NITROGEN NUTRITION OF YOUNG TRITICALE PLANTS GROWN UNDER ALUMINIUM STRESS

ADUBAÇÃO AZOTADA DE PLÂNTULAS DE TRITICALE SOB TOXICIDADE DE ALUMÍNIO

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ABSTRACT

Triticale has proven to be a tolerant crop in many places around the globe, under extreme climatic and edaphic conditions, particularly in Al-toxic soils. To suffice the growing food demand of the world population, one of the most important goal is the sustainable increase of cereal production avoiding the anthropogenic pollution, often air and groundwater contamination by volatilization and leaching of N compounds.

The effects of enhanced ammonia proportion in relation to nitrate nutrition in hydroponics, with or without Al, were investigated on the short-term growth of triticale plants. Three days old plants of the Al-tolerant genotype TTE 9203 were submitted to 0 or 370 μM Al and received different NO_3^-/ NH_4^+ ratios with the four proportions 15:1, 8:1, 3:1 and 1:1 (with fixed total N concentration at 3.2 mM in all treatments).

In relation to the corresponding control solutions, 370 μM Al induced important decreases in root length, ranging from 75.3 % to 47.3 %, reductions in fresh weight from 80 % to 60 % in roots, and from 89 % to 71 % in shoots, depending on the NO_3^-/ NH_4^+ ratio. A decrease in NO_3^- net uptake was shown by plants in the presence of Al. The most detrimental Al effect for young plant growth in nutrient solutions was observed with the 15:1 NO_3^-/ NH_4^+ ratio, which induced the highest reductions of length of the main root (52.7 % reduction relative to control) and of root biomass fresh weight (40.6 %) in four days of treatment. By the contrary, the plants grown in the 8:1 ratio solution with Al suffered the smallest reductions of root length (24.7 % in 370 μM Al treatment relative to control) and of root biomass fresh weight (20.3 %). Taken together the results indicate that NH_4^+ can alleviate Al toxicity in triticale and point out the ideal NO_3^-/ NH_4^+ proportion of 8:1 as the best for these young plants growth and N use efficiency, under acidic and Al toxic condition.

Some economical and ecological advantages of NH_4^+- N sources use in plant fertilization are discussed.

Keywords: Aluminium tolerance, ammonium, nitrate, triticale.

RESUMO

O triticale constitui uma cultura tolerante a condições climáticas e edáficas extremas, estando bem adaptado a solos ácidos, com níveis tóxicos de Al. A satisfação das necessidades cerealíferas crescentes da população mundial é o desafio fundamental da

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agricultura sustentável, evitando a poluição antropogénica, frequentemente por compostos azotados contaminantes de aquíferos e da atmosfera.

Foi estudado o enriquecimento amoniacal na adubação azotada em triticale, na presença de alumínio tóxico. As plantas com três dias do genótipo tolerante ao Al TTE 9203 foram sujeitas à presença de 0 ou 370 μM Al e receberam as quatro proporções de NO_3^-/ NH_4^+ seguintes 15:1, 8:1, 3:1 e 1:1, tendo sido fixada a concentração total de 3,2 mM N nas diferentes modalidades em solução nutritiva.

Relativamente aos correspondentes controlos, os tratamentos com 370 μM Al induziram reduções importantes no crescimento, que variaram significativamente entre 75,3% e 47,3% do comprimento radicular, 80% a 60% da biomassa radicular e 89% a 71% da biomassa aérea, segundo a proporção de NO_3^-/ NH_4^+ presente na solução nutritiva. Efectivamente, o Al tóxico reduziu muito significativamente a absorção de nitrato pelas plantas. O efeito negativo do Al nas jovens plantas foi mais evidente na modalidade com a proporção 15:1 NO_3^-/ NH_4^+, tendo a presença de Al provocado as reduções de 52,7 % do alongamento da radícula mais longa e de 40,6 % da biomassa fresca de raiz produzida em quatro dias de tratamento. Pelo contrário, na modalidade com a proporção 8:1 NO_3^-/ NH_4^+, as raízes das plantas sofreram as reduções mínimas daqueles parâmetros relativamente ao correspondente controlo (24,7 % e 20,3 %, respectivamente). No seu conjunto, os resultados obtidos indicaram que o NH_4^+ pode aliviar a toxicidade do Al em triticale, na fase vegetativa precoce que constitui a mais susceptível à toxicidade daquele metal. Sob stress por Al, a proporção de NO_3^-/ NH_4^+ ideal para o crescimento das plântulas e maior eficiência de uso do azoto será aproximadamente de 8:1.

