SÃO PAULO STATE UNIVERSITY - UNESP
CAMPUS OF JABOTICABAL

YIELD AND QUALITY OF POTATO TUBERS IN RESPONSE TO NITROGEN MANAGEMENT

Camila Seno Nascimento
Agronomist Engineer

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SÃO PAULO STATE UNIVERSITY - UNESP
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Camila Seno Nascimento

Adviser: Prof. Dr. Arthur Bernardes Cecílio Filho

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TÍTULO DA TESE: YIELD AND QUALITY OF POTATO TUBERS IN RESPONSE TO NITROGEN MANAGEMENT

AUTORA: CAMILA SENO NASCIMENTO
ORIENTADOR: ARTHUR BERNARDES CECILIO FILHO

Aprovada como parte das exigências para obtenção do Título de Doutora em AGRONOMIA (PRODUÇÃO VEGETAL), pela Comissão Examinadora:

Prof. Dr. ARTHUR BERNARDES CECILIO FILHO (Participaçao Virtual)
Departamento de Ciências da Produção Agrícola / FCAV / UNESP - Jaboticabal

Dr. HILÁRIO JÚNIOR DE ALMEIDA (Participaçao Virtual)
Agrônomo Autônomo / Jaboticabal/SP

Prof. Dr. LEILSON COSTA GRANGEIRO (Participaçao Virtual)
Universidade Federal Rural do Semiárido-UFERSA / Mossoró/RN

Dr. VÍCTOR MANUEL VERGARA CARMONA (Participaçao Virtual)
Engenheiro Agrônomo Autônomo / Chile

Pesquisador Dr. ROBERTO BOTELHO FERRAZ BRANCO (Participaçao Virtual)
Centro de Análise e Pesquisa Tecnológica do Agronegócio de Horticultura / IAC(APTA) - Ribeirão Preto/SP

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AUTHOR'S CURRICULUM INFORMATION

CAMILA SENO NASCIMENTO was born on September 20, 1990, in Diadema, São Paulo, Brazil to Yaeko Senô and Jorge José de Luna Nascimento. She graduated from São Paulo State University, at the campus of Jaboticabal (FCAV/UNESP) in 2015 with a degree in Agronomic Engineering. Throughout her undergraduate years, she was an intern at Crop Production Science Department, where she acquired knowledge in hydroponics, greenhouse production, agronomic biofortification, intercropping systems, and nutrient management for vegetable crops. During this period, she received three scientific initiation grants from FAPESP (2011/2012) and PIBIC/CNPq (2012/2013 and 2013/2014) to develop projects related to vegetable production. Besides that, since she was a freshman, she was involved with extracurricular activities, such as extension projects, clubs, and volunteer works. From March 2014 to August 2015, Camila participated in the Science Without Borders program in the United States. During her stay in the U.S., she attended academic classes at Michigan State University and did a three-month internship at the University of Wisconsin-Madison. In March 2016, she started her master's degree in Agronomy (Crop Production Science) at São Paulo State University, at the campus of Jaboticabal (Dissertation Title: N: K ratio for phenological growth stages of net melon cultivated in NFT hydroponic system). Her project was sponsored by the Coordination for the Improvement of Higher Education Personnel (CAPES) through the granting of a scholarship. In March 2018, she started her Ph.D. in Agronomy (Crop Production Science) at the same university (Title Ph.D. research project: Yield and quality of potato tubers in response do nitrogen management, CNPq, 141300/2018-3). The funding agency National Council of Scientific and Technological Development (CNPq) supported her Ph.D. project under the supervision of Prof. Dr. Arthur Bernardes Cecílio Filho.

“I may not have gone where I intended to go, but I think I have ended up where I needed to be.”

Douglas Adams

DEDICATION

To my dearest grandparents **Ziro Senô** (*In memoriam*) and **Ana Akemi de Britto Senô** (*In memoriam*) for all the support, teaching, and encouragement, being my great examples in life. To my sister **Carolina Seno Nascimento** for always encouraging me throughout this journey. To my mother **Yaeko Senô** for always believing in me.

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PRODUTIVIDADE DA BATATEIRA E QUALIDADE DE SEUS TUBÉRCULOS EM RESPOSTA AO MANEJO DA ADUBAÇÃO NITROGENADA

RESUMO - O inadequado manejo da adubação nitrogenada pode ocasionar distúrbios fisiológicos na cultura da batata e promover a redução da produção e da qualidade dos tubérculos, afetando a rentabilidade dos cultivos. Desta maneira, o objetivo deste trabalho foi estabelecer doses ótimas de nitrogênio (N) para serem aplicadas em diferentes cultivares de batata, assim como avaliar a influência de fontes e doses de N na performance agronômica e qualidade dos tubérculos. Para isso, os seguintes experimentos foram desenvolvidos em campo: Experimento 1: Foi avaliada a influência de doses de N (0, 70, 140 e 210 kg ha^{-1}) no estado nutricional, fisiologia, crescimento e produtividade das cultivares Ágata (mercado in natura) e Asterix (indústria de fritura). Experimento 2: Foi analisado como a aplicação de N (0, 70, 140 e 210 kg ha^{-1}) nas formas de nitrato de amônio e ureia afetam o desempenho agronômico e o acúmulo de nitrato nos tubérculos de batata. No experimento 1, observou-se que máxima produtividade foi obtida com a aplicação de 131 kg ha^{-1} de N. A aplicação de 210 kg ha^{-1} de N ocasionou uma redução significativa na transpiração, taxas de fotossíntese e massa seca dos tubérculos. No experimento 2, a aplicação de ureia proporcionou maiores produtividades do que o nitrato de amônio. A aplicação de 210 kg ha^{-1} de N, em ambas as fontes, proporcionou máximos teores de nitrato, entretanto não ultrapassou o limite (200 mg kg^{-1}) recomendado para o consumo humano. O método de cozimento promoveu uma diminuição no teor de nitrato nos tubérculos.

Palavras-chave: *Solanum tuberosum*, cultivares, fertilizante nitrogenado, nitrato.

YIELD AND QUALITY OF POTATO TUBERS IN RESPONSE TO NITROGEN MANAGEMENT

ABSTRACT - Inadequate nitrogen (N) fertilizer management can cause physiological disorders in potato crops and promote a reduction in production and quality of tubers, affecting the profitability of the system. In this sense, this study aimed to establish optimal rates of N to different potato cultivars, as well as evaluate the influence of sources and rates of N in the agronomic performance and quality of potato tubers. For this, two experiments were carried out in the field: Experiment 1 evaluated the influence of N rates (0, 70, 140, and 210 kg ha^{-1}) on nutritional status, physiology, growth, and yield of Agata (fresh market) and Asterix (frying industry) cultivars. Experiment 2 assessed how the application of N (0, 70, 140, and 210 kg ha^{-1}) in the forms of ammonium nitrate and urea influence the agronomic performance and nitrate accumulation in potato tubers. In experiment 1, it was noticed the highest yield at 131 kg N ha^{-1}. Application of 210 kg N ha^{-1} led to a remarkable decrease in transpiration, photosynthesis rates, and tuber dry mass. In experiment 2, it was noticed that the application of urea provided a higher yield than ammonium nitrate. The highest nitrate content in potato tubers was achieved at 210 kg N ha^{-1} for both sources, not exceeding the limit (200 mg kg^{-1}) recommended for human consumption. The boiled method promoted a decrease in nitrate content.

Keywords: *Solanum tuberosum*, cultivars, nitrogen fertilizers, nitrate.

LIST OF ABBREVIATIONS

N - nitrogen

P - phosphorus

K - potassium

Ca - calcium

Mg - magnesium

S - sulfur

Cu - copper

Fe - iron

Mn - manganese

Zn - zinc

B - boron

a.s.l - above sea level

K_2O - potassium chloride

P_2O_5 - simple superphosphate

NO_3^- - nitrate

NO_3^-B - nitrate content after boiling

DAP - days after planting

DAE – days after emergence

PAR - photosynthetically active radiation

WUE - water use efficiency

Ci - internal CO_2 concentration

E - transpiration rate

gs - stomatal conductance

A - net photosynthetic rate

SDM - shoot dry biomass accumulation

TDM - tuber dry biomass accumulation

TODA - total dry biomass accumulation

NS - nitrogen content in the shoots

NT - nitrogen content in the tubers

NA - nitrogen accumulation

NAS - nitrogen accumulation in the shoot

NAT - nitrogen accumulation in the tuber

TNA – total nitrogen accumulation

PF - pulp firmness

FM - fresh mass

NUE - nitrogen use efficiency

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CHAPTER 1 - General considerations

1 INTRODUCTION

Potato (*Solanum tuberosum*) is a fast-growing, high-yielding crop, with excellent market acceptance. It represents a cheap and nutritionally rich food source, and it is, therefore, an outstanding cultivation option for farmers. Currently, potato growers find a wide variety of potato cultivars on the market, with different characteristics in relation to pests and diseases tolerance, size and shape of the tuber, skin and flesh color, and culinary aptitude (fresh, boiling, frying, roasting), leaving it up to them to adopt the cultivar that best suits the growing and market condition of their region. In Brazil, Agata (fresh market) is the most grown cultivar, occupying about 50 % of the total cultivated area. Together with Asterix (processing industry), the second most-produced cultivar, it leads the domestic market (Assunção et al., 2020; Oliveira et al., 2020).

Because this crop is a short-cycle species with a high growth rate, it demands a large amount of fertilizers per unit area, especially nitrogen (N), the second most required nutrient by potato crop (Pereira et al., 2020; Zhang et al., 2022). The efficiency of N fertilization in potato plants depends on several factors, such as the cultivar adopted, phenological growth stage, soil attributes, climatic conditions, rate and source of N applied, and plant efficiency in converting absorbed nutrients into biomass (Gitari et al., 2018; Tang et al., 2021). In Brazil, technical guides to mineral fertilizers recommend N rates ranging from 60 to 250 kg ha^{-1}, varying according to the region of potato growth (Fernandes and Soratto, 2012). In São Paulo State, for example, Miranda Filho (1997) recommends rates of N up to 160 kg ha^{-1} splits into two portions, one at planting and one before hilling. However, because N is a main limiting nutrient for potato growth and development, in general, in intensive potato production systems, N is often supplied at rates far greater than those recommended, causing a surplus of this element in soils, that thereafter leads to negative environmental issues (Dong and Lin, 2020; Zhang et al., 2020a).

Establishing the effective management of N fertilization is one of the most difficult and important decisions to be made by producers since insufficient rates of N reduce the development and yield of plants, as well as cause changes in the shape, color, and quality characteristics of tubers (Rens et al., 2018). On the other hand, over-fertilization increases the risk of environmental pollution, causes excessive vegetative growth, reduces starch accumulation, and increases the nitrate content in tubers (Elrys et al., 2021). Nitrates are in nature as part of the N cycle and play an essential role in the process of plant nutrition, growth, and development (Brkić, 2017). However, excess of nitrates in food increases the incidence of Methemoglobinemia (MetHba) and the formation of carcinogenic compounds. MetHba is a clinical syndrome, which can lead to death, caused by increased methemoglobin (MetHb) content (Greer and Shannon, 2005; Chui et al., 2005). This disease is caused by the unbalance in the reactions of reduction and oxidation induced by exposure to nitrite (Nascimento et al., 2008). The nitrite resulting from nitrate reduction promotes the oxidation of iron ions present in hemoglobin, transforming them from Fe^{2+} to Fe^{3+}, which prevents the transportation of oxygen to the cells. About, 4 to 7 % of the total nitrate ingested in food is reduced to nitrite in oral saliva (Walker, 1990). Overall, plant products account for 80 % of the source of nitrates ingested by humans, of which 32 % comes from potato consumption (Santamaria, 2006; Thomson, Nokes and Cressey, 2007; Bryan and van Grinsven, 2013). So, the high accumulation of nitrate in potatoes is a factor that requires a response, since the food industries use this tuber as a basis for preparing ready-to-eat baby foods, which may represent a possible health risk for children (Greer and Shannon, 2005).

The aspects that lead to the difference in nitrate levels in different vegetables are complex, and studies on the mechanisms of nitrate accumulation in vegetables are still scarce. Thus, further research on this topic becomes imperative since, besides the genetic factor, the management adopted (N fertilization, cultivars, light intensity, soil moisture, and temperature) also plays an essential role in the accumulation of nitrates in plants (Hmelak-Gorenjak and Cencič, 2013).

Therefore, establishing the correct management of N fertilization is essential for producers to achieve high yields and obtain tubers with appropriate nitrate content, not offering toxicological risks to human health.

1. 2 LITERATURE REVIEW

1. 2.1 Potato crop

Potato (*Solanum tuberosum*) is a herbaceous perennial plant, grown as an annual for commercial harvest. Its origins date back eight thousand years to South America (Andes Mountains), being later introduced into Europe and, from there, spread as a staple food in most parts of the world. Nowadays, this starchy vegetable is grown in more than 149 countries, showing its high variability and greater adaptation to a broad range of ecological conditions (Birch et al., 2012; Padmanabhan, Sullivan, and Paliyath, 2016).

The growth cycle of potato crop can be roughly divided into four stages. In the first stage (sprout development), sprouts develop from eyes on potato seed tubers and emerge from the soil. In stage II (vegetative growth) there is the development of the root system, leaves, and stems. In this stage, photosynthesis begins, and the potato plant prepares to accumulate nutrients in tubers. In stage III (tuberization) tubers start forming on the end of stolons, and after that, tubers enlarge with the accumulation of sugars and starches. In stage IV (maturation) tubers reach maximum dry matter content, the top of the plant dries out and dies, and the tuber skin set, extending storage life (Filgueira, 2003). In general, the potato growing cycle, in tropical and subtropical conditions, can vary from 90 to 110 days, depending on the cultivar adopted, being classified as an early season (< 90 days), mid-season (90 to 110 days), and late season (> 110 days) (Pereira and Daniels, 2003).

Nutritionally, potato tubers are an important source of energy, in the form of carbohydrates. Because of this, potato plays a fundamental role in the diet of millions of people in developing countries (Beals, 2019), providing in addition to high-quality carbohydrates, good amounts of fiber, phenolic acids, vitamin B1, B2, B6, and C, potassium, protein, iron, magnesium, and carotenoids (Kanter and Elkin, 2019).

Over the last five decades, the global production of potatoes has raised more than 33 %, underlining its importance as a food security crop for 1.3 billion people worldwide (Campos and Ortiz, 2019; Zhang et al., 2021). In fact, in 2019, its production exceeds 370 million tons from over 17,340,986 hectares, being the fifth most expressive crop in terms of production quantity (FAOSTAT, 2019). China, the world's largest producer, accounted for 25 % (91,881,397 tons) of the total amount produced, while Brazil occupied the twentieth position (3,696,930 tons) (Yang et al., 2021; Zhu et al., 2021).

In Brazil, more than 116,682 hectares of farmland are cultivated with potatoes, of which the majority is grown in the Southeast (Minas Gerais, São Paulo, Espírito Santo, and Rio de Janeiro) and South (Paraná, Rio Grande do Sul, and Santa Catarina) regions. Indeed, Minas Gerais (1,199,571 tons), Paraná (748,367 tons), and São Paulo (650,522 tons) account for more than 70 % of the national production (IBGE, 2019).

1.2.2 Potato cultivars

Selecting a proper potato cultivar is an indispensable first step to reaching successful cultivation. Potato growers must consider the agronomic traits and culinary aptitude when evaluating the suitability of a potato cultivar to local growing conditions and intended market (Naumann et al., 2019). Currently, there are on the market hundreds of cultivars that differ in yield, tuber size and shape, skin and flesh color, carbohydrate and dry mass content, dormancy, eye depth, growing cycle, carbohydrate composition, storability, pest and disease resistance, and culinary aptitude (fresh, boiling, frying, roasting) leaving it up to the potato growers to adopt the cultivar that best fit their goals (Assunção et al., 2020; Oliveira et al., 2020; Gustafson et al., 2021; Jiang et al., 2021). In general, potato tubers are consumed fresh or processed and in rare cases, tubers are destined for the extraction of alcohol, starch, dextrin, and glucose (Koch et al., 2019). Despite the world preference for fresh tubers, changes in consumer lifestyles have been shifting consumption patterns (Jayanty, Emragi, and Holm, 2021). Indeed, in Brazil, the consumption of fresh tubers is gradually declining, while the demand for processed tubers (dry, chips, fries, mashed and frozen potato products), has been growing (Oliveira et al., 2020). To properly attend the fresh market and processing industry, public and private sectors have been developing specific cultivars with different characteristics to attending the requirements of each segment.

