Potato tuber greening risk is associated with tuber nitrogen content

Sabine Tanios, Robert Tegg, Alieta Eyles, Tamilarasan Thangavel, Calum Wilson*

ARC Training Centre for Innovative Horticultural Products, Tasmanian Institute of Agriculture, University of Tasmania, New Town Research Laboratories, 13 St. Johns Avenue, New Town, Tasmania 7008, Australia.

* Corresponding author: calum.wilson@utas.edu.au; T: (+61 3) 6226 6381

Keywords: chlorophyll, nitrogen fertilisation, potato, tuber greening, tuber nitrogen.

Running title: Tuber N content is a risk factor for greening

Abstract

Tuber greening is one of the major causes of quality loss in the potato industry, however, the impact of nitrogen in-field fertilisation on this visual defect remains unknown. Two field experiments, one in Tasmania with final N treatment rates of 40, 100 and 190 kg N ha^{-1} for Nicola and 35, 95 and 185 Kg N ha^{-1} for Maranca, and another in South Australia with N rates of 100, 200 and 300 kg ha^{-1} for Maranca, showed a positive linear relationship (R^2 = 0.99, 0.86 and 0.90, respectively) between tuber N content and tuber greening, as determined by chlorophyll content. However, increased N fertilisation rates did not directly translate to increased tuber N content, with responses appearing to be variety specific. We conclude that tuber N content is a risk factor associated with tuber greening, but the manipulation of tuber N content through N fertilisation rate is not straightforward and may depend on variety and location.

Introduction

Potato (*Solanum tuberosum* L.) is the world's third most important food crop for human consumption and is critical for global food security, providing nutrition for over a billion people (Devaux et al. 2014). There is a need to improve potato production and minimize the challenges facing growers and retailers. One of the major reasons for potato wastage is greening of the tuber skin and flesh, which occurs when potatoes are exposed to light in the field or post-harvest, through a dynamic process affected by several genetic, environmental and cultural factors (Bamberg et al. 2015; Tanios et al. 2018). Tuber greening during production has been linked to varietal genetics, insufficient soil coverage, shallow planting depths, re-hilling operations, cracks in the soil or following wind and/or irrigation mediated erosion of light sandy soils (Lewis and Rowberry 1973; Mosley 1975; Stalham et al. 2001; Bohl and Love 2005; Pavek and Thornton 2009; Tanios et al. 2020).

Achieving high yields of potato typically requires high nitrogen (N) inputs (Eyles et al. 2018). Several N fertilisation rates have been advised as optimal for potato production, ranging from 70 to 330 kg ha^{-1}, depending on soil type and variety, though the most economically efficient rates vary from 147 to 201 kg ha^{-1} (Fontes et al. 2010). Excessive N application is not only costly, but also reduces N use efficiency, delays maturity, decreases tuber quality, and causes tuber flesh darkening after boiling (Sharifi et al. 2005; Zebarth et al. 2006; Kumar et al. 2007; Ruza et al. 2013) and potentially may increase tuber greening propensity (Braun et al. 2010). From an environmental perspective, excessive N use increases the risk of groundwater contaminationpollution via the leaching of nitrate (Arriaga et al. 2009; Neumann et al. 2012). ~~However, although the negative effects of excessive nitrogen fertilisers rates are well known, overfertilization may be used by growers as insurance against deficit to ensure maximal yield (Sparrow 2012).~~

In general, increasing N fertilisation rates has been shown to increase tuber N content (Leszczyński and Lisińska 1988, Millard 1986; Ahmed et al. 2009; Saeidi et al. 2009; Ruza et al. 2013), though this relationship can vary with experimental conditions and variety. For example, increasing N fertilizer rates from 40 to 200 kg ha^{-1} increased tuber N content from 1.59% to 2.03% (Leszczyński and Lisińska 1988) and increasing N rates from 0 to 250 kg ha^{-1} increased tuber N from 0.68 to 1.49% (Millard 1986), while increasing N rates from 0 to 120 kg ha^{-1} increased tuber N from 1.09% to 1.53%, but thereafter, remained constant in the higher N treatments of 150, 180 and 210 kg ha^{-1} (Ruza et al. 2013).