São discutidos aspectos económicos e ecológicos da utilização de fontes alternativas de azoto amoniacal na fertilização da cultura.

Palavras-chave: Amónia, nitrato, tolerância ao alumínio, triticale.

INTRODUCTION

Adapted plant genotypes may give economic returns and are specially interesting in developing countries, under stress or suboptimal conditions (Bozzini, 1991; Ceccarelli, 1996). Triticale (x*Triticosecale* Wittm.), which is an amphydiploid of the crossing between wheat and rye, often includes cultivars characterised by resistance to diseases, exhibiting good performance under marginal climatic and edaphic conditions, particularly high grain yield in poor acid soils showing high saturation with Al toxic ions (Aniol, 1996). It constitutes a nutritious staple food for human consumption (Bozzini, 1991). The sustainable increase of agricultural production is the primordial goal to suffice the growing food demand of the world population (Yusuf *et al*., 2003). This includes to increase the efficient use of nutrients by plants and also to reduce the environmental pollution associated, which might be achieved in tropical agro forestry systems (Preto, 1983).

N is available on Earth mainly as the chemically stable diatomic-N (N_2). But there are many reactive N species which concentrations are increasing in the environment (Fields, 2004), as the N oxides (N_2O and NO_x), volatile ammonium (NH_3), ionic-N forms (NO_3^- , NO_2^- and NH_4^+) and biological-N forms (Jones & Willett, 2006). However, in conventional agriculture, undoubtedly mineral NO_3^- and NH_4^+ are the most important N-forms for non-*Leguminosae* plant nutrition (Glass *et al*., 2002). Nitrogen is the most limiting nutrient for plant production, but the incorrect fertilizers applications induce atmospheric and groundwater contamination by this macronutrient. Nitrogen losses may occur by leaching and surface runoff, by volatilisation, and by biological and chemical transformations. Soil N losses are proportional to the excess of supply relative to plants needs (Shaviv, 2000). The nitrate and the toxic nitrite N-forms are most susceptible to leaching and runoff (Silgram & Shepherd, 1999). It must be emphasized that fertiliser production, transport, distribution, and, specially in what concerns NO_3^--N, the mineral nutrient availability to fill up plant stages needs along the cultural cycle are expensive. The N-fertilizers effective uptake by roots and N allocation in the plant is progressive in time, so often the profits of a single or few NO_3^--N applications are exiguous and the effects on environment are negative, especially in many tropical region soils (Rosenzweig & Hillel, 2000).

The optimal NO_3^-/ NH_4^+ ratio applied in N-fertilisation depends on many factors, as the total N-availability (in both mineral and organic forms), plant species preferences, edaphic and climatic conditions (specially water regime and temperature), and agronomic management. Under acidic conditions, enhanced ammonia (NH_4^+) nutrition favoured bigger earnings of wheat (Fleming, 1983), N assimilation in the seeds of barley (Soares & Lewis, 1986) and yield of maize (Alexander *et al*., 1991). The Spring barley yields were similar with applications of NH_4NO_3 or $(NH_4)_2SO4$ (with proportions 1:1 or 0:1 of NO_3^-/ NH_4^+ ratio) in acidic soils, with and without liming (Malhi *et al*., 1988). However, in nutrient solutions, these authors found that the barley plants produced more biomass when they were supplied with only NO_3^--N compared to only NH_4^+–N. In another hydroponic experiment, Vaast *et al*. (1998) found that at 20º C and with 1 mM NH_4^+ (as sole mineral N sources), the N uptake of coffee, *Coffea arabica* L. cv. Catuai Vermelho, doubled between pH 2.75 and 7.25. The NO_3^--N uptake was more reduced than that of NH_4^+-N at temperatures below 16º C and in anaerobic conditions.