In the fresh market, consumers look for tubers with attractive visual characteristics (color, texture, shape, shallow eyes, and resistance to tuber greening) (Pereira and Bortoletto, 2015). These characteristics are different from those desired by the industry, which wants tubers with uniform shape, dry matter content higher than 20 %, high starch content, light color after frying, and low reducing sugars levels (below 0.035 % for chips and 0.12 % for French fries production) (Silva and Carvalho, 2015; Pereira et al., 2020). Industries want cultivars with high starch content and low reducing sugar levels because these traits will directly affect the quality of processed potato products. Usually, 60-80 % of the tubers dry matter is existing as starch, which influences characteristics such as texture, color, oil absorption, and water loss (Wang et al., 2021) in fried products. If tubers present high dry matter, they tend to absorb less oil in the frying process. On the other hand, tubers with high levels of reducing sugars (glucose and fructose) are more susceptible to turning dark brown during the frying process.

In Brazil, Agata is the most grown cultivar, accounting for nearly 50 % of the domestic production (Assunção et al., 2020). The high acceptance of this cultivar is mainly due to its rapid sprouting, high yield potential, early maturation, and tubers with a good visual appearance (uniform oval tubers, yellow skin, shallow eyes, and good resistance to browning caused by impact) (ABBA, 2022). Because this cultivar has a low dry matter content, it is suitable for the fresh market (Evangelista et al., 2011).

Asterix (processing industry) is the second most-produced cultivar in Brazil. It is a red-skin cultivar with yellow flesh, semi-late maturing, medium dormancy, high yield potential, and excellent cooking characteristics. It produces long oval uniform-shaped tubers with yellow flesh and shallow eyes. Due to its culinary versatility, high dry matter, and pale fry colour, Asterix stands out in the processing industry, being well suited to the manufacturing of processed potato products, such as chips, dehydrated potato, and par-fried frozen potato (Mesias et al., 2021).

1.2.3 Overview on the Role of Nitrogen in Potato Mineral Nutrition

The knowledge of the potato nutrient requirements is essential for the proper nutrient management throughout the growing season, since, whenever the soil cannot supply nutrients in amounts compatible with crop needs, it will be necessary to apply synthetic fertilizers (Fernandes and Soratto, 2012). Among the nutrients, N is a major limiting element for plant growth and development, due to its numerous functions in plant metabolism (Rouached and Rhee, 2017). An insufficient supply of N can decrease the development and yield of potato plants, whilst excessive rates delay tuber development, retard leaf senescence, reduce tuber quality and increase environmental problems related to N losses (Assunção et al., 2020; Souza et al., 2020). Thus, establishing strategic N management capable of matching the supply of this input, with the demand of potato crop is crucial to achieve high yields, obtain high-quality tubers and mitigate environmental degradation (Hu et al., 2020).

N has a complex dynamic in the soil and can be lost through several processes, such as volatilization, denitrification, immobilization, and leaching (Assunção, 2020). As a consequence, N in most parts of the world is applied in doses higher than those recommended in technical fertilizers guides, causing an unbalance between N supply and crop demand (Milroy, Wang, and Sadras, 2019). Annually, nearly 109 million tonnes of mineral N fertilizers are applied to the soil, being only less than half absorbed by plants and most of which lost to the environment (Peoples et al., 2019).

In Brazil, N recommendations range from 60 to 250 kg N ha^{-1}, exhibiting variations between the recommended rates in the different states of the country (Fernandes and Soratto, 2012). In São Paulo state, for example, to achieve potential tuber yields (>30 t ha^{-1}), it is recommended to apply 40-80 kg N ha^{-1} at planting and 40-80 kg N ha^{-1} before hilling (Miranda Filho, 1997).

Even though potato crop is highly responsive to N fertilizer, it is known that the N rate which provides the maximum yield and quality of tubers is variable, making the management of N fertilization extremely complex, because it depends on factors such as: cultivar, source of N, soil organic matter, environmental conditions, yield potential, soil type, N use efficiency, culinary aptitude of potato tubers, and crop management (Zhang et al., 2020b; Plauborg et al., 2022).

1.2.4. Synthetic nitrogen fertilizers

Synthetic fertilizers supply some nutrients that might be lacking in the soil and are essential to stimulate the healthy growth and development of potato plants. These fertilizers are generated through manufacturing processes or mined from deposits in the earth, enabling easy handling and application. Because the N cycle includes innumerous routes by which plant-available N can be lost from the soil, the practice of fertilization is widely used by farmers (Norton and Ouyang, 2019). Plants are capable to absorb N in the inorganic forms of ammonium (NH_4^+) and nitrate (NO_3^-). Some crops take up and respond better and quicker to the NO_3^- than the NH_4^+, however, potatoes are equally responsive to both forms (Finch, Samuel, and Lane, 2002).

There are available on the market a wide option of N fertilizers such as urea (45% of N), ammonium nitrate (32 % of N), potassium nitrate (13 % of N), sulfate of ammonia (20 % of N), and calcium nitrate (14 % of N) which have considerable variations in price. Among the chemical N forms, urea is the dominant N fertilizer used in potato production, due to its low cost and high N content (Souza et al., 2020). However, because of its rapid hydrolysis in the soil, there is a high possibility to occurs N losses to the environment (Souza et al., 2019). Taking this into account, farmers often look for other sources which can provide a more consistent soil N availability. In this sense, ammonium nitrate can be an efficient alternative that can assist farmers to improve N use efficiency. Ammonium nitrate is considered one of the most effective and fastest sources of N, since half the nitrogen (as $NO_3^−$) is readily available to the plants, while other sources must pass through a conversion to form NO_3^- in the soil, before being assimilated by the plants.

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CHAPTER 2 - Impact of cultivars and nitrogen rates on nutritional status, physiology, and yield

ABSTRACT – Even though the responses of potato plants to synthetic nitrogen (N) fertilizers have been widely stated in the literature, there is a paucity of information about strategies to improve N management for different cultivars considering its specific demands. In this sense, this study was conducted to assess the influence of N fertilization on the nutritional status, physiology, growth, and yield of Agata and Asterix cultivars. The effectiveness of N supply in each potato cultivar was evaluated by the application of four N rates at side-dressing (0, 70, 140, and 210 kg N ha^{-1}). The increase of N supply led to an increase in N and P contents. Chlorophyll *a* + *b* and carotenoids contents were 31 and 16 % higher in Asterix cultivar than in Agata, respectively. Application of 210 kg N ha^{-1} promoted a notable decrease in transpiration and photosynthesis rates and tuber dry biomass. The highest potato yield was achieved at 131 kg N ha^{-1}.

Keywords: *Solanum tuberosum*, Agata, Asterix, potassium nitrate, mineral nutrition.

2.1 INTRODUCTION

The global potato (*Solanum tuberosum*) production has increased by more than 11 % in the last decade, being the fifth most produced crop in the World (FAOSTAT, 2019). This boost over the years led to an increase in the use and manufacture of synthetic fertilizers, mainly nitrogen (N), the second most required nutrient by potato plants (Pereira et al., 2021; Cecílio Filho et al., 2022). Although essential to plants development, the excessive application of this input causes undesirable impacts on yield, tuber quality, climate change, and even in the profitability of the system, since fertilizers represent about 20 % of operating cost in potato production (CEPEA, 2019; Yang et al., 2021).

Generally, in intensive farming, N fertilizers are being increasingly supplied to potato crops to attempt to achieve target yield, causing an imbalance between N supply and crop demand (Tang et al., 2021). Indeed, potato farmers apply common rates of N for different growing conditions, cultivars, and production purposes, considering only practical aspects and not the nutrient recommendations for potato crop based on the plant requirements, yield potential, and capability of the soil to supply N (Zhang et al., 2020). This constant and sometimes excessive application of N has generated unwanted consequences such as low-N use efficiency, environmental degradation, and reduced yield (Hailu et al., 2017). Fortunately, the current changes in global environmental policies and the ongoing progress in the agriculture sector are devising a potential turning point at which a more conscious N management is significantly advancing across the world (Zhang et al., 2015; Yang et al., 2021).

Potato is a higher nutrient-demanding crop, however, N uptake by this starchy vegetable vary greatly as they depend on several factors such as cultivar, yield, and environmental conditions (Fernandes and Soratto, 2012). For several years, researches conducted over the world have been focused on increasing potato yield and improving its adaptation to biotic and abiotic stresses (Dahal et al., 2019; Zhu et al., 2021), disregarding important factors such as the best approaches to obtain optimal use of N fertilizer for different cultivars.

Even though there is on the market a wide variety of cultivars with different characteristics (size, shape, color, growth cycle, yield, and utilization) nearly most of the fertilizers recommendation guides used in potato production systems are intended for widely grown fresh-market cultivars (Pereira et al., 2021). However, studies have shown that cultivars can take up and respond differently to N supply, evidencing the need for potato fertilization programs to widen their focus from traditional cultivars to also establish strategic nutrient recommendations addressed to new cultivars, especially those destined to frying industry (Milagres, Fontes, and Silva, 2019; Assunção et al., 2020). Therefore, settling a proper dose of N for potato cultivars is crucial for achieving target yield and for enhancing greater profitability (Gálvez et al., 2016).

Based on the above, this study aimed to appraise the influence of N fertilization on the nutritional status, physiology, growth, and yield of Agata (fresh market) and Asterix (frying industry) cultivars. Our hypothesis is that there may be differences between these cultivars in terms of N absorption capacity and responses to N fertilization. So, establishing a specific dose of N for each potato cultivar could reduce the over-fertilization without compromising the plant performance.

2.2 MATERIAL AND METHODS

2.2.1 Experimental site

A field experiment was carried out from 16 April to 11 July 2019 at São Paulo State University (UNESP) in Jaboticabal, São Paulo, Brazil, (21°15'22” S, 48°15'58” W, and 575 m a.s.l.). The total rainfall, mean minimum, mean maximum, and average temperatures throughout the experimental period were 53 mm, 17.3 ºC, 28.2 ºC, and 20.1 ºC, respectively.

The soil was classified as a typical Eutrophic Oxisol with a clayey texture (Santos et al., 2018). The pre-plant soil chemical characteristics were pH ($CaCl_2$): 5.8, organic matter: 23 g dm^{-3}, P (resin): 60 mg dm^{-3}, K: 5.6 $mmol_c$ dm^{-3}, Ca: 25 $mmol_c$ dm^{-3}, Mg: 12 $mmol_c$ dm^{-3}, H + Al: 16 $mmol_c$ dm^{-3}, cation exchange capacity: 58.2 $mmol_c$ dm^{-3}, and base saturation of the soil: 73 %.

2.2.2 Experimental design and treatments

The experimental design was a randomized complete block design, in a 2 x 4 factorial scheme, with four replicates. The treatments consisted of two potato cultivars (Agata and Asterix) and four N rates at side-dressing (0, 70, 140, and 210 kg N ha^{-1}) applied as potassium nitrate. The concentration of potassium was balanced between the treatments, using potassium chloride. The fertilizer was applied along the rows, 3 cm apart, at the beginning of tuber formation, before performed hilling.

The experimental plot consisted of a 5.0-m-long row with 0.80 m row spacing, containing fourteen potato plants. Data were collected from the ten central plants, disregarding two plants at each end.

2.2.3 Experimental setup

The experimental land was prepared by plowing and harrowing before opening the furrows. According to the chemical analysis, it was unnecessary to perform liming prior to planting, once the soil base saturation was above 60 % (Granja et al., 2014).

The fertilization at planting consisted of 40 kg N ha^{-1} (potassium nitrate), 100 kg K_2O ha^{-1} (potassium chloride), 200 kg P_2O_5 ha^{-1} (simple superphosphate), and 1 kg B ha^{-1} (boric acid) applied and incorporated into planting furrows at an average depth of 0.15 m. Subsequently, uncut seed tubers were planted at 0.05 m buried into the soil, and spaced 0.35 m apart in the furrows. The seed tubers of Agata and Asterix cultivars had an average weight of 76 g (69 to 94 g) and 63 g (51 to 76 g) and an average diameter of 38 mm (33 to 46 mm) and 35 mm (28 to 38 mm), respectively. In the side-dressing fertilization, N was applied at the rates established in the treatments 20 days after emergence (DAE) for both cultivars.

Weeds were controlled by manual hoeing, and pests and diseases were prevented by phytosanitary products recommended for potato crops. Irrigation was performed by sprinkler at a rate of 5 mm day^{-1}.

The harvest was carried out 70 DAE.

2.2.4 Nutritional analysis

For the appraisal of the nutritional status of potato plants, the macronutrients (g kg^{-1}) and micronutrients (mg kg^{-1}) were determined in the third leaf from the apical tuft, (one per plant of 10 plants) at 30 DAE, as described by Lorenzi, Monteiro, and Miranda Filho (1997)**.** The materials were washed, dried, ground, and prepared to ascertain N, P, K, Ca, Mg, S, Cu, Fe, Mn, B, and Zn contents, according to the methodology recommended by Miyazawa et al. (2009).

2.2.5 Gas exchange parameters

Gas exchange parameters were assayed 40 DAE on the upper third of the newly developed leaf of potato plants, between 8:00 - 11:30 a.m. Internal CO_2 concentration (Ci; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs; mmol H_2O m^{-2} s^{-1}), and net photosynthetic rate (A; µmol m^{-2} s^{-1} CO_2) were measured using a portable gas exchange equipment (LC-PRO+, ADC Bioscientific Ltda Herts England) with photosynthetically active radiation (PAR) of 1200 µmol m^{-2} s^{-1}. Water use efficiency (WUE; µmol CO_2 $mmol^{-1}$ H_2O) was determined by A/E.

2.2.6 Photosynthetic pigment content analysis

The photosynthetic pigments content (µg g^{-1}) was analyzed spectrophotometrically. Fresh tissue samples, collected from the upper third of the first newly physiologically mature leaf at 30 DAE, were added in tubes with 1.5 ml acetone (80 %). After shaking for 48 h at 4 °C, the samples were read in 470, 645, and 662 nm for carotenoids, chlorophyll *a*, and chlorophyll *b*, respectively. The pigment contents were estimated according to Lichtentlaler (1987).

2.2.7 Dry biomass and N accumulation

Dry biomass accumulation (g per plant) was determined 63 DAE. Two plants randomly collected were cut and separated into shoots and tubers. Then, the samples were washed, dried (65 ^{o}C ± 5), and weighed. The dry biomass accumulation in the entire plant was obtained by the sum of the individual amounts of the shoot and tuber dry biomass. The percentage of dry biomass distribution among the potato organs was calculated through the relation of the dry biomass of each organ (shoot and tubers) with the total dry biomass (whole plant) (Benincasa, 2003).

The assessment of the N content in shoots and tubers was determined at 63 DAE (two plants per plot). The N accumulation was expressed in g per plant and was obtained by the product of the N content and the respective dry biomass of each organ of the plant. Total N accumulation by plant corresponded to the sum of the quantities present in the shoot and tubers multiply by total dry biomass.

2.2.8 Potato yield

Potato yield (t ha^{-1}) was estimated from the total mass of tubers harvested in the useful area of the plot.

2.2.9 Statistical analysis

The dataset was submitted to analysis of variance (F-test, $p < 0.05$) using the Agroestat software (Barbosa and Maldonado Júnior, 2015). Polynomial regression analysis was performed for N rates, choosing the equation with the highest level of significance and coefficient of determination.

2.3 RESULTS

Magnesium (Mg) was not influenced by N supply or the interaction between the traits, but was influenced by the cultivars (Table 1). Asterix plants (3.8 g kg^{-1}) taken up 5.6 % more Mg than Agata plants (3.6 g kg^{-1}).

