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RESEARCH ARTICLE

IMPORTANCE OF NITROGEN IN MAIZE PRODUCTION

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ABSTRACT

Among several functions, the nitrogen plays a key role on plants metabolism, this element takes part in different metabolic path ways of great importance to plants and participates in the protein synthesis. Most of the cultivated soils allow plants growth without addition of nutrients in mineral form. However, to seek higher production levels, the application of nutrients in mineral form is required, the current recommendation of nitrogen fertilization to define the amount to be applied. More than 95% of the nitrogen in the soil is organic, this organic form is not assimilated by plants, requiring to go through a mineralization process, in other words, the transformation from organic to mineral nitrogen called amination and ammonification. The identification of the character of highest contribution to maize yield is an important tool which assists identifying the critical period of the crop development, allowing the adoption of management practices in order to optimize the conditions at the moment of the main grain yield component definition. Generally, nitrogen is present in the soil solution on nitrate or ammonium forms, however the plants are physiologically responsive only to nitric nutrition, there were verified different responses among genetic materials regarding on nitrogen utilization efficiency

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INTRODUCTION

The inadequate management of nitrogen (N) is considered a major limiting factor for maize grain yield. N is important for the plant metabolism as it participates of proteins and chlorophyll biosynthesis, being necessary since the early phenological stages of the plant development (Basso and Ceretta, 2000), and according to Sangoi *et al*. (2008) it participates in several major metabolic pathways of plants biochemistry (Andrade *et al.,* 2003). Results obtained by Coelho (2004) and Lemaire and Gastal (1997) demonstrated that, under appropriate levels of other nutrients in the soil, nitrogen provides the greatest increment to maize yield. Due to several transformations the N is subjected in the soil, this nutrient is considered a dynamic and complex element generating debates and controversies regarding on its best source and moment of application in maize.

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On soil-plant system, the N dynamic is influenced by many features of the cropping systems (tillage or no-tillage farming), crop management techniques, edaphoclimatic conditions (Santos *et al.,* 2010), and the fertilizer type. Currently, urea and ammonium sulfate are the main nitrogen sources utilized in maize. According to Alva *et al*. (2006), both are subject to loss in the soil by leaching, runoff, ammonia volatilization and microbial biomass immobilization. Therelease of nitrogen to plants occurs according to its source. Urea [$CO(NH_2)_2$] presents itself in the amidic form being rapidly hydrolyzed in the soil producing ammonium[NH_4^+], which is retainedby negative charges of soil colloids becoming available to the plants (Novais *et al*., 2007). However, some authors state that the NH_4^+ from urea tends to nitrify faster than the NH_4^+ from ammonium sulfate due to pH increase during hydrolysis (Mcinnes and Fillery, 1989; Silva and Valle, 2000). When ammonium sulfate [$(NH_4)_2SO_4$] is applied to the soil, its dissolution process generates ammonium ions, which will be oxidized to nitrate through nitrification process (Silva and Valle, 2000). On the other hand, depending on climatic conditions, the ammonium sulfate may have a delayed N release because of its protection with organic sulfur.

According to Queiroz *et al*. (2011) the coverage by organic and inorganic substances allows the nutrient gradual release, and as stated by Trenkel (2010), the N release may be delayed due to slow hydrolysis of water soluble compounds. Another factor that may influence the nitrification of ammonium sulfate and urea is the calcium concentration in the soil. Work performed by Aurora *et al*. (1986) observed that ammonium from urea nitrified faster than ammonium from ammonium sulfate in the absence of calcium carbonate. The nitrogen assimilation might vary among hybrids. According to Machado (1992), genetic differences may influence the nitrogen activity and assimilation by maize plants. Tests conducted by this author concluded that hybrids which incorporate ammonium ion into amino acids through the glutamine synthetase enzy me have greater nitrogen efficiency of utilization when compared to enzymes present in other hybrids. This result is similar to the one found by Fernandes *et al*. (2005), expressing different efficiencies of hybrids to N doses. Other factors that may influence the nitrogen fertilization response are the genetically modified hybrids, which represented 42% of the commercialized seeds in the 2010 off-season. This is reflected in the growth of maize yield through utilization of hybrid cultivars of greater genetic potential and the increase of fertilizer applications (Cruz *et al*., 2010).

