according to recommendations as 70% in the base dressing and the rest as side dressing at 22 days after emergence. Each experimental unit was 7.5 m² and consisted of four rows (2.5 × 0.75 m). Before planting, all plots received macro nutrients (phosphorus, potassium and magnesium) with the amount of 420, 220 and 200 kg ha⁻¹, respectively. Micro nutrients (boron, copper, zinc-10 kg ha⁻¹ of each) and 250 g ha⁻¹ of molybdenum were supplied as well. The above-mentioned nutrients were blended and broadcasted inside the furrows before planting (Fontes, 2005).

‘Ágata’ pre-sprouted tubers (35-50 mm diameter) were planted manually in the furrows (25 cm apart) then covered by 10-15 cm of soil. At 21 days after emergence, nutritional indexes, SPAD index, total chlorophyll and total nitrogen content, were measured in the fourth expanded leaf from the apex. SPAD readings were collected using the hand-held SPAD 502 from five representative plants. Five readings were collected in each leaflet of the fourth leaf from plant apex, avoiding the midrib, and the mean value represents one plant. Moreover, the mean value of five plants represents one replicate. Measurements were collected in the morning between 8 and 11 o’clock. Then, two discs from each leaflet were removed, frozen and transferred to the lab for total chlorophyll determination following the method described by Lichthenthaler, (1987). In addition, the same leaves were removed, dried and subjected to total nitrogen determination following Kjeldahl method.

At harvest (103 DAP), seventeen plants of the two central rows were manually harvested. Tubers were left two hours for curing then graded according to the standards of Brazilian Ministry of Agriculture, (1995). Subsequently, tubers were weighted to calculate total and marketable yield considering 50 thousand plants per hectare. The sum of grades with a diameter higher than 3.3 cm represented the marketable yield. The obtained results were subjected to statistical analysis, regression and correlation using SAEG software (Version 9.1).

**Results**

After emergence, the measured leaf nutritional indexes, SAPD, total chlorophyll and N-total content, within the fourth leaf showed linear increases by increasing GM nitrogen dose (table 1). SPAD readings varied between 36.9 and 39.3. The extracted total chlorophyll (g kg⁻¹) in the fresh matter bases ranged from 6.75 to 7.32 in the low and high GM nitrogen doses, respectively. Moreover, N-total content (g kg⁻¹) within the dry matter bases ranged from 53.28 to 56.34. As the adjusted regression models for nutritional indexes were linear, it was impossible to determine a threshold or critical value. Total and marketable yields as influenced by GM nitrogen doses followed the same linear trend, as well. The highest total and marketable tuber yields were 47.89 and 45.95 t ha⁻¹ from the highest GM nitrogen dose, respectively.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Equation</th>
<th>Significance</th>
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<tbody>
<tr>
<td>SPAD</td>
<td>Y = 36.1125 + 0.0107x N</td>
<td>R² = 0.98 **</td>
</tr>
<tr>
<td>chlorophyll</td>
<td>Y = 6.475 + 0.00262xN</td>
<td>R² = 0.90 *</td>
</tr>
<tr>
<td>N-total</td>
<td>Y = 52.2031 + 0.0144883xN</td>
<td>R² = 0.96 *</td>
</tr>
<tr>
<td>T yield</td>
<td>Y = 32.383,9 + 53.5937xN</td>
<td>R² = 0.95 **</td>
</tr>
<tr>
<td>M yield</td>
<td>Y = 31.123,9 + 52.2453xN</td>
<td>R² = 0.94 **</td>
</tr>
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</table>

*, ** significant at 5% and 1 % probability, respectively

The correlations between nutritional indexes and total yield component were positive and significant (Table 2). Correlations between nutritional and marketable yield were positive and significant as well.

**Table 2. Pearson's correlation and linear regression between nutritional indexes and total and marketable yield of**
Compared to the mineral nitrogen control treatment, higher GM nitrogen doses (75 and 150 kg ha⁻¹) produced lower yields than the control. However, the elevated doses (225 and 300 kg ha⁻¹) produced higher yields. **

**Discussion**

The increases of the estimated nutritional indexes, within GM treatments, after emergence can be attributed to the rapid nitrogen release from the sunhemp residue after incorporation, which was matched with potato nitrogen acquisition. Odhiambo et al. (2010) reported that approximately 135 kg N ha⁻¹ of the sunhemp biomass nitrogen content were released after five weeks of incorporation. As nitrogen is the precursor of amino acids and other active metabolic components, the increased nitrogen absorption was reflected in increased leaf N content and consequently, chlorophyll content and SPAD index, leading to optimum light interception, dry matter accumulation and yield increases (Taiz et al. 2009).

Taking into account the thresholds of the fourth leaf dry matter N-total content (40 to 65 g kg⁻¹) which were mentioned by Rodrigues et al. (2000) for potato economic yields, the GM sunhemp provided potatoes with adequate nitrogen supply as leaves’ nitrogen content was within that range. The positive correlations between nutritional indexes and yield components are similar and consistent with that obtained by Coelho et al. (2010). The correlation values (R) of 0.63, 0.64 and 0.42 for total yield and 0.59, 0.6 and 0.35 for marketable yield with SPAD index, N-total and total chlorophyll content, respectively.

The lower values of SPAD index of GM treatments when compared with the control could be attributed to other factors such as soil water supply. As green manures incorporation increases soil organic matter leading to improved water-holding capacity and stable water supply then decreased SPAD index (Haverkort et al., 2006). However, the insignificant differences of total chlorophyll and N-total content within GM treatments with the control could be attributed to the potential of green manures to increase N uptake (Wivstad, 1997) and regarded as physiological adaptation by plants to optimize light interception and dry matter accumulation.

The higher yields of GM nitrogen doses are consistent with that obtained by (Campiglia et al., 2009) as they reported similar yield of potatoes after incorporating legume green manures to that of mineral nitrogen. Moreover, the higher yields can be attributed to the beneficial effects, besides the added nitrogen, of the GM sunhemp incorporation prior to potato as mentioned by Bath, (2000).
Conclusion
Nutritional indexes can be valid estimates to determine nitrogen nutrition status and predict yield when sunhemp is used as a sole nitrogen source in organic potato crop.

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