Table 1. Summary of analysis of variance for leaf macronutrient contents (g kg^{-1}) of potato cultivars (Agata and Asterix) submitted to nitrogen rates (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	N	P	K	Ca	Mg	S
Cultivar (C)	33.01^{**1}	4.22^{ns}	201.86^{**}	23.05^{**}	5.61^{*}	44.26^{**}
N Rates	6.76^{**}	4.93^{**}	3.30^{*}	9.99^{**}	1.19^{ns}	12.02^{**}
C x N	2.12^{ns}	0.41^{ns}	7.64^{**}	12.53^{**}	1.18^{ns}	4.46^{*}
Blocks	0.19^{ns}	2.24^{ns}	0.16^{ns}	0.65^{ns}	2.49^{ns}	1.34^{ns}
Error	0.47	0.45	0.79	0.21	0.15	0.18
CV(%)	3.21	13.48	5.79	5.25	8.20	9.57
Average - cultivars						
Agata	$28.58b^{2}$	6.41a	31.36a	8.40a	3.56b	3.27b
Asterix	30.51a	7.07a	23.39b	7.68b	3.81a	4.11a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

N application of 210 kg ha^{-1} increased the N content by 6.6 % (28.6-30.5 g kg^{-1}) compared to the control plants (no N applied at side-dressing) (Fig. 1a). However, regardless of the dose of N applied, potato plants displayed N content beneath the adequate range for the crop (40-50 g kg^{-1}) proposed by Lorenzi, Monteiro, and Miranda Filho (1997). Plants of Asterix cultivar (30.1 g kg^{-1}) exhibited N content higher than Agata cultivar (28.6 g kg^{-1}).

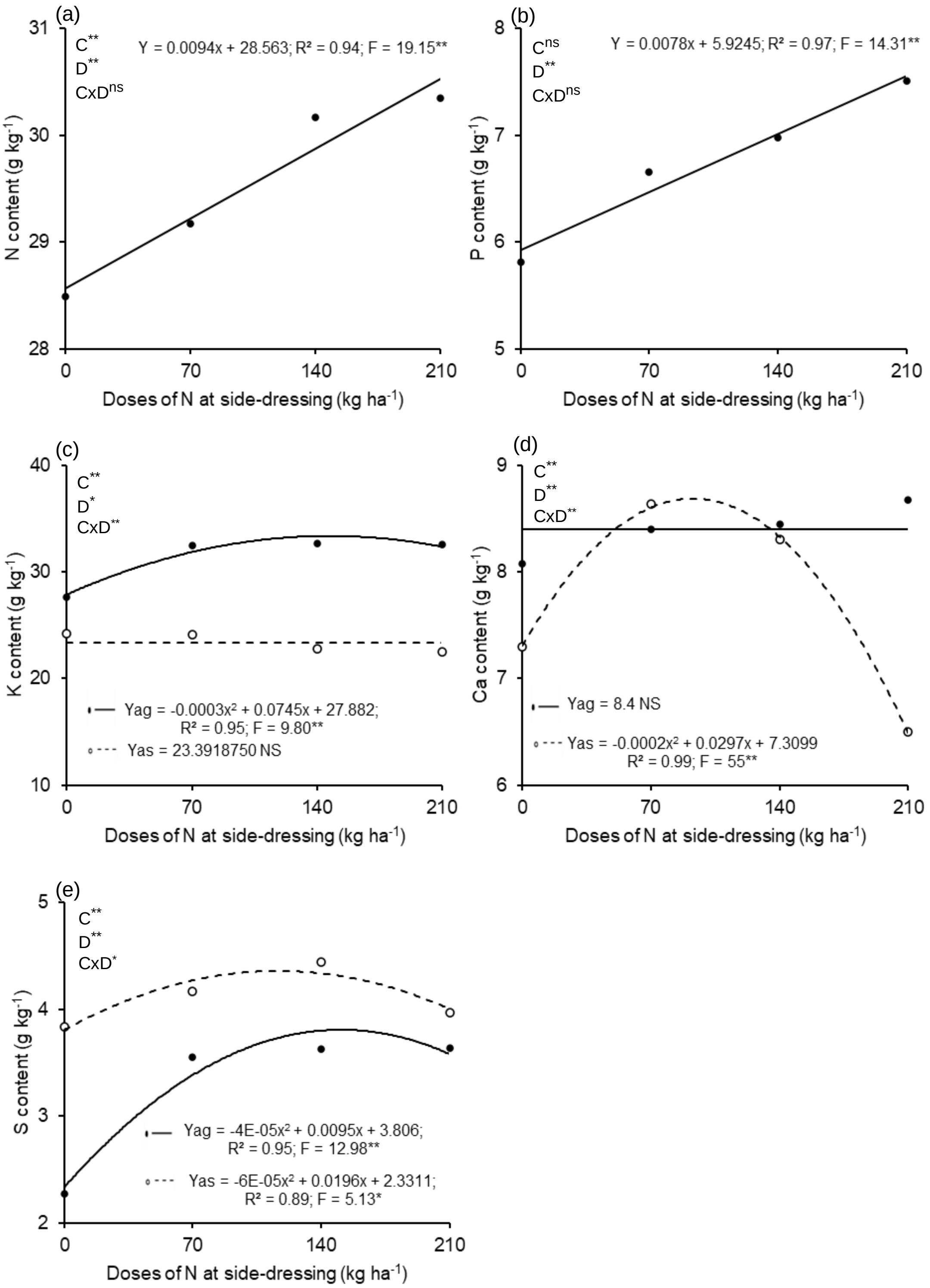

Fig 1. Nitrogen fertilization at side-dressing on nitrogen (a), phosphorus (b), potassium (c), calcium (d), and sulfur (e) contents in potato leaves. * $p < 0.05$; ** $p < 0.01$; ^{NS} non-significant by F test. C to cultivar, D to rates of N at side-dressing, and CxD to interaction.

Phosphorus (P) content was affected only by N application rates. N supply led to a linear increase in P content, which varied from 5.9 to 7.6 g kg^{-1} (Fig. 1b).

Interactions were observed between cultivars and N application dose for potassium (K), calcium (Ca), and sulfur (S) (Fig. 1c-e). In Agata cultivar, the applied N rates lead the K and S contents to increase following a quadratic function, reaching maximum contents at 147 (33.4 g kg^{-1}) and 151 kg N ha^{-1} (3.8 g kg^{-1}), respectively. No difference was observed for Ca content in this cultivar (Fig. 1d). Likewise, in the Asterix cultivar, N supply influenced Ca and S contents, resulting in quadratic functions. The highest contents were achieved at 93 and 117 kg N ha^{-1} for Ca and S contents, respectively, representing an increase of 19.2 (7.3 - 8.7 g kg^{-1}) and 15.8 % (3.8 - 4.4 g kg^{-1}) in relation to untreated plants. K content was not influenced by N fertilization in this cultivar (Fig. 1c).

Manganese (Mn) was not influenced by N supply or the interaction between the traits, but was influenced by the cultivars (Table 2). The Mn foliar content of the Asterix plants (79.9 mg kg^{-1}) was 14.1 % higher than Agata plants (70.0 mg kg^{-1}).

Table 2. Summary of analysis of variance for leaf micronutrient contents (mg kg^{-1}) of potato cultivars (Agata and Asterix) submitted to nitrogen rates (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	Cu	Fe	Zn	B	Mn
Cultivar (C)	88.65^{**1}	33.12**	97.98**	0.37^{ns}	17.04**
N Rates	5.63**	15.69**	16.39**	1.12^{ns}	2.18^{ns}
C x N	0.26^{ns}	6.75**	0.73^{ns}	14.39**	0.83^{ns}
Blocks	0.75^{ns}	0.63^{ns}	0.91^{ns}	0.85^{ns}	3.19*
Error	0.66	18.34	1.20	1.48	3.41
CV(%)	11.76	16.53	9.22	23.75	9.09
Average - cultivars					
Agata	8.97b[2]	184.59b	21.84b	12.78a	70.01b
Asterix	13.33a	259.23a	30.23a	12.14a	79.96a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

The Cu and Zn contents rely on the cultivars and rates of N applied during the growing season (Table 2). N application caused a quadratic effect, declining the contents of both nutrients (Cu and Zn) as increasing in N rates (Fig. 2a and 2c).

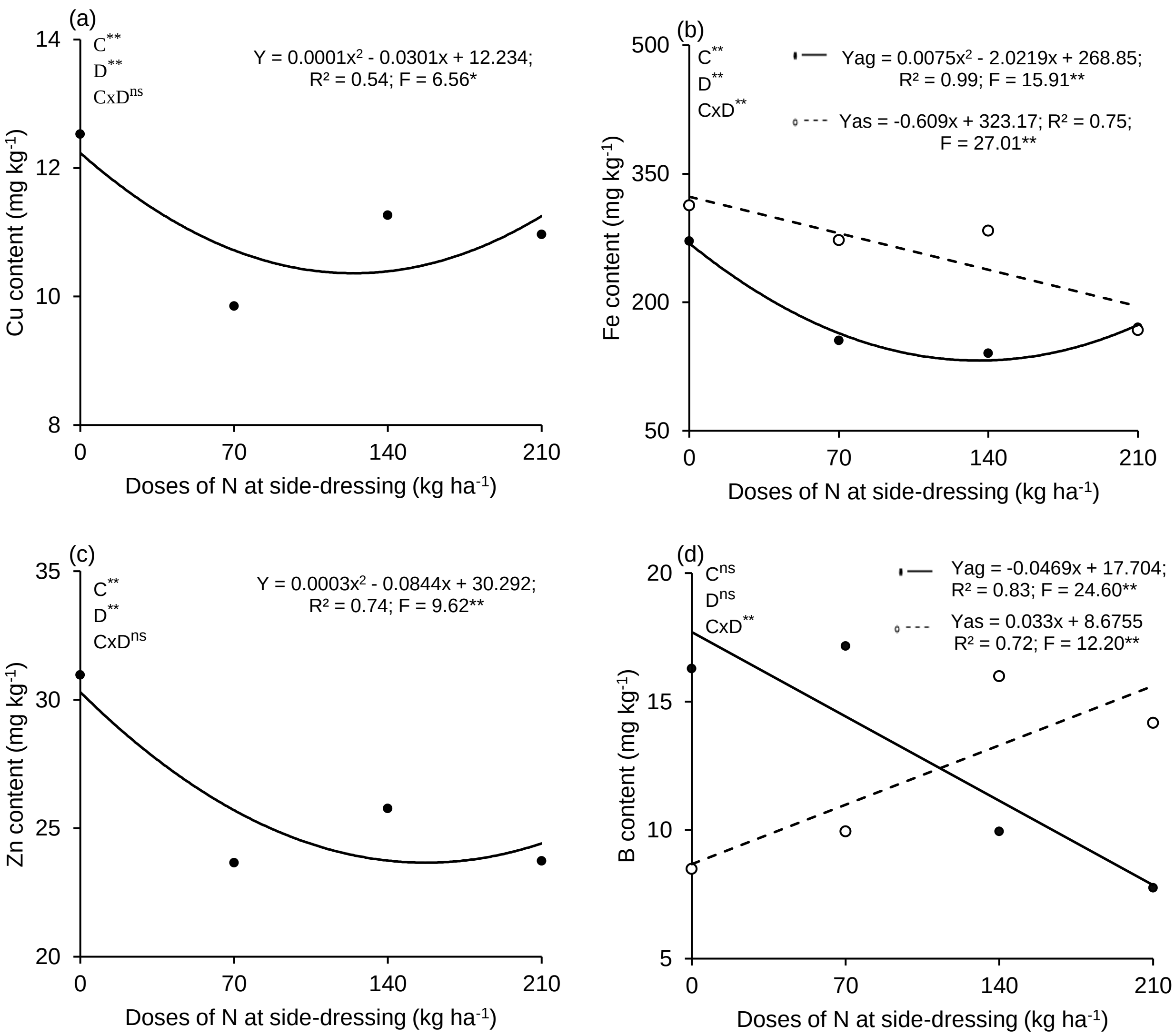

Fig. 2 Nitrogen fertilization at side-dressing on copper (a), iron (b), zinc (c), and boron (d) contents in potato leaves. * $p < 0.05$; ** $p < 0.01$; ^{NS} non-significant by F test. C to cultivar, D to rates of N at side-dressing, and CxD to interaction.

The lowest contents of Cu and Zn in the potato leaves were recorded with the application of 124 and 157 kg N ha^{-1}, respectively, representing a reduction of 14.8 and 21.8 % when compared to control. Potato plants of the Asterix cultivar had higher contents of Cu and Zn than plants of the Agata cultivar.

An interaction was observed between cultivars and rates of N in Fe and B contents (Table 2). The Fe content of Agata cultivar quadratically decreased as N rates increase, reaching the lowest point at 135 kg N ha^{-1} (Fig. 2b), while B content decreased linearly (Fig. 2d). In Asterix cultivar, there was a linear decrease in Fe content (Fig. 2b) and a linear increase in B content as N rates increase (Fig. 2d).

Appraisal of the gas exchange parameters of potato plants unveils different responses to cultivars and rates of N (Table 3).

Table 3. Summary of analysis of variance for intracellular CO_2 concentration (Ci; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs; mmol H_2O m^{-2} s^{-1}), net photosynthesis rate (A; µmol m^{-2} s^{-1} CO_2), water use efficiency (WUE; µmol CO_2 $mmol^{-1}$ H_2O), chlorophyll *a* + *b* (*µ*g g^{-1}), and carotenoid (*µ*g g^{-1}) contents of potato cultivars (Agata and Asterix) submitted to nitrogen rates (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	Ci	E	gs	A	WUE	Chlorophyll *a* + *b*	Carotenoid
Cultivar (C)	0.77^{ns1}	22.09^{**}	7.31^{*}	163.46^{**}	0.39^{ns}	37.49^{**}	18.13^{**}
N Rates	8.35^{**}	6.06^{**}	9.60^{**}	7.83^{**}	6.17^{**}	9.03^{**}	7.05^{**}
C x N	1.32^{ns}	0.82^{ns}	5.60^{**}	1.79^{ns}	1.33^{ns}	2.77^{ns}	1.84^{ns}
Blocks	8.23^{**}	6.09^{**}	2.68^{ns}	2.29^{ns}	8.32^{**}	1.14^{ns}	0.91^{ns}
Error	14.51	0.24	0.018	0.44	0.68	0.02	0.01
CV(%)	11.50	16.39	11.37	4.45	18.46	12.49	10.20
				Average - cultivars			
Agata	$256.83a^{2}$	2.50b	0.29b	17.92b	7.49a	0.23b	0.12b
Asterix	247.83a	3.28a	0.33a	21.94a	7.19a	0.30a	0.14a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

Intracellular CO_2 concentration (Ci) and water use efficiency (WUE) were influenced only by N fertilization. N supply had a quadratic effect in Ci (Fig. 3a) and linearly increased WUE (Fig. 3e). Ci and WUE varied from 242.2 µmol m^{-2} s^{-1} CO_2 and 6.0 µmol CO_2 $mmol^{-1}$ H_2O at untreated plants to 295.2 µmol m^{-2} s^{-1} CO_2 and 8.6 µmol CO_2 $mmol^{-1}$ H_2O at the highest rate (210 kg N ha^{-1}), respectively.