Acid conditions shift the chemical stability of the ubiquitous Al compounds in soils and dramatically rise the concentration of soluble forms of Al. Blamey *et al*. (1983) considered that the activities of $AlOH^{2+}$, $Al(OH)_3^0$, and $AlSO_4^+$ were low in solution and suggested that generally the Al^{3+} and $Al(OH)_2^+$ ions might be the predominant species responsible for decreased root elongation. The Al concentration as low as 37 to 74 mM in soil solutions can damage the plant roots and disturb the metabolic systems, e.g., provoke impaired cation nutrients uptake (Roy *et al*., 1988). Moreover, monomeric and polymeric Al forms might induce very detrimental rhizotoxic effects (Blamey *et al*., 1983; Andrade *et al*., 1996).

After 3-4 days of treatment with 74 m*M* Al in nutrient solution, initially at pH 4.5 and containing 4 mM N at 12.4:1 of NO_3^-/ NH_4^+ ratio, the differential Al susceptibility of wheat cultivars (*Triticum aestivum* L.) was evident by visual symptoms in the roots (Taylor & Foy, 1985). Al tolerance negatively correlated with the high rate of NH_4^+ depletion and it was positively correlated with the moderate rate of NO_3^- depletion and, consequent, gradual rise of the pH root media. The authors concluded that cultivars preference for NO_3^--N, and the corresponding raise in the pH of the solution, significantly contributed to Al tolerance of those genotypes. Nevertheless, varieties of bread wheat grown hydroponically with mixed N-forms (1mM NO_3^- plus 0.3 mM NH_4^+) accumulated less Al in the roots compared to those grown only with 1.3 mM NO_3^--N (Andrade *et al*., 1996).

The nitrate reductase and nitrite reductase activities of roots and shoots of pearl millet (*Pennisetum typhoids L.* ou *Pennisetum glaucum* (L.) R. Br.) seedlings declined, as well the NO_3^--N, soluble protein and chlorophyl contents in tissues, in Al-treated plants (Albassam, 2001). The presence of Al in the root media reduced uptake and reduction of nitrate, particularly in the more susceptible cultivar of rice (Justino *et al*., 2006).

The objectives of this study were to investigate the effects of different proportions of NO_3^-/ NH_4^+ in nutrient solutions with 3.2 mM N, added with 0 or 370 mM Al, on the early growth and N net uptake of triticale. The nutrient solution method used in a short-term experiment, under strictly controlled conditions, pretends to contribute to a better adjustment of the nitrogen fertilisation needs of Al-tolerant triticale germplasm grown under very acidic and Al-toxic conditions in sustainable agriculture.