The impact of nutrition, particularly N, on tuber greening propensity however remains unclear, with to our knowledge, only one reported study. It found that an increase of N fertiliser rate, from 0 to 300 kg ha^{-1} resulted in an increase in greening of washed tubers of four varieties, after 25 days of exposure to light (Braun et al. 2010), however tuber N was not quantified. Where N levels have been measured, such as from leaf tissue, there exists a well-defined positive relationship between leaf N and chlorophyll content (Evans 1989; Mauromicale et al. 2006; Güler 2009), and it remains to be seen as to whether such relationships occur at a tuber level, and whether N fertilisation and subsequent changes in tuber N content could influence tuber greening.

The aim of this study was to determine how N fertilisation rates affects tuber N content, and subsequently, tuber greening. We hypothesise that tuber N content positively correlates with tuber greening propensity, as evidenced by increases in tuber chlorophyll concentration. To this end, two field trials in major potato production regions of two Australian states were conducted.

Materials and Methods

Field trial 1

Potato seed tubers of cvs. Nicola and Maranca were planted in a commercial potato field with a loamy sand soil at Buckland, Tasmania (42° 36'.03.1" S; 147° 42'.08.8" E). Row spacing was at 0.85 m and there was negligible soil N (0.2%) prior to planting. Three N treatments were established in a randomised block design in three separate plots of 0.1 ha for each variety. As per standard grower practice, calcium nitrate was applied in the furrow at planting at a rate of 5 kg N ha^{-1} for Maranca and 10 kg N ha^{-1} for Nicola across all three treatments. During the growing season, a topdressing of either 30 (1 application), 90 (3 applications) and 180 (3 applications) kg N ha^{-1} was applied to treatment plots in the form of calcium nitrate or urea, to give a final N treatment rate of 35, 95 and 185 Kg N ha^{-1} for Maranca and 40, 100 and 190 kg N ha^{-1} for Nicola. Tubers were harvested on two sampling dates; *c.* two-weeks before (i.e. 90 and 100 days after planting for Maranca and Nicola respectively), and 2-4 weeks following herbicide-induced (Reglone 2L/ha, a.i. 200g/L diquat, Syngenta Australia) haulm (vine) destruction of each variety, referred in this study as early-harvest and late-harvest, respectively. For yield assessment, all tubers from six randomly selected plants, from three replicates plots from each N treatment were harvested, the excessive soil brushed off, and weighed. Washed tubers of similar size and free of visible damage, from each treatment and replicate, were stored in the dark, for further analysis, as detailed below.

Field trial 2

Seed tubers of Maranca, were planted in a commercial potato field, with a free-draining deep sandy soil at Parilla, South Australia (35° 09'.28.7" S; 140° 35'.43.8" E). Row spacing was at 0.85m and there was negligible soil N prior to planting preparation. Three N treatments were established in a randomised block design with three replicates of 0.02 ha per treatment. As per standard grower practice, mono ammonium phosphate was broadcast pre-planting at a rate of 25 kg N ha^{-1} across all three treatments. During the growing season, a fertigation of either 75 (1 application), 175 (two applications) and 275 (3 applications) kg N ha^{-1} was applied to treatment plots in the form of calcium nitrate or urea, to give a final N treatment rate of 100, 200 and 300 Kg N ha^{-1}. Tubers were sampled *c.* four-weeks after plant senescence (~130 days after planting). For yield assessment, all tubers from five randomly selected plants were harvested, the excessive soil brushed off and weighed from each of the three replicate plots of each N treatment. Tubers were gently washed and those of approximately similar size and free of visible damage, from each treatment and replicate, were stored in the dark, for further analysis, as detailed below.

Light exposure treatment

Three tubers from each treatment per replicate in each field trial were exposed to a fluorescent light source (12 µmol/m^2/s at tuber surface) for 120 hours at room temperature (20 ±3 °C). Tubers were arranged in rows and their places within the row were repositioned daily to avoid any possible bias of positioning from variation in light intensity, ensuring that the orientation of the tuber remained the same.

Chlorophyll analysis

Three periderm disks from each tuber (1.5 mm thick and 1 cm diameter) were cut using a cork borer from the stem, the middle and the bud end of each tuber periderm. The disks were frozen in liquid N and ground to a fine powder using a mortar and pestle. 5 mL of N, N-dimethylformamide was added per sample, then incubated at 4 °C in the dark for 24 hours.