Currently, the nitrogen recommendation for maize to the Rio Grande do Sul state is from 20 to 30 kg $ha^{-1}$of N at sowing, and the rest in to pdressing between V4 and V6phenological stages (CQFS-RS/SC, 2004), however, Escosteguy *et al*. (1997) affirm the moment of topdressing may vary from four to eight completely expanded leaves. An extremely important factor in maize nitrogen management is the quantity applied. As stated by Kappes *et al*. (2013), maize productivity ranges according to the increase of N doses. On this regard, it is observed several concerns related to application techniques and the losses evidenced in certain weather conditions due to several chemical reactions, affecting nitrogen release and absorption by plants. Considering the reactions that N is subject in the soil, works related to nitrogen sources and application timing in maize have generated controversies and several debates (Meira *et al*., 2009).

Soratto *et al*. (2012), studying nitrogen sources and application timing in maize found no difference for timing, but observed an increase in productivity among sources. Meanwhile, Schiavinatti *et al*. (2011) found negative effects on productivity when urea was applied in a single time at the eight leaves stage, however, they found no differences among nitrogen sources. Results found in the literature are conflicting for nitrogen application timing in maize, therefore the hypothesis underlying this work is the splitting of application and the source of nitrogen may affect certain plant parameters and the final grain yield, considering the possibility of different responses among genetically modified hybrids. In addition, the current recommendation is based on row spacing of 80-90 cm, however reducing the spacing will directly influence on fertilizer utilization by maize plants. Thus, splitting the application may improve nitrogen assimilation efficiency by plants resulting in significant grain yield gains, requiring techniques to reduce losses, set the best nitrogen source, linking to the most appropriate moment of application. Therefore, the objective of this study is to evaluate the effect of nitrogen application timing on some plant parameters, and the grain yield of two hybrids of maize using urea and ammonium sulfate.

Nitrogen-maize relation

Among several functions, nitrogen plays a key role on plants metabolism. This element takes part in different metabolic pathways of great importance to plants (Sangoi *et al*., 2008), and participates in the protein synthesis (Vieira *et al*., 1995). It constitutes molecules such as ATP, NADH, NADPH, storage proteins, nucleic acids and enzymes (Harper, 1994), cytochro me molecules, and chlorophyll (Bull, 1993), evidencing that nitrogen is directly related to the plant development and productivity. Most of the cultivated soils allow plants growth without addition of nutrients in mineral form. However, to seek higher production levels, the application of nutrients in mineral form is required. Among the crops of agronomic interest, maize express nutritional dependence, especially of nitrogen (Cancellier, 2011). On favorable weather conditions, in order to achieve high grain yields, maize demands amount of nitrogen superior to 150 kg ha^{-1}, requiring mineral supplementation from nitrogen fertilization sources (Amado 2002; Scalco *et al*., 2002). Studies conducted by Taiz and Zeiger (2009) show a direct dependence of maize on mineral nutrients, as large quantities applied reflect positively on grain yield. Work performed by Silva *et al*. (2005) demonstrates that nitrogen is a limiting nutrient for the crop establishment, and according to Calonego (2012) the efficiency of N absorption and translocation to the grains is a factor of extreme importance because it directly impacts the productivity.