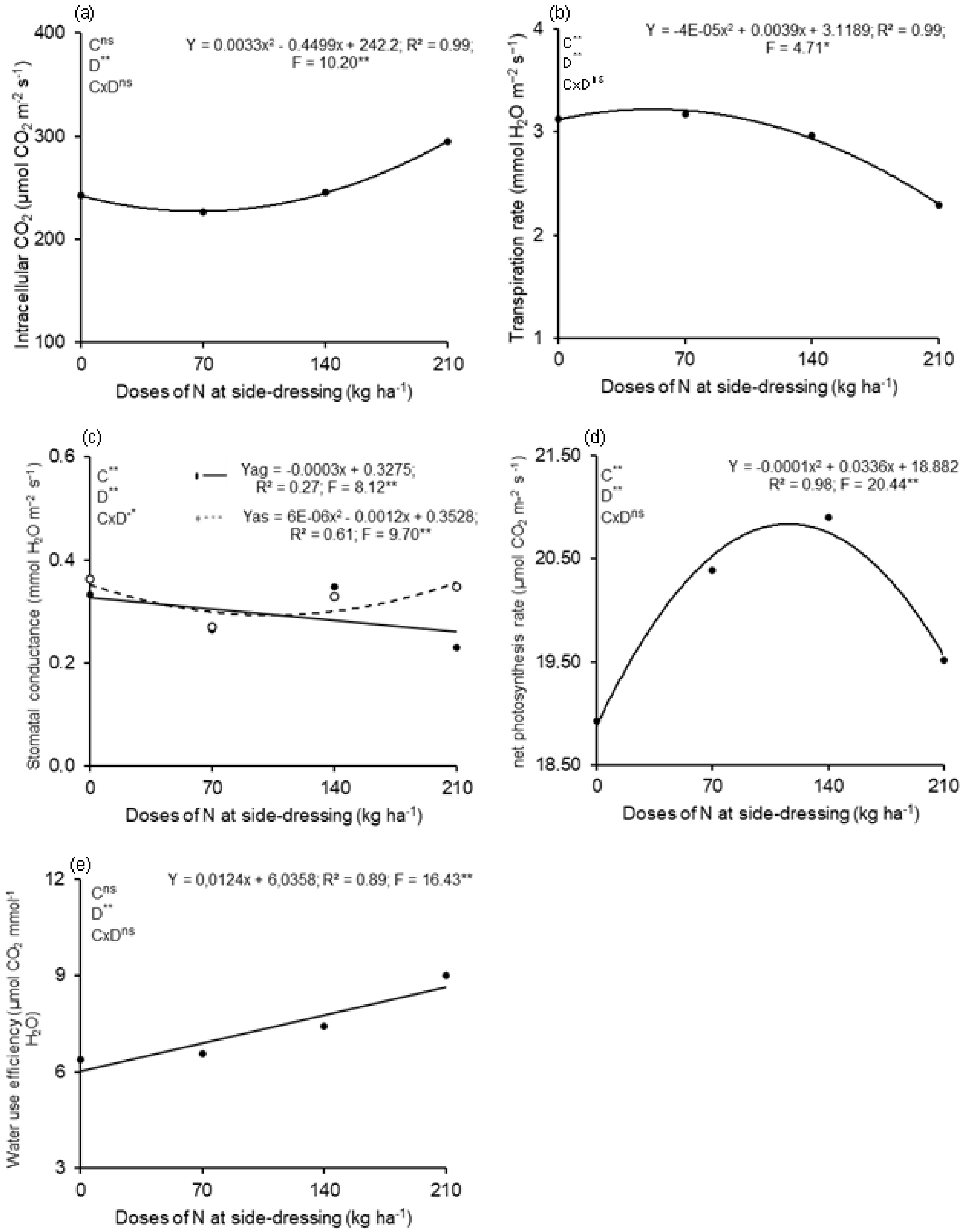

Fig. 3 Nitrogen fertilization at side-dressing on internal CO_2 concentration (a), transpiration rate (b), stomatal conductance (c), net photosynthetic rate (d), and water use efficiency (e) in potato leaves. * $p < 0.05$; ** $p < 0.01$; ^{NS} non-significant by F test. C to cultivar, D to rates of N at side-dressing, and CxD to interaction.

Transpiration rate (E) and net photosynthesis rate (A) were significantly influenced by cultivars and N supply. Asterix plants displayed higher E and A (3.2 mmol m^{-2} s^{-1} H_2O and 21.9 µmol m^{-2} s^{-1} CO_2) than Agata plants (2.5 mmol m^{-2} s^{-1} H_2O and 17.9 µmol m^{-2} s^{-1} CO_2). The N supply caused the E to decrease following a quadratic equation, attaining the maximum of 3.2 mmol m^{-2} s^{-1} H_2O at 52 kg N ha^{-1}, value 2.6 and 40.6 % higher than those obtained in control plants and plants treated with the highest dose of N, respectively (Fig. 3b). The A in potato plants increased with increasing N supply to a maximum of 20.8 µmol m^{-2} s^{-1} CO_2 in plants fertilized with 116 kg N ha^{-1}, then decreased as N fertilization was further increased (Fig. 3d).

An interaction was noticed between cultivars and rates of N on stomatal conductance (gs). N fertilization led to a linear decrease in gs in the Agata cultivar whilst causing a quadratic increase in the Asterix cultivar (Fig. 3c). At the highest dose of N (210 kg N ha^{-1}), Agata plants showed the lowest gs, although the inverse was observed on Asterix plants. Considering gs in comparison to untreated plants, the highest N application dose provided a decrease of 21.2 % (0.33-0.26 mmol m^{-2} s^{-1} H_2O) in Agata plants and an increase of 2.9 % (0.35-0.36 mmol m^{-2} s^{-1} H_2O) in Asterix plants.

Asterix plants exhibited chlorophyll *a* + *b* and carotenoid contents 31.4 and 16.1 % higher than Agata plants (Table 3). N application in potato plants linearly increased the content of the photosynthetic pigments. N dose that resulted in the highest increments of chlorophyll *a* + *b* and carotenoids was 210 kg N ha^{-1} (Fig. 4a-b), which corresponded to an increase of 36.2 % (0.22 – 0.31 µg g^{-1}) and 23.7 % (0.11 - 0.14 µg g^{-1}), respectively, in relation to untreated plants.

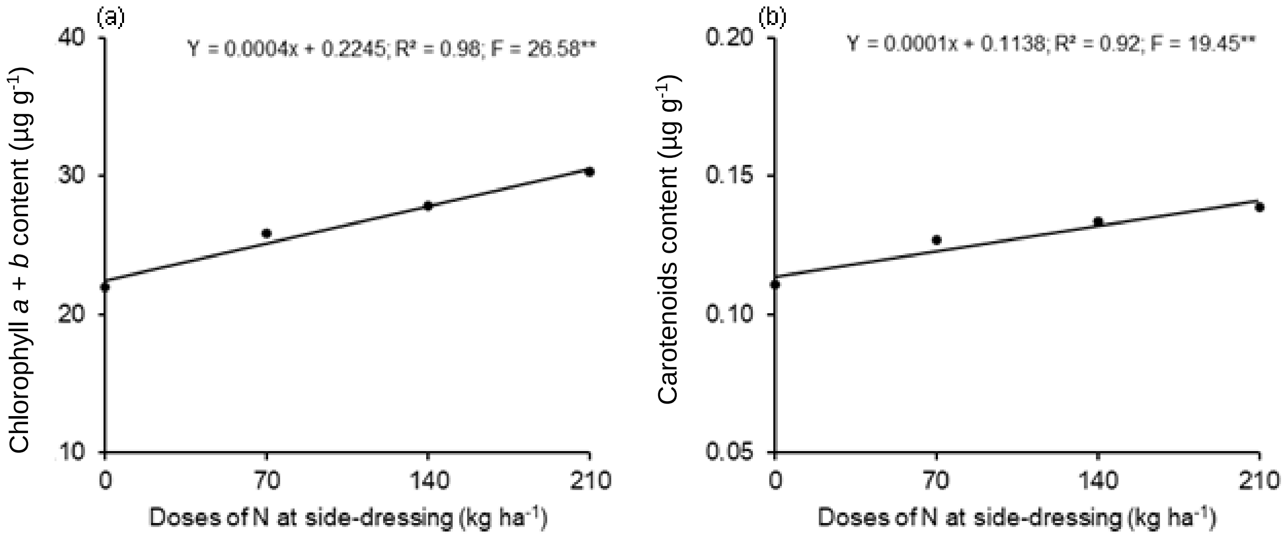

Fig. 4 Nitrogen fertilization at side-dressing on chlorophyll *a* + *b* (a), and carotenoids (b) contents in potato leaves. * $p < 0.05$; ** $p < 0.01$; ^{NS} non-significant by F test. C to cultivar, D to rates of N at side-dressing, and CxD to interaction.

N fertilization linearly increased shoot dry biomass (SDM) (Fig. 5a) and quadratically increase tuber dry biomass (TDM) (Fig. 5b) of potato plants. Maximum SDM (53.8 g per plant) and TDM (172.9 g) was reached at 210 and 143 kg N ha^{-1}, respectively, representing an increase of 30.4 and 31 % relative to the control. SDM was unaffected by cultivars, while Agata plants (172.6 g) presented greater tubers than Asterix plants (142.9 g) (Table 4).

Table 4. Summary of analysis of variance for shoot dry biomass (SDM; g per plant), tuber dry biomass (TDM; g per plant), total biomass accumulation (TBA; g per plant), biomass partitioning to the shoots (BPS; %), and biomass partitioning to the tubers (BPT; %) of potato cultivars (Agata and Asterix) submitted to nitrogen rates (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	SDM	TDM	TODA	BPS	BPT
Cultivar (C)	3.54^{ns1}	12.68^{**}	5.66^{*}	23.88^{**}	23.88^{**}
N Rates	3.73^{*}	4.72^{*}	5.36^{**}	0.61^{ns}	0.61^{ns}
C x N	1.79^{ns}	3.06^{ns}	3.14^{*}	0.83^{ns}	0.83^{ns}
Blocks	1.33^{ns}	0.51^{ns}	0.72^{ns}	0.94^{ns}	0.94^{ns}
Error	4.178	11.78	14.32	1.67	1.67
CV(%)	1.760	14.93	13.95	14.32	4.34
			Average – cultivars		
Agata	44.70a[2]	172.59a	217.29a	20.48b	79.52a
Asterix	50.26a	142.95b	193.21b	26.26a	73.74b

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

The interaction of cultivars and rates of N was significant for the total dry biomass accumulation (TODA). Plants of Agata cultivar presented higher biomass than Asterix plants (Table 4). Based on the regression coefficients, the estimated maximum TODA for Agata (244.9 g) and Asterix (218.2 g) were acquired with the application of 210 and 126 kg N ha^{-1}, respectively. The biomass partitioning to the shoots and tubers was only affected by cultivars. In Agata plants, tuber accounted for 79.5 % of the total plant biomass, while in Asterix plants it corresponded for 73.7 % (Table 4).

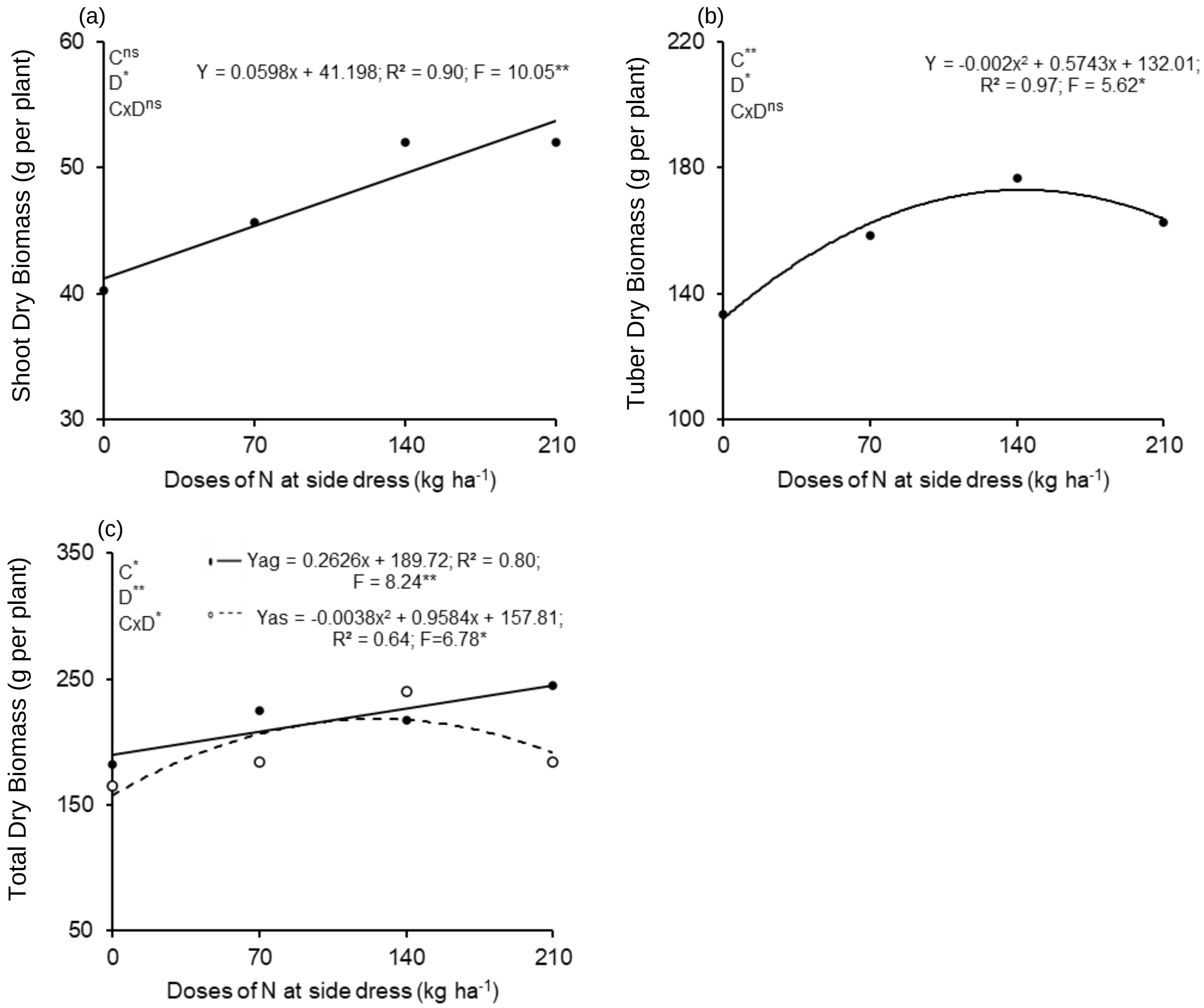

Fig. 5 Nitrogen fertilization at side-dressing on shoot dry biomass (a), tuber dry biomass (b), and total dry biomass accumulation (c) of potato plants. * $p < 0.05$; ** $p < 0.01$; NS non-significant by F test. C to cultivar, D to rates of N at side-dressing, and CxD to interaction.

The N content in the shoots and tubers of Agata plants was affected by the interaction of cultivars and rates of N (Table 5).

Table 5. Summary of analysis of variance for N content in the shoots (NS; g kg^{-1}), N content in the tubers (NT; g kg^{-1}), N accumulation (NA; g per plant), and yield (t ha^{-1}) of potato cultivars (Agata and Asterix) submitted to nitrogen rates (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	NS	NT	NA	Yield
Cultivar (C)	19.26^{**1}	15.75**	5.38*	124.73**
N Rates	4.13*	4.28*	9.00**	5.83**
C x N	4.25*	3.69*	3.48*	2.76^{ns}
Blocks	3.13*	3.18*	2.55^{ns}	0.45^{ns}
Error	0.06	0.43	0.17	1.30
CV(%)	10.69	11.67	16.69	6.86
		Average - cultivars		
Agata	1.24a^{2}	8.00a	2.15a	43.05a
Asterix	1.05b	6.79b	1.88b	32.78b

CV – coefficient of variation; ^{1} **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. ^{2} Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

The applied N rates lead the N content in these organs to increase ensuing a quadratic function (Fig. 6a-b) with maximums of 1.4 and 8.9 g kg^{-1} for shoot and tubers, respectively, acquired at 130 and 133 kg N ha^{-1}. No significant differences in N content in shoot and tubers were observed in the Asterix cultivar (Fig. 6a-b).

A similar trend was noticed for N accumulation which also was influenced by the interaction of the traits. The plants of Agata cultivar exhibited linear increments in this parameter (Fig. 6c) with the increase of N supply, representing a rise of 68.7 % (1.6-2.7 g per plant) compared to untreated plants. On the other hand, plants of Asterix cultivar increase quadratically in response to N application (Fig 6c). The highest N accumulation in this cultivar (2.2 g per plant) was reached at 112 kg N ha^{-1}.