MATERIALS AND METHODS

Seeds of the genetic line of triticale (x*Triticosecale* Wittm.; amphydiploid hybrid from Family *Poacea*) TTE 9203 from Estação Nacional de Melhoramento de Plantas (Elvas), were surface sterilised with 5% sodium hypochlorite for eight minutes, washed with distilled water and were germinated over water imbibed cotton covered with filter paper, at 20 ± 3º C. Seedlings having 15 ± 5 mm root length were selected and placed floating on well aerated solution, in a controlled water bath at 25º ± 1ºC, with a relative humidity of about 60% and irradiance intensity of 150 mmoles Q $m^{-2}s^{-1}$. Seedlings were grown for three days in a basal nutrient solution (modified from Camargo & Oliveira, 1981). The pH of the basal solution was set at 4.1 ± 0.1 and adjusted daily with 0.1 N HCl. After complete induction of nitrate and ammonium transport systems (Glass *et al*., 2002), the plants were transferred to different treatment solutions for four days. After treatment, the plants were transferred to the corresponding control treatment solution (without Al), added by 125 µM KH_2PO_4 and all micronutrients, and kept at constant pH 4.1 ± 0.1, for a recovery period of three days. The macronutrients composition of basal and ten treatment solutions is described in Table 1. The solutions were supplemented with micronutrients as follows: 7.5 µM NaCl; 2.5 µM H3BO3; 0.5 µM MnSO4.4H2O; 75 nM CuSO4; 200 nM $ZnSO_4$; 25 nM Na2MoO4.2H2O; and 2.5 µM $FeCl_3$.6H2O (Camargo & Oliveira, 1981). The ten treatment solutions differed mainly in NO_3^-/ NH_4^+ ratios (15:1, 8:1, 3:1 and 1:1) and were added or not by 370 µM Al, as $Al_2(SO_4)_3$.16 H_2O - A_0 to E_0 were control solutions without Al and treatment solutions A1 to E1 had Al added. All treatment solutions were set initially at pH 4.1 ± 0.1, they were supplemented with the micronutrients, except for iron, and were unprovided of phosphorus, to avoid Al precipitation. The pH was measured periodically.

The roots were measured with accuracy of 0.5 mm. Separately, shoots and roots were weighted, at the end of each growth period (after three, seven and ten days of assay). The relative reduction (RR) of root elongation or biomass increment of plants grown in a treatment solution was defined as the difference of the unit minus the ratio between the values obtained in Al treatment (x_1) and those in the corresponding control (x_0), expressed as percentage, i.e., $RR = (1 - x_1 / x_0)\ 100$. The net acquisition of NO_3^- and NH_4^+ from solutions was calculated by the depletion of these ions determined spectrophotometrically in solutions in the beginning and at end of each growing period, respectively, according to procedures adapted from Cataldo and collaborators (1975) and Solozano (1969).

There were two recipients with 90 plants in 3.8 litres of aerated basal solution. During the treatment and recovery periods there were, respectively, 12 and 6 plants, in pots with 300 ml of aerated solutions. The experiment included two factors, ratio of N mineral forms and Al doses, and three blocks (which had ten recipients completely randomised in each). ANOVA was applied and means compared by the Student test.

RESULTS

Root and shoot growth

Table 2 shows the daily absolute increment of root length during four days of treatment with 0 or 370 µM Al. The increment of root length of young plants was not differently affected by N-form nutrition without Al, but under Al stress there were significant differences. So, the highest reduction relative to the corresponding control (52.7%) was observed in the solution with the 15:1 NO_3^-/ NH_4^+ ratio (A1). The Al presence in the solution with 1:1 NO_3^-/ NH_4^+ ratio (E1) induced a great reduction of daily root length increment also (44.2 %). Both solutions with 3:1 NO_3^-/ NH_4^+ ratio (C1 and D1) showed about one third of reduction relative to control and the lowest reduction (24.7%) was fund in the proportion 8:1 (B1). Unexpectedly, during the last three days of recovery, the plants grown with 3:1 (D0) and 1:1 (E0) NO_3^-/NH_4^+ ratio evidenced significantly lower root elongation than the other control plants. The recovery of root elongation was reduced due to Al stress in all modalities.

In the control solutions, the shoot biomass production with the 15:1 NO_3^-/NH_4^+ ratio (A0) was higher than the observed for the 3:1 (C0 and D0) and 1:1 (E0) proportions. And the production of root biomass was significantly smaller with the 1:1 NO_3^-/NH_4^+ ratio (E0) compared with other control solutions (Figure 1). After four days of treatment, the production of shoot biomass was not significantly affected by Al presence. But Al presence affected negatively the root fresh weight in all modalities (Figure 1). The reductions relative to the corresponding controls were by decreasing order 40.6% (A1), 35.8% (E1), 24.2% (C1), 21.8% (D1), and 20.3% (B1).