Researchers report numerous results regarding the importance of nitrogen to maize crop. According to Basi *et al*. (2011), N has influence on the quality of corn silage. They affirm that grain quality is positively affected when nitrogen is applied, as well-nourished plants producea silage of higher nutritional value. Studying agronomic traits with nitrogen utilization, Ferreira *et al*. (2001) concluded that nitrogen fertilization improved grain quality increasing protein and mineral nutrients content, intervening positively in the number of ears per plant, weight of ears, as the mass of a thousand seeds increased according to the nitrogen doses. Another important factor for the determination of nitrogen fertilization in maize is the difference in N use and assimilation among hybrids (Nunes *et al*., 2013).

Nitrogen in the soil-plant system

The N in the soil is associated to organic matter (OM), hence this is one of the criteria considered in the current recommendation of nitrogen fertilization to define the amount to be applied. More than 95% of the nitrogen in the soil is organic. This organic form is not assimilated by plants, requiring to go through a mineralization process, in other words, the transformation from organic to mineral nitrogen called amination and ammonification (Rangel and Silva, 2007). According to Mary *et al*. (1996), time and amount of mineralized nitrogen may vary with species, amount of organic residue, temperature, aeration and humidity. Therefore, the total reserve of N in the soil does not represent the nutrient availability to the plants (Amado *et al*., 2002). Since the adoption of no-tillage system, nitrogen fertilization has become even more important, especially when grass is the preceding crop, due to the large amount absorbed and the low rate of decomposition. According to Ceretta *et al*. (2002), decomposition of plant residue is directly associated to its carbon/nitrogen ratio (C/N), which will reflect on the residue breakdown and N immobilization, influencing on the nutrient availability. Nitrogen stands out among the essential nutrients for plants, since it goes through several transformations in the soil-plant system (Stevenso, 1982). These reactions are performed by microorganisms (Victória *et al*., 1992) resulting in extremely complex dynamics in the soil. Depending on environmental conditions, the N in the soil may be subject to losses especially when applied in large amounts, resulting in environmental harm. According to Silva (2005), the N output from the soil occurs through several ways, highlighting gaseous losses by ammonia volatilization, NO_3^- to N_2denitrification, leaching, soil erosion and nutrient uptake by plants.

Volatilization is the loss of N in gaseous form. This process occurs both to urea and ammonium sulfate under the influence of factors such as temperature, soil pH, and the application form. Another cause of loss is denitrification. In this process, the absence of oxygen may be supplanted by microorganisms converting the nitrate ion (NO_3^-) to nitrite (NO_2^-). Nitrogen losses may also occur due to the leaching through the movement in depth, being dragged by water while in NO_3^-, NH_4^-forms or humified organic compounds (Reichardt and Timm, 2012).Coelho (2003) affirms that NO_3^- leaching occurs because of its high mobility. There are two ways that nitrogen is present in the soil and available to plants. One is the nitric form (N-NO_3^-) and the other is the ammonia cal form (N-NH_4^+) (Araújo *et al*., 2012), which are absorbed by roots and translocated into stems and leaves (Reichardt and Timm, 2012). Several studies show the success of nitrogen utilization rarely exceeds 50% when applied in inappropriate environmental conditions (Scivittarro *et al*., 2000). The N absorption by roots begins after the maize emergence, however, the N absorbed on initial stages has structural function, and only a small part will be stored or translocated (Ta and Weiland, 1992).

Tests conducted by Ferreira *et al*. (2001) report the N quantified in leaves at 25 DAE (days after emergence) has no correlation with the grain yield, however at 45 and 63 DAE it shows a positive correlation to grain yield. Franca *et al*. (2011) observed that nitrogen absorption increased reaching the maximum value at 74 DAE, in the milk stage. After that, it was detected a reduction of the N in the biomass. This result differs from the one found by Fornasieri (1992), where the demand for N increased two and three weeks before flowering and decreased at 82 DAE due to the N mobilization to grains, leaching of N to leaves, and falling of senescent leaves and stems. As well as nitrogen, other nutrients accumulated in plant tissues are translocated to grains during the grain filling stage, demonstrating a positive correlation with the leaves chemical composition, and with the accumulation of protein and nutrients in the grains (Ferreira *et al*., 2001).