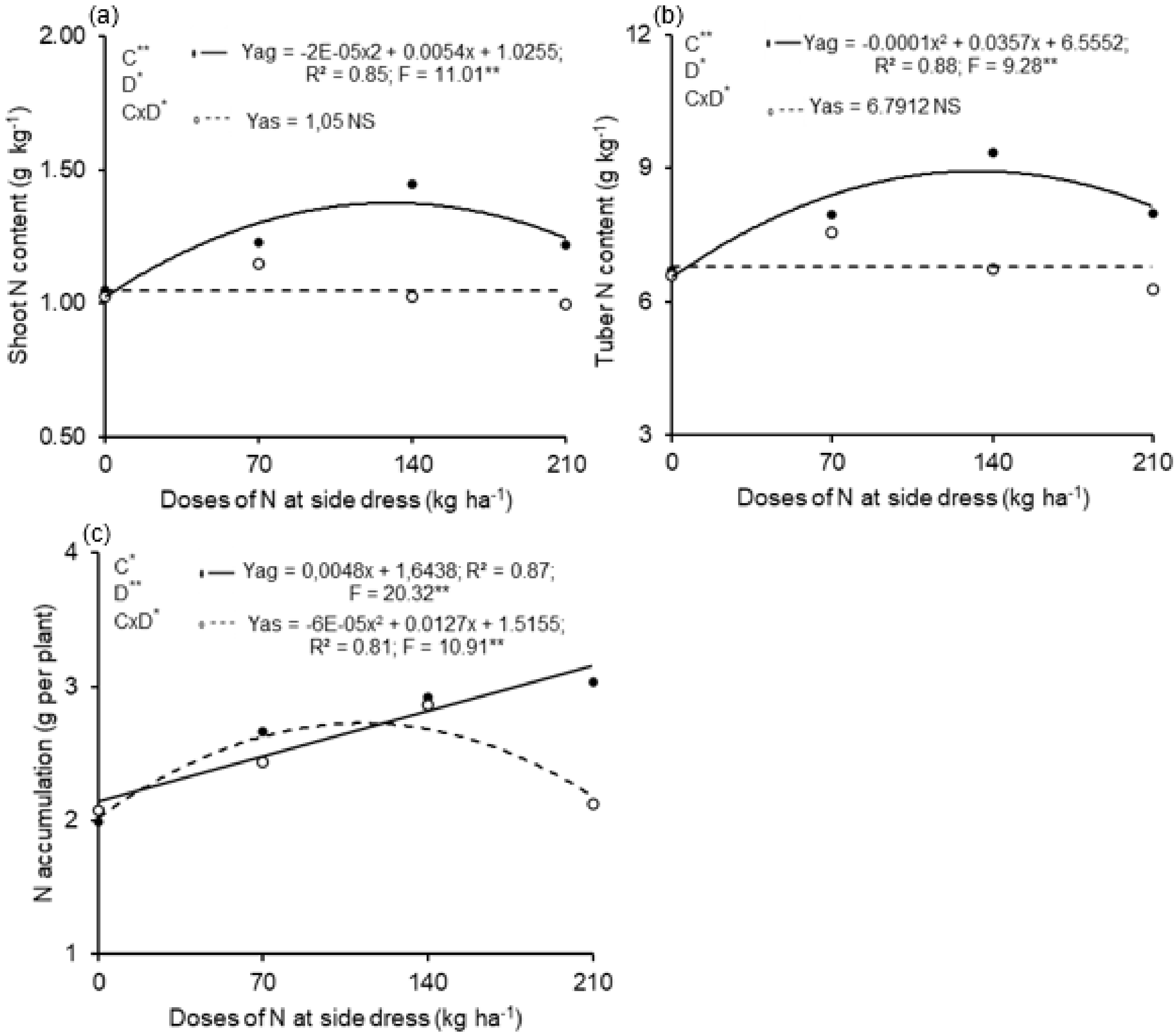

Fig. 6 Nitrogen fertilization at side-dressing on shoot N content (a), tuber N content (b), and N accumulation (d) of potato plants. * $p < 0.05$; ** $p < 0.01$; ^{NS} non-significant by F test. C to cultivar, D to rates of N at side-dressing, and CxD to interaction.

The yield of potatoes was affected by cultivars and N fertilization, individually (Table 5). Plants of Agata cultivar (43.0 t ha^{-1}) produced more than Asterix cultivar (32.8 t ha^{-1}). N application promoted a quadratic effect, declining the yield as the N supply increased, peaking (39.9 t ha^{-1}) at 131 kg N ha^{-1} (Fig. 7).

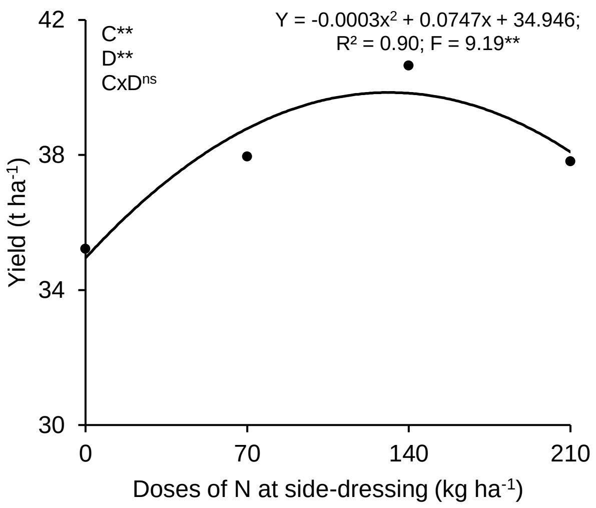

Fig. 7 Nitrogen fertilization at side-dressing on the yield of potato plants. * $p < 0.05$; ** $p < 0.01$; ^{NS} non-significant by F test. C to cultivar, D to rates of N at side-dressing, and CxD to interaction.

2.4 DISCUSSION

In this study, we appraised the effects of N fertilization rates on nutritional status, gas exchange, photosynthetic pigments, growth, and potato production of the Agata and Asterix cultivars. Our hypotheses were partially corroborated since through the results was possible to establish an optimal N dose reducing the over-fertilization without affecting cultivars' performance.

In all the evaluated treatments, regardless of the N fertilization dose, potato plants exhibited N, K, Ca, and B contents in the potato leaves beneath the adequate range stated by Lorenzi, Monteiro, and Miranda Filho (1997), however, both cultivars reached productivities beyond the national average (31.7 t ha^{-1}) (IBGE, 2021). These findings suggest that the satisfactory productivities obtained in this study are related to the greater nutrient use efficiency of these cultivars (Grzebisz et al., 2017). Most of the fertilizers recommendation guides used in Brazil were formulated based on field research with cultivars grown in the past. However, studies have shown that over the last years there were significant improvements in the ability of current cultivars to convert absorbed nutrients into production, this way the quantities extracted by plants to produce a ton of tubers are smaller than in some old cultivars (Fernandes and Soratto, 2012).

As expected, P content linearly raised with increasing N content, staying above the range recommended by Lorenzi, Monteiro, and Miranda Filho (1997) (2.5-5.0 g kg^{-1}). High concentrations of P decrease the uptake and use efficiency of micronutrients, due to its precipitation in the conductive vessels affecting the transport of micronutrients to the shoots.

Among the micronutrients Fe content was extremely high, ranging from 131.9 to 268.9 mg kg^{-1} for Agata cultivar and from 195.3 to 323.2 mg kg^{-1} for Asterix, being above the recommended range (50 to 100 mg kg^{-1}) described by Lorenzi, Monteiro, and Miranda Filho (1997). Such values can be attributed to the high availability of Fe in the soil (13 mg dm^{-3}) (Raij et al., 1996).

The application of doses higher than 52 and 116 kg N ha^{-1} lead to the decrease of E and A, respectively. This reduction may be correlated with a concomitant increase in shoot dry biomass as N supply increased, leading to an increase in water requirements. The deprivation of water by potato plants, mainly during the hottest hours, leads to stomata closure and decline of photosynthesis (Olivera-Viciedo et al., 2021). The gs of plants decreases to prevent the leaf's water potential from reaching critical levels that could be harmful to the stability of the water transport (Oren et al., 1999). As an aftermath of reduced gs, E, one of the main factors for leaf temperature regulation also decreases. Consequently, with the reduction of E, the WUE increases, as occurs a reduction in the loss of water vapor to the environment (Habermann et al., 2021).

Increasing N input after potato plants reached the maximum yield (39.9 t ha^{-1}) at 131 kg N ha^{-1} promoted a considerable decline in production. In fact, the application of 210 kg N ha^{-1} (38.1 t ha^{-1}) resulted in a reduction of 4.5 %. Probably, this result can be attributed to the vigorous vegetative growth favored by the increase in N supply, inhibiting the tuberization (Milroy, Wang, and Sadras, 2019). It is fundamental to highlight that this decrease in yield is economically unfavorable for potato growers, since means that they will fail to earn R$ 3,960.00 per ha, considering an average potato price of R$ 2.2 per kg (Ceagesp, 2021). Despite that, they will also have an increase in the production costs, since considering the mean price of R$ 6,200.00 per ton of potassium nitrate, the value to apply 210 kg N ha^{-1} (R$ 1,302.00), would be 60.3% higher than supplying 131 kg N ha^{-1} (R$ 812.20), decreasing the profitability of farmers. Studies in the literature, also reported that the overuse of synthetic N fertilizers is not necessarily a guarantee to steadily increase yield, on the contrary, the N supply beyond crop's demand might culminate in undesirable consequences as low N use efficiency, excessive shoot growth, environmental degradation and reduced yield (Assunção et al., 2020; Yousaf et al., 2016).

2.5 CONCLUSION

The results provided strong evidence that N fertilizer management can influence the performance of different cultivars of potato, revealing useful information to further optimize potato fertilizer programs. In this way, analyzing the responses of both cultivars over the use of N fertilizers, the results indicated that the most promising dose to enhance greater yield, profitability, and maintain environmental sustainability without imperiling crop performance is the supply of 131 kg N ha^{-1}. Analyzing the effect of cultivars on potato responses, the results demonstrated that Agata exhibited higher yields than Asterix plants.

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Chapter 3 - Optimizing potato agronomic performance through nitrogen fertilization with minimal nitrate accumulation

ABSTRACT – Enhancements in nitrogen (N) fertilization programs for potato crop are crucial for addressing the defiance of food security, quality of food, environmental degradation, and human health. Such enhancements are dependent not exclusively on understanding the metabolic pathways of N in potato plants, but also on establishing an optimum N fertilization approach to match the N supply with crop demand, maximizing potato agronomic performance, and improving its quality through the reduction of nitrate accumulation in tubers. In this context, this study aimed to appraise how the supply of N (0, 70, 140, and 210 kg N ha^{-1}) in the forms of ammonium nitrate and urea affects the nutritional composition, gas exchange, growth, yield, and quality of potato tubers. The decline in stomatal conductance and net photosynthetic rate associated with lower yield reveal that the raise in N supply above the optimum rate disturbs physiological mechanisms, reflecting in biomass production. Application of 136 kg N ha^{-1} as urea provided a remarkable increase in yield compared to the other rates and sources. The highest nitrate content in potato tubers was achieved at 210 kg N ha^{-1} for both sources, not exceeding the limit (200 mg kg^{-1} of fresh mass) recommended for human consumption. These results demonstrated that the over-fertilization worsened the yield as well as tuber quality of potato plants, providing valuable information to establish an effective fertilization program for the potato crop.

Keywords: *Solanum tuberosum*, Agata, ammonium nitrate, mineral nutrition, urea.

3.1 INTRODUCTION

Potato (*Solanum tuberosum*) is one of the most produced crops worldwide after sugar cane, maize, rice, and wheat, underlining its enormous importance to ensure global food security (FAOSTAT, 2019). Over the past twenty years, the production of this staple food has increased more than 25 %, promoting a significant rise in the use of synthetic fertilizers, particularly nitrogen (N), one of the most limiting elements for biomass production (Giletto et al., 2020; Pereira et al., 2021).

Potato is a high N-demanding crop with low N-uptake efficiency, due to its poorly developed root system (Milroy, Wang, and Sadras, 2019). Because of that, investments in N fertilizers are high, in order to avoid nutritional deficiencies and obtain high yields (Kawakami, 2015). N has a complex dynamic in the soil and can be lost by nitrate leaching, volatilization, denitrification, and immobilization (Assunção et al., 2020). As a result, N in most parts of the world is often applied using rates higher than plant requirements. The over-fertilization that has been occurring over the years has increased the concern on the future demand and supply of mineral N fertilizers in the world, since global consumption of this input increased about 244 % in the last fifty years and will further boost by 2050, putting an unprecedented pressure on the agriculture sector to use N in a more conscious way to avoid environmental degradation and food safety issues (Abdo et al., 2020; Devaux et al., 2021; Cecílio Filho et al., 2022).

The negative impact of excessive N application on food safety is due to the consumption of tubers containing nitrate content higher than 200 mg kg^{-1} of fresh mass (Elrys et al., 2021), which can be detrimental to human health. Under excessive N supply, plants uptake nitrate above its assimilation capacity, occurring the accumulation of the element in plants tissues (Colla et al., 2018). Nitrate when consumed by humans is converted to nitrite, which promotes the oxidation of iron ions present in hemoglobin, transforming them from Fe^{2+} to Fe^{3+}, which prevents the transportation of oxygen to the cells, causing the methemoglobinemia (Santamaria, 2006). Therefore, investing in research that simultaneously approaches factors such as N management, yield, and nitrate accumulation is urgently needed since nearly 80% of the total nitrate intake of the human population comes from the ingestion of vegetables, representing a possible threat to human health (Bryan and van Grinsven, 2013; Rosier et al., 2020).

Establishing the correct N management is one of the most important and difficult decisions to be made by farmers, since the N status in the soil is very dynamic, being almost impossible to predict its availability over time. Several studies have been performed to establish proper N rates for potato crop, however, the relationship between rates and sources is often overlooked, decreasing the N management efficiency (Oliveira et al., 2020; Zhang et al., 2020). Mineral N fertilizers are available on the market in many forms with significant price differences and characteristics. The application of the right chemical N form could influence how quickly the nutrient would be available to be used by the crop. In this way, if farmers choose the proper N source, they could maximize N use efficiency.

In the light of the foregoing, this study aimed to appraise the effect of ammonium nitrate and urea application on nutritional status, photosynthesis, yield, and quality of field-grown potato plants. The hypothesis tested herein is that the establishment of the optimum N rate combined with the right source could minimize the over-fertilization, traditionally used by potato growers, enabling maximum return on N supply and maximizing potato agronomic performance with minimal nitrate accumulation in tubers.

3.2 MATERIAL AND METHODS

3.2.1 Experimental conditions

The experiment was conducted in the field from 16 April to 4 July 2019 at São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil (21°15'22” S, 48°15'58” W, and 575 m a.s.l.). The rainfall, mean maximum, mean minimum, and average temperatures during the experimental period were 48.7 mm, 28.7 ^{o}C, 18.1 ^{o}C, and 20.5 ^{o}C, respectively.

The soil was classified as a typical Eutrophic Oxisol with a clayey texture (Santos et al., 2018). The pre-planting soil chemical properties from the 0-20-cm layer were pH ($CaCl_2$): 5.8, organic matter: 23 g dm^{-3}, P (resin): 60 mg dm^{-3}, K: 5.6 $mmol_c$ dm^{-3}, Ca: 25 $mmol_c$ dm^{-3}, Mg: 12 $mmol_c$ dm^{-3}, H + Al: 16 mmolc dm^{-3}, cation exchange capacity: 58.2 $mmol_c$ dm^{-3} and base saturation of the soil: 73 %.

3.2.2 Experimental design and treatments

The experiment was designed in a complete randomized block, in a 2 x 4 factorial scheme, with four replicates. The treatments consisted of two N sources (ammonium nitrate and urea) and four N rates at side-dressing (0, 70, 140, and 210 kg ha^{-1} N) applied along the rows, 3 cm apart, at the beginning of tuber formation, before hilling.

Each experimental plot consisted of a 5.0-m-long row, with 0.80 m spacing between rows, containing fourteen potato plants. Data were collected from the ten central plants, disregarding two plants at each end.

3.2.3 Experimental setup

The experimental site was prepared by plowing and harrowing before opening the furrows for planting fertilization and distribution of seed tubers. According to the chemical analysis, it was not necessary to perform liming before planting, since the soil base saturation was above 60 % (Granja et al., 2014). At planting, fertilization consisted of 1 kg B ha^{-1} (boric acid), 100 kg K_2O ha^{-1} (potassium chloride), 200 kg P_2O_5 ha^{-1} (simple superphosphate), and 40 kg N ha^{-1} (using the source established in the treatments) applied in the planting furrows at an average depth of 0.15 m. Afterward, whole seed tubers of the cultivar Agata were distributed 0.05 m above and spaced 0.35 m apart in the furrows. The seed tubers used in the experiment had an average weight and diameter of 76 g (69 to 94 g) and 38 mm (33 to 46 mm), respectively.