The ratio between the root and shoot fresh biomass of seven days old plants grown in solution with 15:1 NO_3^-/NH_4^+ ratio decreased about 20% due to Al stress (from 0.655 to 0.523), whereas the other proportions induced insignificant changes of this parameter.

NO_3^- and NH_4^+ net acquisition

In control solution having the proportion 15:1 (A_0), the NO_3^- depletion was almost complete (Table 3). Al stress reduced NO_3^- net uptake, but not NH_4^+ net acquisition by plants. The NO_3^- net acquisition was also limited by the higher NH_4^+ net acquisition (Table 3). The control plants grown with the proportions 3:1 and 1:1 of NO_3^-/NH_4^+ ratio depleted NO_3^-, respectively, 60% (C_0 or D_0) and 68.7% (E_0) of the availability in solutions. The Al treated plants depleted only 47.7% (C_1 or D_1) and 48.9% (E_1) of the NO_3^- available in solutions in accordance with others (Antunes, 1998; Jerzykiewiez, 2001).

The ammonia depletion observed was almost complete in solutions with 15:1, 8:1 and 3:1 proportions (Table 3). In solutions with the 1:1 proportion (E0 and E1 with concentration of 1.6 mM NH_4^+-N), the plants had 10 mmoles NH_4^+-N plant^{-1} day^{-1} available inside each pot. The net acquisition of NH_4^+-N exceeded that of NO_3^--N, with and without Al stress. So, the ratio between NO_3^- and NH_4^+ uptaken by plants was lower than the unit (0.86 for E0 and 0.73 for E1).

The nitrogen use efficiency (NUE) corresponding to solution with 8:1 ratio were the highest. Whereas, the 1:1 solution ratio induced significantly lower NUE than the other proportions of NO_3^-/NH_4^+, both with and without Al (Table 3). In all N-forms proportions, NUE was slightly affected by Al stress (Table 3).

Consistently, the presence of Al induced higher net acquisition of N per unit of root fresh weight, particularly in solutions with the proportions 15:1 and 1:1 (Figure 2). The NH_4^+-N uptake per unit of root fresh weight in the proportion 1:1 were the highest (see E0 and E1, in Figure 2), which might have induced NH_4^+ accumulation and toxicity in plant tissues.

Solution pH variation

In the first two days during the treatment period decreases of the pH values of 0.3 to 0.6 were observed in all the ten modalities. Thereafter, differential pH rises were observed in solutions containing different N-forms proportions or Al treatments. The control solutions with the proportions 15:1 and 8:1 changed to pH 6.0 and 5.8, respectively, while the other controls reached initial pH values of 4.0 ± 0.1, at the end of treatment period. In the presence of 370 μM Al, the solutions containing the proportions 15:1 and 8:1 reached also the initial pH values, but in those containing 3:1 and 1:1 null increases of pH could be detected in the last two days of treatment.

DISCUSSION

In the present experiment, the root length and root fresh biomass in 370 μM Al treatments showed more important reductions relative to controls in the plants grown in the root media with the highest NO_3^-/ NH_4^+ ratio (15:1), followed by the treatment with the highest NH_4^+ concentration (the 1:1 NO_3^-/ NH_4^+ ratio, containing 1.6 mM NH_4^+). Therefore, the Al toxicity to roots was smaller in the solutions containing the proportions 3:1 and, principally, 8:1 of NO_3^-/ NH_4^+ ratio. The effect of low ionic strength that enhanced the free Al ions activity observed by Pintro & Taylor (2004) could not explain the greater Al toxicity observed in our solutions A_1 and E_1 (see RR in Al treatment – Table 2). The electric conductivity of the control and 370 μM Al nutrient solutions rose gradually from A to E (as in Table 1), respectively, from 656 to 818 μS cm^{-1} and from 720 to 920 μS cm^{-1}.

Previous results (Antunes, 1998) obtained with the Al-sensitive cv. Beagle treated with the proportions of 15:1 or 8:1 of NO_3^-/ NH_4^+ ratio, and 0 or 185 μM Al, also led to the conclusion that the young plants, once submitted to Al stress, grow better with enhanced ammonia nutrition with evidence for cation amelioration effect of NH_4^+ over Al toxic ions (Klotz & Horst, 1988).