After centrifugation for 15 min at 2500 × g, the absorbance was measured with a spectrophotometer (Thermo Scientific Spectronic 200E, Madison, Wisconsin, USA) at 647 and 664 nm. Chlorophyll concentrations were~~as~~ determined before and after light exposure according to Porra et al. 1989, using the equation below and expressed as Δchlorophyll (the difference between chlorophyll concentrations after and before light exposure) in mg kg^{-1} FW:

Total chlorophyll = 17.67 (A647) + 7.12 (A664)

Tuber N analysis

From each separate field trial, three tubers from each individual replicate treatment were pooled. Sub-samples from sliced tuber tissues were oven-dried at 60 °C overnight, then ground to a fine powder using a mortar and pestle and sieved to 2 mm, prior to analysis. Total N analysis was conducted using the Dumas high temperature combustion method (Leco analyser; CSBP Soil and Plant Analysis Laboratory, Australia). Samples were loaded into a combustion tube at 950 °C and flushed with oxygen. The gases generated from this process were measured using a thermal conductivity cell for N. Tuber N was calculated as percentage of tuber dry weight (DW).

Statistical analysis

For the field trial 1, the effect of fertilisation and maturity and their interaction on tuber N and Δchlorophyll concentration were examined using two-way ANOVA and the effect of fertilisation on yield was examined using one-way ANOVA. For field trial 2, the effect of fertilisation on yield, tuber N, and Δchlorophyll concentration was assessed using one-way ANOVA. Treatment means were separated using Tukey's HSD test ($p < 0.05$). A linear regression approach was used to assess the influence of N fertilisation on tuber N and tuber N on Δchlorophyll concentration. All analyses were conducted using R version 3.6.1 (R Core Team 2019).

Results

Field trial 1

Tuber yields were significantly influenced by N rate with higher yields recorded for both Nicola and Maranca at 95-100 and 185-190 kg N ha^{-1} compared to 35-40 kg N ha^{-1}. Increasing N rate from 95-100 to 185-190 kg N ha^{-1} did not significantly improve tuber yield (Table 1).

The interaction between N fertilisation and harvest date was not significant for any of the studied variables (Table 1). The effect of N rate on tuber N and Δchlorophyll concentration varied between varieties. For Nicola, the highest tuber N and Δchlorophyll concentration were noted under 40 kg N ha^{-1} compared to 190 kg N ha^{-1}, while for Maranca, tubers fertilised with 185 kg ha^{-1} had the highest N and Δchlorophyll concentration (Table 1).

Tuber N was significantly greater from the later harvested tubers compared to those harvested early for *cv.* Nicola, but no differences were found for *cv.* Maranca (Table 2). Both varieties showed no significant differences in Δchlorophyll concentration between the two harvests.

Negative and positive relationships were found between N fertilisation and tuber N for Nicola ($R^2 = 0.92$) and Maranca ($R^2 = 0.95$), respectively (Fig. 1). Positive relationships were found between tuber N and Δchlorophyll concentration for both Nicola ($R^2 = 0.998$) and Maranca ($R^2 = 0.862$) (Fig. 2~~1~~).

Field trial 2

The lower N treatment (100 kg ha^{-1}) produced a significantly reduced yield approximately half that of the higher N treatments (200 and 300 kg ha^{-1}). There were no significant differences in tuber N and Δchlorophyll concentration between different N treatments (Table 3). A negative relationship was found between N fertilisation and tuber N (R^2 = 0.95) (Fig. 3), while ~~however,~~ a positive relationship was found between tuber N and Δchlorophyll concentration (R^2 = 0.90) (Fig. 4~~2~~).

Discussion

This study found a positive relationship between tuber N content and greening propensity, the first such report of this association. Central to identifying this positive relationship was the measurement of individual tuber N content as the relationship between N fertilisation rate and individual tuber N content was inconsistent and variety and location dependant. For field trial 1, the increase in N fertilisation rates from 35 to 185 kg ha-1 increased tuber N in Maranca, while the converse was observed for Nicola, leading to a decrease in tuber N. The lack of consistent association between N fertilisation and tuber N content could potentially be explained by N mobilisation with potato plants. Excessive N supply is known to increase dry matter yield in parts of the plant other than the tubers. For example, high N can reduce the proportion of assimilates supplied to the below ground plant parts (Oparka et al. 1987). Similarly, N content was shown to be twice as high in potato foliage as in tubers, with these differences continuously increasing with N fertiliser rate (Ruza et al. 2013). While Kursanov (1984) showed that shoot and root growth was more rapidly stimulated with excessive N rates, becoming active consumers of photosynthesis products. Therefore, the application of excessive N rates could result in reduced N partitioning to tubers, which may be affected by variety.