In the side-dressing fertilization, N was supplied at the rates and sources established in the treatments, 30 days after planting (DAP).

Weed control was carried out by manual hoeing and pests and diseases were prevented by phytosanitary products registered for the potato crop. Irrigation was performed by sprinkler at a rate of 5 mm day^{-1}.

The harvest was carried out at 80 DAP.

3.2.4 Mineral analysis

For the assessment of the nutritional status of potato plants, the third leaf from the apical tuft (one per plant of 10 plants) was collected at 40 DAP, as described by Lorenzi, Monteiro, and Miranda Filho (1997). The samples were washed, dried, ground, digested, and analyzed to determine the N, P, K, Ca, Mg, and S contents (g kg^{-1}) following Miyazawa et al. (2009).

3.2.5 Gas exchange parameters

Gas exchange parameters were appraised 50 DAP on the upper third of the newly developed leaf of potato plants, from 8:00 to 11:30 a.m. Internal CO_2 concentration (Ci; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs; mmol H_2O m^{-2} s^{-1}), and net photosynthetic rate (A; µmol m^{-2} s^{-1} CO_2) were evaluated using a portable gas exchange device (LC-PRO$^+$, ADC Bioscientific Ltda Herts England) with photosynthetically active radiation (PAR) of 1200 µmol m^{-2} s^{-1}. Water use efficiency (WUE; µmol CO_2 $mmol^{-1}$ H_2O) was calculated through A/E.

3.2.6 Content of total chlorophyll and carotenoids

Evaluations of total chlorophyll (Chl *a* + *b*) and carotenoids were determined spectrophotometrically at 40 DAP. Fresh tissue samples collected from the upper third of the first newly developed leaf of potato plants were added in tubes with 1.5 ml acetone (80 %). After shaking for 48 h at 4 °C, the samples were read in 470, 645, and 662 nm for carotenoids, chlorophyll *a*, and chlorophyll *b*, respectively. The pigment contents were estimated according to Lichtentlaler (1987). The results were expressed in µg g^{-1}.

3.2.7 Dry biomass

Two potato plants randomly collected at 73 DAP were cut and separated into shoots and tubers. Subsequently, the material was washed, dried (65 ^{o}C ± 5), and weighed. The dry biomass accumulation (g per plant) in the entire plant was calculated by the sum of the individual amounts of the shoot and tuber dry biomass. The percentage of dry biomass distribution between potato constituent parts was determined by the relation of the dry biomass of each organ (shoot and tubers) with the total dry biomass (whole plant) (Benincasa, 2003).

3.2.8 N accumulation and N use efficiency

N accumulation in the shoots and tubers was determined at 73 DAP (two plants per plot). The N accumulation (g per plant) in the whole plant (shoots and tubers) was estimated following the formula: N accumulation = ((N content g kg^{-1}) * (Potato dry biomass g per plant)) / 1000. N use efficiency was calculated by (total dry biomass accumulation)^2 / (total N content in the plant) as described Siddiqi and Glass (1981).

3.2.9 Nitrate content in tubers (mg kg^{-1})

At the end of the experiment, tubers (four per plot) were randomly collected from 5:00 to 6:30 a.m. Then, the samples were washed, dried, ground, and prepared to appraise the nitrate content following the procedure of Mantovani et al. (2005). The nitrate content obtained in the dry biomass was converted into mg NO_3^- kg^{-1} in the fresh mass.

3.2.10 Nitrate content after cooking process

To evaluate the effect of the boiling process on the nitrate content, 100 grams of homogeneous pieces of potato tubers were immersed in 800 mL of boiling water for 15 minutes. Then, shortly after, to stop the cooking process, the samples were cooled in 800 ml of water and ice for 7 minutes. Afterward, the samples were drained off (65 ºC ± 5) until they reached constant masses and prepared for the determination of nitrate following the procedure of Mantovani et al. (2005).

3.2.11 Potato yield, tuber classification, pulp firmness, and pH of tuber

At the end of the experiment, tubers in the useful area were collected and weighed. The total yield (t ha^{-1}) was estimated from the total biomass of tubers from each plot. Subsequently, the potato tubers were classified according to the transverse diameter: I (> 70 mm), II (> 42 and ≤ 70 mm), III (>33 and ≤ 42 mm), IV (>28 and ≤ 33 mm), and V (≤ 28 mm) classes (CEAGESP, 2015). The data of classes percentage were transformed in ArcSin √x/100.

Pulp firmness (Newton) was determined using a penetrometer in the central part of potato tubers (with peel). Two readings were taken on each side of the potato tuber (three tubers per plot). pH was measured in 10 g of tuber pulp diluted in 100 mL of deionized water through a digital pH meter (Zenebom and Pascuet, 2005).

3.2.12 Statistical analysis

The dataset was subjected to analysis of variance (F-test) using the Agroestat software (Barbosa and Maldonado Júnior, 2015). Polynomial regression analysis was conducted for N rates, choosing the equation with the highest level of significance and coefficient of determination.

3.3 RESULTS

Nitrogen (N) and phosphorus (P) increased at the individual effect of N application rates reaching maximum contents of 31.3 and 6.9 g kg^{-1} at 135 and 117 kg N ha^{-1}, respectively (Fig. 1a-b).

Potassium (K) was unaffected by the traits evaluated, alone or in an interaction, exhibiting an average content of 31 g kg^{-1} (Table 1).

Table 1. Summary of analysis of variance for macronutrients contents (g kg^{-1}) of potato plants submitted to nitrogen sources (ammonium nitrate and urea) and N rates at side-dressing (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	N	P	K	Ca	Mg	S
Source (S)	1.52^{ns1}	0.00^{ns}	3.41^{ns}	22.05**	5.71*	3.99^{ns}
N Rates	20.05**	5.19**	0.30^{ns}	3.54*	1.58*	3.52*
S x N	2.97^{ns}	0.61^{ns}	0.07^{ns}	1.55^{ns}	0.18^{ns}	9.17**
Blocks	2.61^{ns}	0.45^{ns}	0.47^{ns}	0.17^{ns}	1.38^{ns}	0.40^{ns}
Error	0.66	0.39	1.10	0.31	0.16	0.13
CV(%)	4.45	12.40	7.05	8.41	9.70	5.82
Average - sources						
Ammonium nitrate	29.86a^{2}	6.29a	30.53a	7.83a	3.36a	4.41a
Urea	29.29a	6.28a	31.96a	6.81b	3.09b	4.23a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

Calcium (Ca) and magnesium (Mg) contents varied in response to N sources and supply (Table 1). The applied N rates caused the Ca and Mg to raise following a quadratic function, peaking at 98 (7.7 g kg^{-1}) and 110 kg N ha^{-1} (3.4 g kg^{-1}), respectively (Fig 1c-d). Potato plants have taken up 8.7 and 14.9 % more Mg and Ca when fertilized with ammonium nitrate than urea (Table 1).

An interaction between traits was noticed for sulfur (S) content (Table 1). Ammonium nitrate application led to a linear decrease of S, whilst urea promoted a quadratic increase (Fig. 1e). The highest content was obtained with the application of 127 kg N ha^{-1} from urea, representing an increase of 21 % compared to control plants (no N applied at side-dressing).

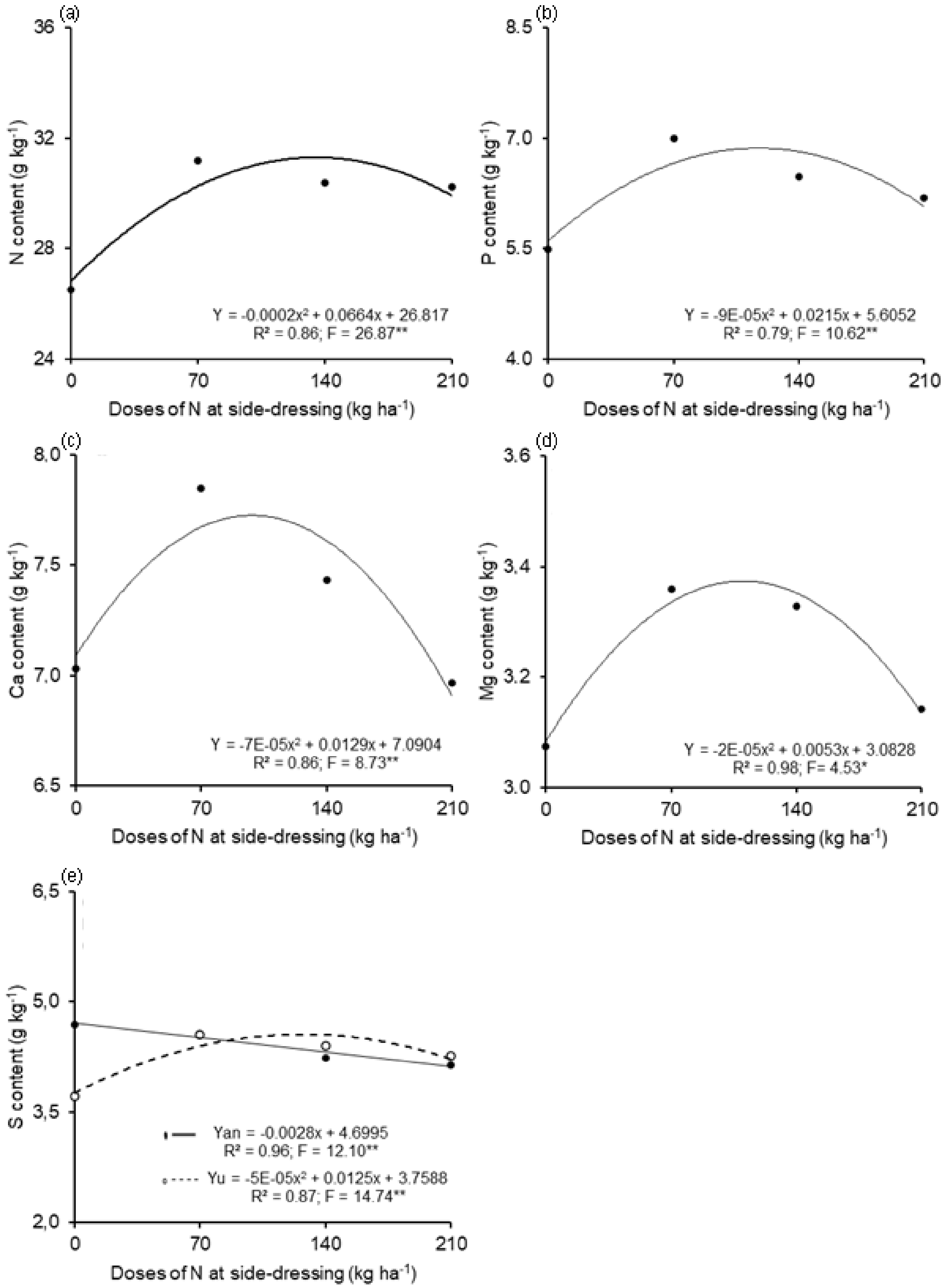

Fig. 1 Effects of nitrogen fertilization at side-dressing on nitrogen (a), phosphorus (b), calcium (c), magnesium (d), and sulfur (e) contents in potato leaves. *, ** significant at 5 % and 1 % probability by F test, respectively.

The gas exchange parameters were significantly influenced by N management (Table 2).

Table 2. Summary of analysis of variance for intracellular CO_2 concentration (Ci; µmol CO_2 m^{-2} s^{-1}), transpiration rate (E; mmol H_2O m^{-2} s^{-1}), stomatal conductance (gs; mmol H_2O m^{-2} s^{-1}), net photosynthesis rate (A; µmol m^{-2} s^{-1} CO_2), water use efficiency (WUE; µmol CO_2 $mmol^{-1}$ H_2O), chlorophyll *a* + *b* (µg g^{-1}), and carotenoid (µg g^{-1}) contents of potato plants submitted to nitrogen sources (ammonium nitrate and urea) and N rates at side-dressing (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	Ci	E	gs	A	WUE	Total Chlorophyll (*a* + *b*)	Carotenoid
Sources (S)	4.80[*1]	0.00[ns]	0.72[ns]	0.91[ns]	0.44[ns]	3.52[ns]	1.28[ns]
N Rates	3.77[*]	13.21[**]	7.31[**]	2.19[**]	4.41[*]	40.56[**]	18.38[**]
S x N	6.59[**]	5.27[**]	2.62[ns]	1.48[ns]	7.43[**]	2.43[ns]	0.08[ns]
Blocks	10.13[**]	16.08[**]	1.03[ns]	1.34[ns]	20.67[**]	1.92[ns]	2.12[ns]
Error	6.89	0.1	0.02	0.66	0.32	0.01	0.06
CV(%)	5.69	7.72	12.26	6.88	8.75	11.44	10.71
		Average - sources					
Ammonium nitrate	247.21a[2]	2.69a	0.31a	18.91a	7.20a	0.23a	0.12a
Urea	236.54b	2.69a	0.30a	19.35a	7.35a	0.21a	0.11a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; [ns] - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

There was a quadratic increase in stomatal conductance (gs) and net photosynthetic rate (A) as N rates rose (Fig. 2c-d). Based on the regression coefficients, the estimated maximum gs (0.4 mmol H_2O m^{-2} s^{-1}) and A (19.8 µmol m^{-2} s^{-1} CO_2) were achieved with the supply of 110 and 91 kg N ha^{-1}, respectively.

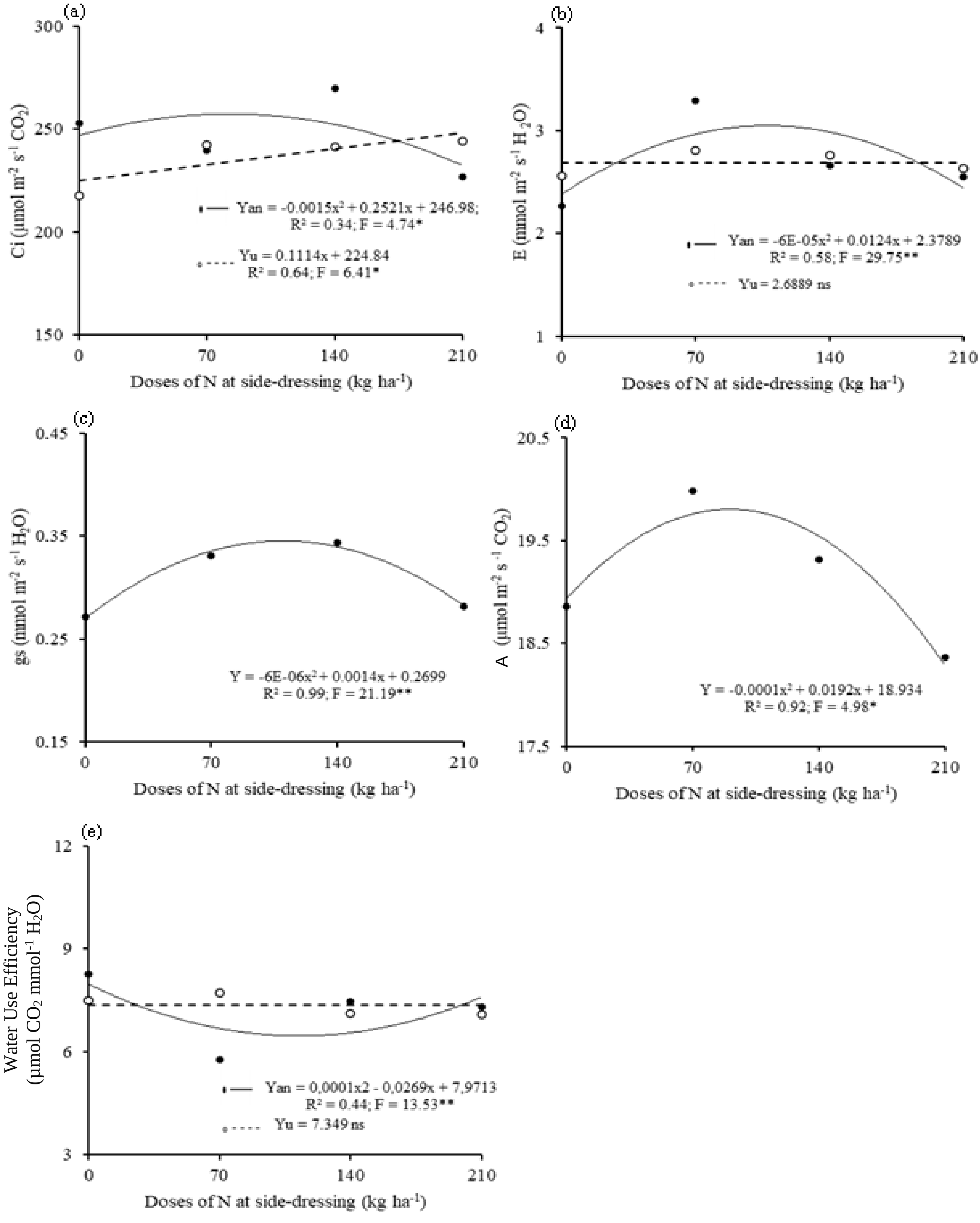

Fig. 2 Effects of nitrogen fertilization at side-dressing on internal CO_2 concentration (a), transpiration rate (b), stomatal conductance (c), net photosynthetic rate (d), and water use efficiency (e) in potato leaves. *, ** significant at 5 % and 1 % probability by F test, respectively.