In an experiment with a basic diluted nutrient solution, containing 2,4 mM NO_3^- and 0,16 mM NH_4^+, with the pH fixed at 6,0 and 4,0 and, in this last modality with 0 or 185 μM Al added, there were very significant differences statistically (P= 0.05) among the three treatments for the growth triticale and wheat cultivars (Antunes, 1998). The seven days old plants of Beagle and principally Anza (*Triticum aestivum*) were H_3O^+- and Al-susceptible (Table 4) by the criteria of the relative elongation rate (RER) of the main root. The root elongation of young plants is considered one of the most sensitive indicators for plant proton and metals toxicities, in short term experiments. The roots elongation and their ability to explore water and nutrients in deeper soil layers are decisive to plant development, particularly to rain fed cereal crops. Potentially, the maintenance of high RER of plant roots in very acid root media added with Al toxic concentration might allow improved nutrition, long-term plant health and growth, and higher grain yields under acidic and Al-toxic soil conditions of the selected germplasm (Camargo & Oliveira, 1981; Antunes, 1998).

In this triticale experiment, the pH reduction observed and the following pH increase were consistent with uptake from the nutrient solutions of NH_4^+ (phase I – external acidification) and of NO_3^- (phase II – external alkalisation), as stated by Taylor and Foy (1985). Indeed, the pH changes could be explained, at least partially (as other ions were also involved), by the N-ions influx by the roots. The passive influx of NH_4^+ by root cells is permitted by the efflux activity of proton pumps and NH_4^+ accumulation in tissues could be cytotoxic (Cruz, 1994). On the other hand, the uptake of NH_4^+ might inhibit the NO_3^- uptake, possibly because of the plasmalemma electrochemical potential disruption, which prevents the active symport of NO_3^- and H^+ (Cruz, 1994). The measured NO_3^--N uptaken by the plants grown in the proportions 3:1 and, principally, 1:1, under Al stress, were the lowest, which might had turned unfeasible the rising of the low pH found the in these pots, during the last two days of treatment. Other authors suggested that the hydrolysis of NH_4^+-N could directly increase the concentration of H_3O^+ and indirectly raise the concentration of the Al soluble forms that induce higher rhizotoxicity (Andrade *et al.*, 1996). This is a possibility, in the present conditions, at the highest NH_4^+ concentration (solution E_1, with 1:1 ratio) with formation of H_3O^+ and free NH_3, which would be phytotoxic (Cruz, 1994). Also, high NH_4^+ concentration could induce antagonism to other nutrient cations uptake by roots (Roy *et al.*, 1988).

In all solutions, iron and phosphate were totally omitted during the treatment period to avoid the rapid precipitation of Al (Camargo & Oliveira, 1981). The presence the of highest sulphate concentration (900 μM SO_4^{2-}) in solutions C_1 and E_1 could have induced Al chemical speciation with reduced rhizotoxicity, but this effect was not observed.

Although the enhanced sulphate could partially explain the slightly better recovery of the plants grown in solution C over the plants from D (both containing 3:1 NO_3^-/ NH_4^+ ratio).

The solutions D (with 3:1 NO_3^-/ NH_4^+ ratio) and E (1:1) had higher concentration of Cl^- ions (respectively, 1415 and 2215 μM) in comparison with the other three solutions A (15 μM), B (165 μM) and C (615 μM). Britto and collaborators (2004) considered increased NaCl sensitivity and tissue Cl^- accumulation under NH_4^+ nutrition. The recovery of root elongation of seven days old plants grown in control treatments D0 and E0 possibility indicates that the chloride might have exerted some interactions with the NO_3^- and NH_4^+ ions uptaken by the roots, in spite of the fact that the potentially toxic external level (100 mM Cl^-) was not reached.