Morphological components and corn yield

The identification of the character of highest contribution to maize yield is an important tool which assists identifying the critical period of the crop development, allowing the adoption of management practices in order to optimize the conditions at the moment of the main grain yield component definition (Balbiton *et al*., 2005). The number of grains produced per area is the character that most affects grain yield, which depends on the plant's ability of producing and distributing assimilates to supply the demand after the silk stage (Sangoi *et al*., 2005). This assertion is supported by Souza *et al*. (2014), who reported similar results studying the relation among maize characters and grain yield. They concluded the character height of ear is positively correlated to grain yield, being a suitable trait for indirect selection of grain yield. In addition, it was found that the character diameter of stem had a direct negative influence on grain yield, as plants with larger stem presented lower productivity.

Studying the yield variation among open pollinated varieties (OPV) of maize, Balbiton *et al*. (2005) found variability in grain yield and yield components among maize varieties. In this job, the number of kernels per row was the yield component that most contributed for productivity. Another result that demonstrates the importance of grain yield and yield components correlation is presented by Sangoi *et al*. (2011), who evaluated the influence of N on morphogenesis and tilleringprocesses in maize, concluding that tillering does not directly contribute ingrain yield.

Nitrogen in no-tillage system

Since 1990, the utilization of no-tillage system has been intensified. This system has the premises of preventing soil revolving and maintaining straw on its surface, hence several actions were taken regarding the nitrogen management in maize in order to develop strategies to improve nitrogen efficiency. According to Basso and Ceretta (2000) in no-tillage system, the availability of nitrogen in mineral form must be increased, especially when the preceding crop is a grass. For ground covering in winter period, oat stands out among the grasses, as affirmed by Amado (2002), this crop has the capability of absorbing and accumulating nitrogen in the aerial part. However, study conducted by the same author to evaluate oat decomposition noticed that only 20% of the N present in the plants was released during the first four weeks. This behavioris correlated to the C: N ratio and brings some benefits such as keeping plant residues longer on the ground, but causing lower nitrogen availability to the successor crop (Collier *et al*., 2011). In contrast, leguminous plants have a lower C: N ratio providing more nitrogen to the successor crop. Asdeclare Santos *et al*. (2010), it occurs because the legumes fix and add nitrogen to the system. As affirmed byAmado (2002), leguminous crops have the capacity to recycle and synchronize the slow release of nutrients, increasing the medium and long term nitrogen availability in the soil. Aita *et al*. (2001), studying the nitrogen release in winter legumes and cruciferous plants, concluded that 70% of the phytomass nitrogen was released in four weeks.

Aiming to improve nitrogen efficiency in no-tillage system, several researchers have adapted the nitrogen management in maize. Ceretta *et al*. (2002) evaluated the possibility of transferring part or the entire topdress nitrogen application to tillering oats or pre-sowing maize, and concluded that it is not the best strategy. This result confirms those obtained by Basso and Ceretta (2000) studying the application of N in maize in succession to different cover crops, reporting that it is safer to apply N at sowing and topdressing instead of applying it in pre-sowing. The increase of N availability for maize can occur through the use of cover crops as a nitrogen source (Aita *et al*., 2001; Aita and Giacomini, 2003). A study conducted by Heinrichs *et al*. (2001) demonstrates that the mix of black oat + vetch preceding the maize is able to replace urea application. In addition, the maize productivity after 90% vetch + 10% black oat intercropping was equivalent to the one verified after fallowing + 75 kg ha^{-1} of urea.