Alternatively, reduced N content in tubers may also simply reflect relative tuber mass. Although not directly measured, smaller sized tubers for Nicola were observed in the 40 kg ha^{-1} treatment, and for Nicola, reduced tuber mass may have led to a greater relative concentration of N per gram of tuber tissue than seen in larger tubers at higher N rates. For field trial 2, where overall higher N rates were applied, the increase in N fertilisation rates had no significant effect on tuber N. Similarly, Ruza et al. (2013) showed tuber N remained constant in the higher N treatments of 150, 180 and 210 kg ha^{-1}.

Nitrogen fertilisation also affected tuber yield in accordance with previous studies (Fontes et al. 2010; Srek et al. 2010; Ruza et al. 2013). For field trial 1, yields increased with increasing N fertiliser from 35-40 to 95-100 kg ha^{-1}, with further increases in N rates not significantly affecting yield, although data trends suggested improved yield at 185-190 N kg ha^{-1}. Similarly, for the field trial 2, with applied N rates at or greater than 100 N kg ha^{-1}, no significant effects of N fertiliser on yield were observed. In similar studies, Ruza et al. (2013) showed that potato yields improved with an increase in N fertilizer rate up to 120 kg ha^{-1}, but further increases in N did not significantly affect yields. A rate of 140 N kg ha^{-1} of mineral fertilizers has been suggested as optimal, resulting in a tuber yield above 30 t ha^{-1} (Srek et al. 2010).

Nitrogen is an essential component of the chlorophyll molecule and a positive correlation between N concentration and photosynthetic capacity of leaves has been previously reported (Evans 1983; Field and Mooney 1986; Uchida et al. 1980; Yoshida and Coronel 1976; Eyles et al. 2012). In leaves, low foliar N causes damage to the chloroplast structure (Shah et al., 2017), affects the number of thylakoids per unit leaf area (Terashima and Evans 1988), the synthesis of Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) and several other

key enzymes involved in the Calvin cycle (Lu and Zhang 2000; Huang and Jiang 2004). We therefore, propose that the increase in tuber N concentration facilitates chlorophyll formation, resulting in higher tuber greening propensity.

In summary, the results of our study indicate potato greening propensity is associated with tuber N content as hypothesised, however, the relationship between N fertilisation rates and N tuber content was inconsistent and appeared variety specific. Future research with a wider range of varieties and N treatments, combined with a detailed analysis on N accumulation and distribution within potato plants could extend our understanding on the relationship between N fertilisation, tuber N and greening.

Acknowledgments

Funding: This work was supported by the Australian Research Council's Industrial Transformation Training Centres scheme under Grant IC140100024. ST is the recipient of a tuition fee scholarship from the University of Tasmania.

Conflicts of Interest: The authors declare no conflicts of interest.

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Figure legends

Fig. 1: Relationship between N fertilisation rates and tuber N for *cvs*. Nicola and Maranca. R^2 values represent regression coefficient. Data was collected from field trial 1 in Buckland, Tasmania. Each data point represent the mean ± SE of six replications.

Fig. ~~1~~2: Relationship between tuber N and Δchlorophyll concentration for *cvs*. Nicola and Maranca. R^2 values represent regression coefficient. Data was collected from field trial 1 in Buckland, Tasmania. Each data point represent the mean ± SE of six replications. Chlorophyll concentrations were calculated before and after 120 hours of light exposure.

Fig. 3: Relationship between N fertilisation rates and tuber N for *cv*. Maranca. R^2 values represent regression coefficient. Data was collected from field trial 2 in Parilla, South Australia. Each data point represent the mean ± SE of three replications.

Fig. ~~2~~4: Relationship between tuber N and Δchlorophyll concentration for *cv*. Maranca. R^2 values represent regression coefficient. Data was collected from field trial 2 in Parilla, South Australia. Each data point represent the mean ± SE of three replications. Chlorophyll concentrations were calculated before and after 120 hours of light exposure.