The interaction between the treatments (S×D) was significant for intracellular CO_2 concentration (Ci), transpiration rate (E), and water use efficiency (WUE) (Table 2). The applied N rates as ammonium nitrate induced the Ci and E to increase following a quadratic function, with a maximum rate of 257.4 µmol CO_2 m^{-2} s^{-1} and 3.1 mmol H_2O m^{-2} s^{-1} for the rates of 82 and 108 kg N ha^{-1}, respectively (Fig. 2a-b). On the other hand, the application of N as urea lead to a linear increase in Ci whilst no difference was observed between the rates in E (Fig. 2a-b). Ci in potato plants treated with urea ranged from 224.8 to 248.4 µmol CO_2 m^{-2} s^{-1}.

Regarding water use efficiency (WUE), the increase of N rates as ammonium nitrate caused a quadratic effect in this attribute, reaching minimum efficiency at 112 kg N ha^{-1} (6.5 µmol CO_2 $mmol^{-1}$ H_2O), representing a decrease of 23.4 and 17.6 % in relation to untreated plants and plants at the highest N dose (210 kg N ha^{-1}), respectively (Fig. 2e). WUE in potato plants was not significantly different for urea source.

The photosynthetic pigments of potato plants depend on the N rates applied throughout cultivation (Table 2). A remarkable increase in total chlorophyll (*a* + *b*) and carotenoid contents was observed in response to N application. At the highest dose applied (210 kg N ha^{-1}), total chlorophyll (*a* + *b*) and carotenoid were 82.6 (0.283 µg g^{-1}) and 43.8 % (0.138 µg g^{-1}) higher than untreated plants, respectively (Fig. 3).

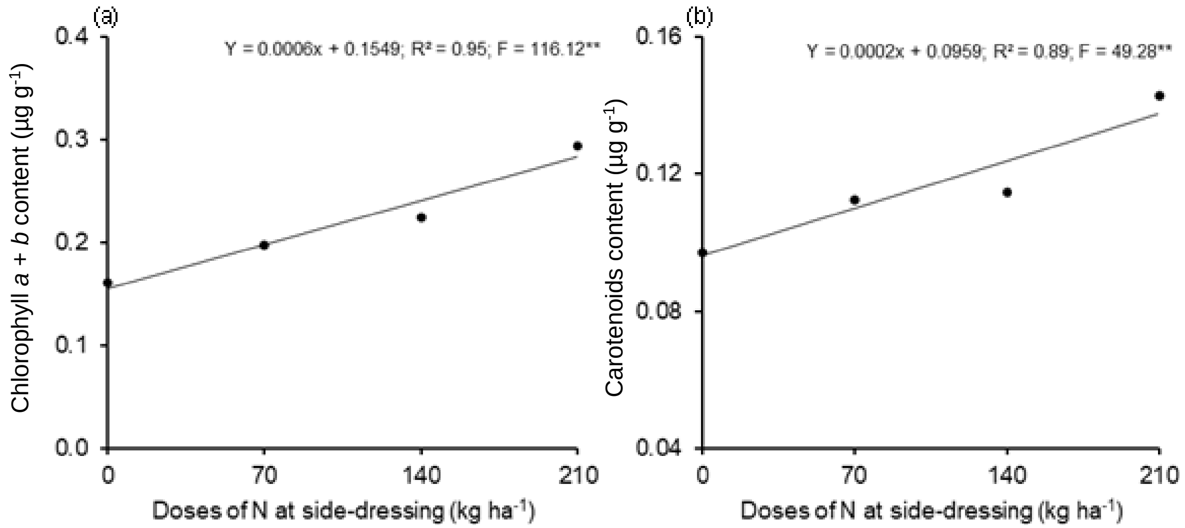

Fig. 3 Effects of nitrogen application at side-dressing on chlorophyll *a* + *b* (a), and carotenoids (b) contents in potato leaves. *, ** significant at 5 % and 1 % probability by F test, respectively.

The shoot dry biomass (SDM) and total dry biomass accumulation (TODA) were influenced by the interaction of N sources and rates (Table 3). SDM and TODA increased quadratically with increasing N rates from ammonium nitrate (Fig. 4a, c), peaking at 114 (54.4 g) and 108 kg N ha^{-1} (252.7 g). respectively, representing an increase of 90.3 and 47.9 % compared to untreated plants. Further, for the urea source, the SDM had a quadratic effect, reaching a maximum (58.1 g) at 147 kg N ha^{-1}, while TODA reached the highest accumulation (251.4 g) at the highest dose studied (210 kg N ha^{-1}) (Fig. 4a, c). N fertilization led to a quadratic effect in tuber dry biomass (TDM) (Fig. 4b) of potato plants.

The percentage of dry biomass in the shoots and tubers was influenced only by N rates (Table 3). N application caused a quadratic effect to increase biomass partitioning to the shoots (BPS) until 132 kg N ha^{-1} and, also a quadratic effect in the biomass partitioning to the tubers (BPT) of potato plants as increasing N rates (Fig. 4d-e). At the highest dose studied BPT contributed to nearly 78.80 % of the total plant biomass, while shoots accounted for 21.20 %.

Table 3. Summary of analysis of variance for shoot dry biomass (SDM; g per plant), tuber dry biomass (TDM; g per plant), total dry biomass accumulation (TODA; g per plant), biomass partitioning to the shoots (BPS; %), and biomass partitioning to the tubers (BPT; %) of potato plants submitted to nitrogen sources (ammonium nitrate and urea) and N rates at side-dressing (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	SDM	TDM	TODA	BPS	BPT
Sources (S)	1.05^{ns1}	0.45^{ns}	0.15^{ns}	2.13^{ns}	2.13^{ns}
N Rates	27.11**	4.57*	9.54**	5.51**	5.51**
S x N	3.69*	2.35^{ns}	3.19*	0.48^{ns}	0.48^{ns}
Blocks	2.34^{ns}	5.57**	6.18**	1.16^{ns}	1.16^{ns}
Error	3.65	16.00	16.93	1.64	1.64
CV(%)	16.80	19.05	16.36	15.66	4.15
			Average - sources		
Ammonium nitrate	42.13a^{2}	167.30a	209.42a	20.08a	79.62a
Urea	44.77a	159.95a	204.72a	21.77a	78.23a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

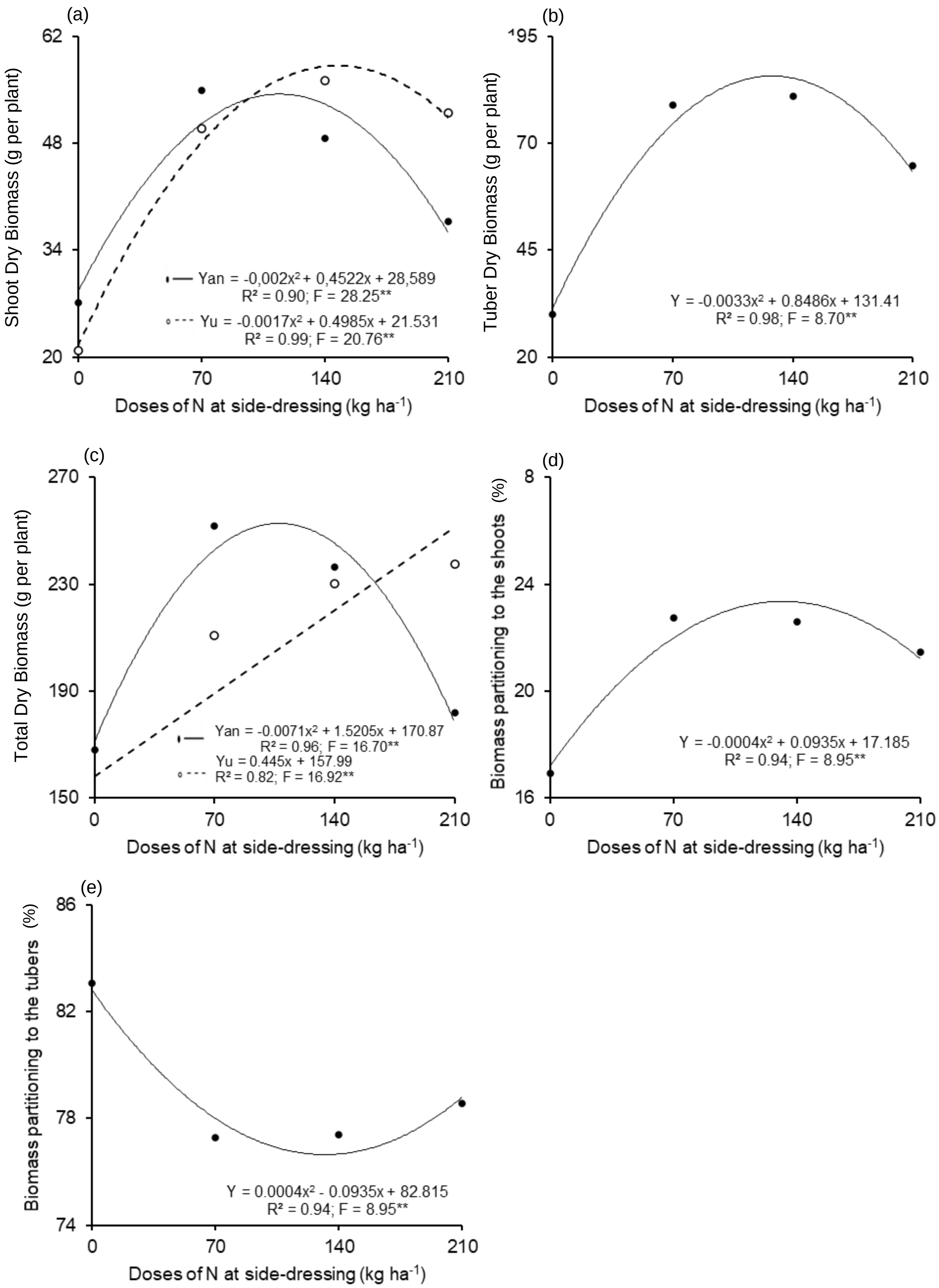

Fig. 4 Effects of nitrogen fertilization at side-dressing on shoot dry biomass (a), tuber dry biomass (b), total dry biomass accumulation (c), biomass partitioning to the shoots (d), and biomass partitioning to the tubers (e) of potato plants. *, ** significant at 5 % and 1 % probability by F test, respectively.

The N accumulation in potato plants was affected by the N rates applied at side-dressing (Table 4), being higher amounts accumulated in the tubers than in the shoot of the plant (Fig 5).

Table 4. Summary of analysis of variance for N accumulation in the shoots (NAS; g per plant), N accumulation in the tubers (NAT; g per plant), total N accumulation (TNA; g per plant), and N use efficiency (NUE; kg N absorbed) of potato plants submitted to nitrogen sources (ammonium nitrate and urea) and N rates at side-dressing (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	NAS	NAT	TNA	NUE
Sources (S)	0.00^{ns1}	0.55^{ns}	0.33^{ns}	2.20^{ns}
N Rates	21.29^{**}	9.68^{**}	17.97^{**}	2.28^{*}
S x N	2.3^{ns}	2.21^{ns}	3.06^{ns}	2.26^{ns}
Blocks	1.87^{ns}	4.51^{*}	4.47^{*}	2.92^{ns}
Error	77.59	151.06	194.92	2115.48
CV(%)	19.99	23.11	18.71	20.49
Average - sources				
Ammonium nitrate	775.58a[2]	1267.97a	2043.55a	21754.72a
Urea	776.45a	1346.86a	2123.31a	19534.34a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

N accumulation in the shoots (NAS), tubers (NAT), and whole potato plant (TNA) had a quadratically effect increasing in response to N supply (Fig 5a-c). N fertilization significantly raised the NA up to 135 kg N ha^{-1}, then decline as N application was further increased.

N use efficiency (NUE) was significantly influenced by N supply (Table 4). Increasing rates of N fertilizer promoted a quadratic raise in NUE (Fig. 5d), varying from 18230.4 to 19793.1 kg of total dry mass per kg^{-1} of N absorbed.

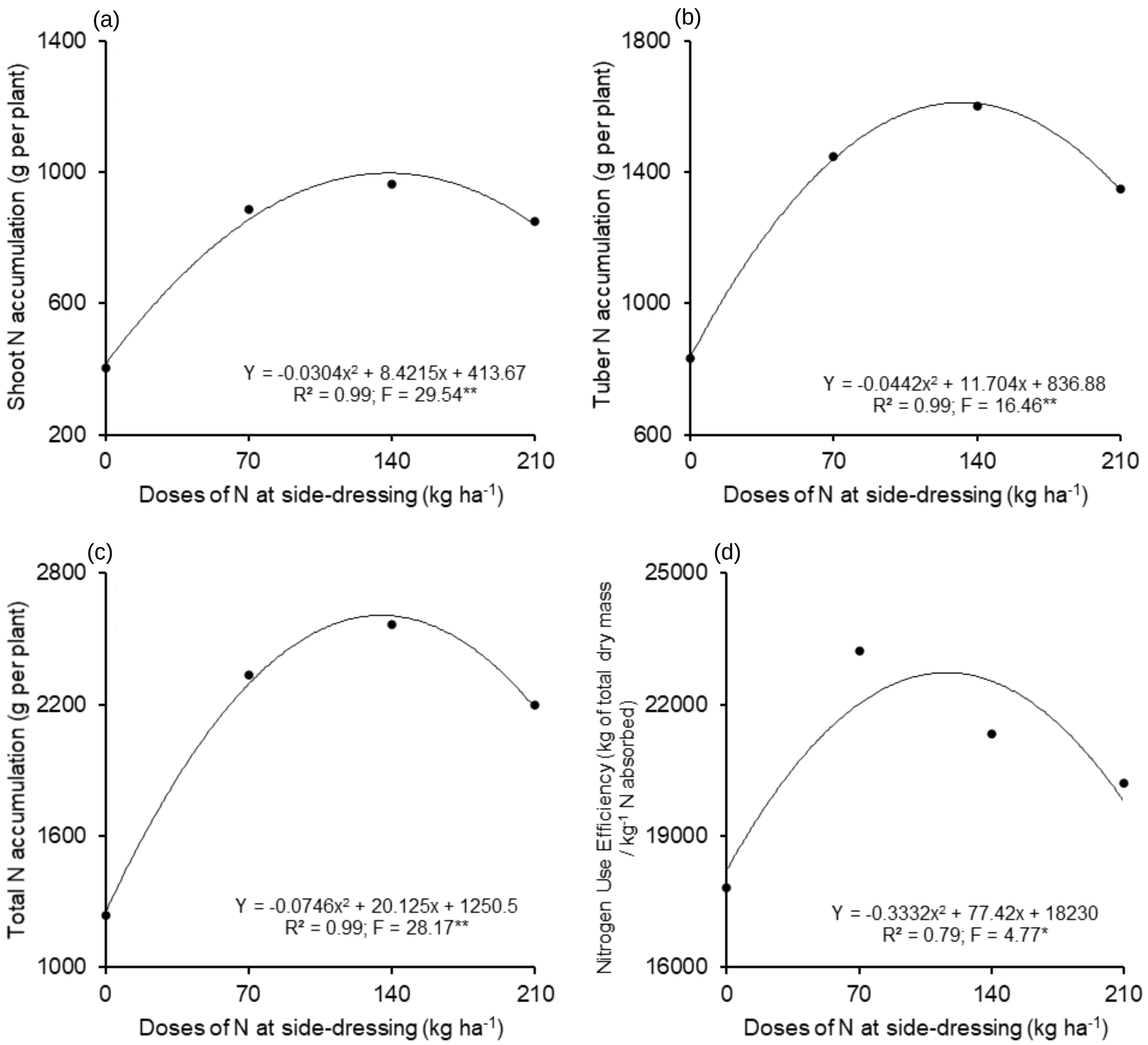

Fig. 5 Effects of nitrogen fertilization at side-dressing on shoot N accumulation (a), tuber N accumulation (b), total N accumulation (c), and nitrogen use efficiency (d) of potato plants. *, ** significant at 5 % and 1 % probability by F test, respectively.