The recommendation of nitrogen fertilization in maize to Rio Grande do Sul state is based on the content of organic matter in the soil, preceding crop, and expected yield (CQFS-RS / SC, 2004). It is recommended for no-tillage system with a grass as preceding crop the dosage of 20 to 30 kg de N ha^{-1}, and 10 to 15 kg de N ha^{-1} when the preceding crop is a leguminous. The topdressing nitrogen is to be applied from four to six leaves stage. Currently, it is being adopted strategies in the nitrogen fertilization management seeking to improve nitrogen use and efficiency by maize plants in order to increase yields (Cruz *et al*., 2008). The greatest nitrogen efficiency is directly linked to the moment of application, because very early, and/or late applications will be poorly used by plants (Silva *et al*., 2005). According to Mengel and Barber (1974), the timing of application directly influences on nitrogen utilization by maize plants. This statement may be linked to the results found by Silva *et al*. (2005), reporting higher profitability index (PI) when nitrogen was applied half at sowing and the remaining at four to six leaves stage. However, results obtained by Raganin *et al*. (2010) and Arf *et al*. (2007) found no difference among nitrogen application timing.

Nitrogen may present different behavior among maize hybrids influencing the fertilization timing. Tests carried out by Fernandes *et al*. (2005) revealed similarity in nitrogen efficiency between two of three hybrids. In contrast, test conducted by Junior *et al*. (2008) observed differences between maize hybrids regarding on their efficiency of nitrogen utilization. Generally, the lack of nitrogen during initial growth stages reduces crop yield. As stated by Pereira *et al*. (2013), the emission of the fourth to sixth leaf occurs in this period, as well as the floral differentiation, and the end of the leaf differentiation phase. As affirms Magalhães *et al*. (2003), the differentiation of leaves and cobs begins during V5 stage, in V8 stage the number of rows and grains are set, and the phenological stages are defined by the apparent formation of a line in the leaf sheath insertion with the stem, dividing the stages in vegetative and reproductive.

Nitrogen sources for maize

There are many sources of nitrogenous fertilizer available, however, in Brazil the most used are urea and ammonium sulfate (Meira *et al*., 2009). The urea $[CO(NH_2)_2]$ has a high nitrogen concentration, high solubility, and lower cost/unit of nutrient, but higher volatilization (Calonego, 2012). The high volatilization index occurs because urea goes through hydrolysis when it gets in contact with the urease enzyme present in plant residues, producing ammonium carbonate $[(NH_4)_2CO_3]$ and releasing ammonia gas (NH_3) into the atmosphere (Costa, 2001). When urea is applied on suitable conditions, the hydrolysis reaction utilizes a H^+ ion, promoting the pH elevation (Singh and Nye, 1984; Kiehl, 1989). The elevation of the soil pH in no-tillage system promoted by acidity correctives applied to the surface layers is correlated with nitrogen losses. According to Tasca *et al*. (2011), the NH_3volatilization from urea increases alongside the nitrogen dose and the soil pH.

The ammonium sulfate $(NH_4)_2SO_4$, in addition of being a nitrogen supply, also provides sulfur, showing advantages when soils are deficient (Malavolta, 1989). The ammoniacal form fixes to the clay particles of the soil (Zhou *et al*., 2011), and at pH levels below seven, it does not evidence losses by ammoniacal nitrogen volatilization (N-NH_3) (Filho *et al*., 2010). Working with different nitrogen sources, Carmo *et al*., (2012) found no significant differences for the evaluated components among sources. Sangoi (2009) analyzed the effect of germination and initial development of maize plants by using different doses of urea and ammonium sulfate. It was observed that urea is detrimental to germination and initial development when compared to ammonium sulfate. Cabezasand Souza (2008) reported lower grain yield when urea was used as fertilizer compared to ammonium sulfate. On the other hand, Eurides *et al*. (2008) reported the lower cost of urea when compared to other nitrogen sources.

Generally, nitrogen is present in the soil solution on nitrate or ammonium forms, however the plants are physiologically responsive only to nitric nutrition (Duete *et al*., 2009). There were verified different responses among genetic materials regarding on nitrogen utilization efficiency (Cancellier *et al*., 2011).

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