The yield of potato plants was affected by N sources and rates interaction (Table 5).

Table 5. Summary of analysis of variance for yield of potato plants and classification of tubers of potato plants submitted to nitrogen sources (ammonium nitrate and urea) and N rates at side-dressing (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	Yield	Class II	Class III	Class IV	Class V
Sources (S)	1.36^{ns1}	6.79*	0.54^{ns}	0.23^{ns}	1.25^{ns}
N Rates	2.49^{ns}	1.80^{ns}	1.79^{ns}	1.90*	1.93^{ns}
S x N	4.10*	0.39^{ns}	3.66*	2.75^{ns}	1.69^{ns}
Blocks	0.90^{ns}	6.97**	0.69^{ns}	0.63^{ns}	1.78^{ns}
Error	4.29	0.03	0.05	0.05	0.04
CV(%)	19.28	68.27	18.14	17.14	27.59
		Average - cultivars			
Ammonium nitrate	46.29a^{2}	0.12a	0.57a	0.53a	0.26a
Urea	42.75a	0.06b	0.54a	0.54a	0.29a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ^{ns} - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

The increase in N rates from the ammonium nitrate source did not affect the yield of potato plants (Fig. 6). On the other hand, the increasing N from the urea promoted a quadratic effect by increasing yield, reaching a maximum of 51.45 t ha^{-1} at 136 kg N ha^{-1}, representing an increase of 79.3 and 14.9 % in comparison to untreated plants and plants fertilized with the highest dose of N.

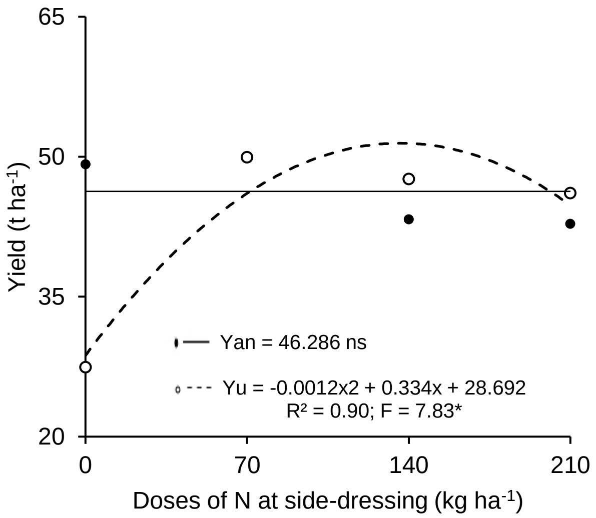

Fig. 6 Effects of nitrogen fertilization at side-dressing on the yield of potato plants. *, ** significant at 5 % and 1 % probability by F test, respectively.

No interaction between sources and rates of N was observed for the II, IV, and V classes (Table 5). In terms of the total production, fewer potato tubers fell into the II class. Most of them had a diameter between >28 and ≤ 42 mm, fitting into III and IV classes.

In all the evaluated treatments, potato plants did not produce tubers with a diameter greater than 70 mm (class I).

The II class of potatoes was influenced only by N sources. Potato plants fertilized with urea exhibited a lower number of tubers in this class than those fertilized with ammonium nitrate, likely due to the vigorous vegetative growth, causing a decrease in tuber development (Table 5).

The III class quadratically increased with increasing N from the urea, peaking at 135 kg N ha^{-1}, whereas it was not significantly different for the ammonium nitrate source (Fig. 7a).

N application at a dose of 210 kg N ha^{-1} led to a lower number of tubers with less than 28 mm of diameter (Fig. 7b) compared to the other rates.

V class of potato was unaffected by N sources and rates, alone or in an interaction.

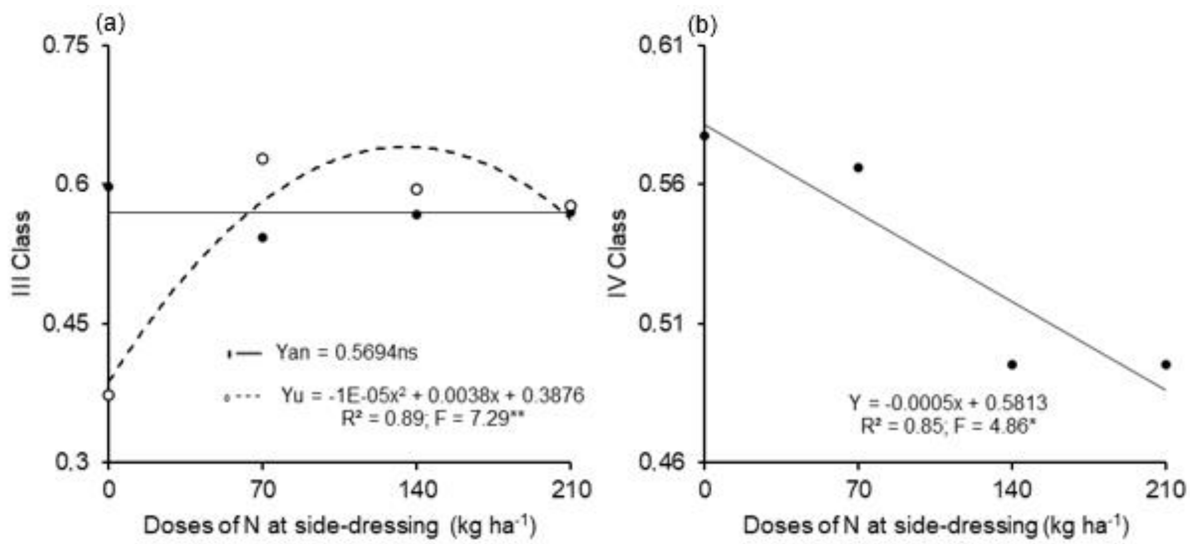

Fig. 7 Effects of nitrogen fertilization at side-dressing on the classification of tubers in the III class (a) and IV class (b). *, ** significant at 5 % and 1 % probability by F test, respectively.

N management did not affect the pulp firmness (PF) and pH, showing an average of 8.6 N and 6.1, respectively (Table 6).

Table 6. Summary of analysis of variance for pulp firmness (PF; Newton), potential of hydrogen (pH), nitrate content in tubers (NO_3^- FM), nitrate content in tubers after boiling (NO_3^-B) of potato plants submitted to nitrogen sources (ammonium nitrate and urea) and N rates at side-dressing (0, 70, 140, and 210 kg N ha^{-1}).

Variation source	PF	pH	NO_3^- FM	NO_3^- B
Sources (S)	1.22^{ns1}	0.11^{ns}	10.68^{**}	46.78^{**}
N Rates	2.48^{ns}	2.82^{ns}	47.21^{**}	5.98^{**}
S x N	1.89^{ns}	2.82^{ns}	3.14^{*}	7.93^{**}
Blocks	7.77^{**}	0.18^{ns}	2.83^{ns}	0.14^{ns}
Error	0.22	0.05	1.74	2.26
CV(%)	5.15	1.75	6.45	10.24
Average - sources				
Ammonium nitrate	8.69a[2]	6.09a	51.83b	38.71b
Urea	8.51a	6.10a	55.84a	49.65a

CV – coefficient of variation; [1] **, * - Significant at 1 and 5 %, respectively; ns - not significant by F test at 5 % probability. [2] Means in the column followed by equal letters do not differ at 5 % probability by Tukey test.

The applied N sources and rates lead the nitrate content to raise following a quadratic equation, with maximums of 65.2 and 64.9 mg NO_3^- kg^{-1}, acquired at 210 kg N ha^{-1} from both sources (Fig. 8a). The lowest nitrate accumulation in potato tubers was reached at 64 (44.1 mg kg^{-1}) and 45 kg N ha^{-1} (50.8 mg kg^{-1}).

A similar trend was observed for nitrate content after boiling, which also was influenced by the interaction of N sources and rates (Table 6). Potato plants exhibited linear increments in this characteristic with the increase of N supply from ammonium nitrate, representing a rise of 56.53 % (30.2 – 47.4 mg kg^{-1}) in relation to untreated plants. On the other hand, no difference was observed between the N rates from urea (Fig. 8a-b).

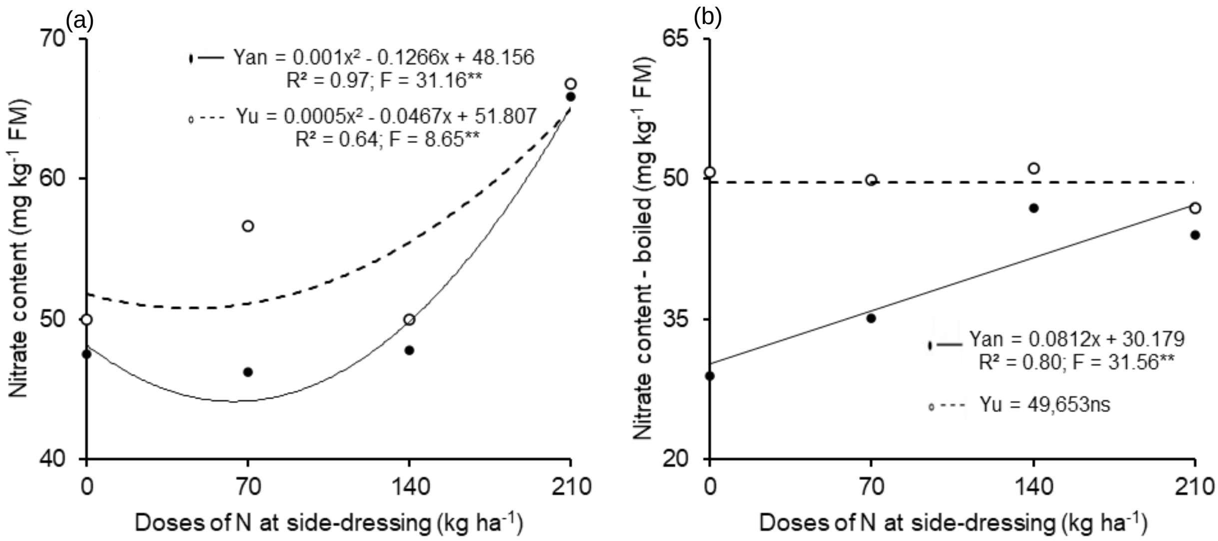

Fig. 8 Effects of nitrogen fertilization at side-dressing on the nitrate content (a) and nitrate content after boiling (b). *, ** significant at 5 % and 1 % probability by F test, respectively.

3.4 DISCUSSION

Despite the chemical form and dose of N applied, potato plants exhibited N, K, and Ca contents beneath the appropriate range for the crop proposed by Lorenzi Monteiro, and Miranda Filho (1997), however, no visual symptoms were noticed, and plants achieved yields higher than the national average (31.7 t ha^{-1}) (IBGE, 2021).

The results observed in this study, indicated that N fertilization may benefit potato growth and quality or cause detrimental effects, depending on the N source and dose applied.

N application rates and sources promoted different effects on gas exchange parameters. The supply of rates above 110 and 91 kg N ha^{-1} for gs and A, associated with lower yield reveals that the increase in N supply above the optimum rate disturbs physiological mechanisms, reflecting in tubers biomass production. Lower stomatal conductance was observed with increasing N application rates, particularly when surpassing crop demand, promoting a decrease in E and creating a barrier to the acquisition of atmospheric CO_2. Consequently, the reduction of Ci caused a decrease in A, affecting the yield of potato plants (Giletto et al., 2020). These results can be explained from two perspectives. First, it might be associated with increments in N-compounds to the detriment of starchy accumulation, causing vigorous vegetative growth. As a result, an increase in the shoot/root ratio occurred, impairing the development of the root system and reducing plant resistance to dry periods (stomata closure) (Yang et al., 2022). Secondly, it can be attributed to lower K contents in potato plants, which influences the optimal turgor of the stomatal guard cells (Prado, 2014).

The application of rates greater than 116 kg N ha^{-1} reduced N use efficiency, affecting potato development and dry mass accumulation. The results observed in this study corroborate with those found for several other authors who also observed a decrease in N use efficiency as N supply increased (Hailu et al., 2017; Wang et al., 2020).

Notably, differences in potato production were noticed under ammonium nitrate and urea fertilization. Even though ammonium nitrate was the most appropriate source for reducing nitrate content in tubers, it was not the better chemical form to maximize potato yield. The best treatment that led to the highest production consisted of the application of 136 kg N ha^{-1} using urea as a source, which resulted in a nitrate content equivalent to 49.24 mg kg^{-1}, below the limit recommended for human consumption (200 mg NO_3^- kg^{-1} of fresh mass). These findings may be related due to the fact that when ammonium nitrate (34 % of N constituted of 50 % NO_3^- and 50 % NH_4^+) is supplied, nitrate is immediately available into the soil to plants, increasing the risk of N loss by leaching, mainly under excessive rainfall and irrigation conditions (Prasad and Hochmuth, 2016). In addition, high levels of ammonium can be toxic to plants and negatively affect the uptake of important cations such as K^+, $Mg^{2+,}$ and Ca^{2+}, influencing plants' development and production (Nascimento, Nascimento, and Cecílio Filho, 2020).

Brazil does not have proper legislation regarding the nitrate content in vegetable crops, however, regardless of the sources and rates applied, the nitrate contents recorded in this study were within the limit established by the German standards, which allows levels up to 200 mg NO_3^- kg^{-1} of fresh mass (Elrys et al., 2021).

The boiling method significantly reduced nitrate content in potato tubers. Nitrate is highly soluble in water, thereby, the boiling process, enhances its diffusion into the cooking water, reducing its content in the tuber (Boari et al., 2013). This result proves that cooking tubers thought boiling can be an excellent alternative to deplete nitrate, reducing its health-related risks.

Considering a daily consumption of 88.5 g of potato (FAOSTAT, 2019) and a nitrate$^-$ content of 55.1 mg kg^{-1} FM (nitrate content obtained at 136 kg N ha^{-1} from urea - highest yield), then the daily intake of nitrate would be 4.9 mg NO_3^- kg^{-1} in raw tubers and 2.7 in boiled tubers.

Determining the proper N source and dose to potato plants, besides benefitting the yield and quality of tubers also decrease the total operational costs of the system. Based on an average price of R$ 3,395.05 per ton of urea (IEA, 2021), the cost of applying 136 kg N ha^{-1} (R$ 461.72) would be 193.4 % lower than applying the same dose as ammonium nitrate (R$ 1,354.56).

3.5 CONCLUSION

The findings obtained in this study unveiled that the screening of sources and rates of N is important for building an effective potato nutrition program since the management of N fertilization can alter the performance of potato plants, influencing the agronomic outcomes and quality of tubers. In this manner, based on potato yield, the best strategy to maximize profits in the agricultural sector without compromising the quality of tubers is to apply 136 kg N ha^{-1} using urea as a source, which gives a nitrate content equal to 49.24 mg kg^{-1}, below the limit, recommended for human consumption (200 mg NO_3^- kg^{-1}).

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