Many authors found reduced toxicity of Al organic complexes with carboxylic acids and phenols (Roy *et al*., 1988; Hue & Licudine, 1999), as well as of Al-sulphates and Al-phosphates in solutions (Mora *et al.*, 2005). In fact, the manure, like other organic sludges and slurries, used as N source for plant nutrition (Yusuf *et al*., 2003), constitute an important valorisation of effluents of animal production. The organic fertilizers exhibit variable composition and unsteady NO_3^-/ NO_3^--N ratio (Van Kessell *et al*., 2000), due to their origin, storage and environmental conditions. They are also enriched or contain important amounts of compounds easily convertible into P_2O_5, K_2O, and sulphate, that are available to plants. The surface applications of chicken manure and sewage sludge were superior to lime in increasing the soil pH and exchangeable Ca, and reducing Al toxicity, especially at lower soil depths (Hue & Licudine, 1999).

In soils with abundant water disposal (from rain or irrigation), ammonia is diluted and its toxicity to plants becomes limited or inexistent. On the other hand, cations in soil solution are less susceptible than anions of being dragged superficially or leached downward, as, the cation exchange pool of soil is adsorbed by negatively charged radicals of soil particles (Fernando *et al.*, 2005). The N remaining in the top 60 cm of soils after fall-applied fertilisers on wheat were 9-20% for $Ca(NO_3)_2$ or 30-59% for $(NH_4)_2SO_4$ (Huber *et al*., 1980). In Spring time, the greatest growths and biggest crop yields were obtained with $(NH_4)_2SO_4$, especially when addicted with specific nitrification inhibitor (Huber *et al*., 1980). Naturally, under acidic or reducing conditions the nitrification of NH_4^+-N is low. To slow NO_3^--N release, a localised system of ammonia fertilisation, like band application, nests or large granules might be used, always preventing the risks of root or shoot direct damage (Shaviv, 2000). Alternatively, synchronisation of mixed N application with plant demand might be achieved by adding salts (stabilising and complementing the organic fertilisers with mineral nutrients) to the sludge applied directly on the soil to reduce NH_3 volatilisation from and toxicity effects on the installed crop.

The amount and long distance transport of free reactive N species (like ammoniacal and nitric fertilizers not uptaken by plant roots) must be reduced in the environment. The peaks of NO_3^--N and NH_4^+-N uptake by the plant occurred in late afternoon, at the end of daylight hours (Glass *et al*., 2002). However, the night application of NH_4^+-N sources (after sunset and before sun rise), when the air temperature falls, under favourable weather (absence of wind) and moderate wet soil conditions, might be advisable to reduce volatilisation. Since the initial N losses by volatilisation are high in field crops, the recommended rates of organic NH_4^+-N sources applied to crops might be reduced. An effective agronomic useful use of N applied was achieved by intercropping rye cover with maize (Rasse *et al.*, 2000). The circumscription of field plots with living borders, particularly of arboreal type (Preto, 1983), limits the long distance transport and the acidic deposition of nitrogen compounds else where, and reduces atmospheric acid pollution. This practice avoids the reduction of spontaneous plant diversity, which is susceptible to Al excess (Roem *et al*., 2002), specially the vanishing of slow growing plant species adapted to low N environment. Also, the deeper root systems permit to recycle N in percolating waters. The riverain vegetation might contribute to the depletion of soluble N lost downstream. The reduction of environmental pollution, and the increase or maintenance of NUE by applications of mineral, organic or bio-amended N-sources might be conjugated by the observation of proper agricultural techniques (Silgram & Shepherd, 1999; Kouyaté *et al*., 2000; Yusuf *et al*., 2003). Social and economical profits, and ecological conservation, by the maintenance of natural diversity of microorganisms, flora and fauna, might be satisfied in traditional/ or adapted integrated agro-ecosystem management (Preto, 1983).

Moreover, the plant breeding methods used to produce better cultivars might allow the conservation of the population genetic variability and plasticity (Ceccareli, 1996; Antunes, 1998), which are necessary to face crop constraints, like the soil vertical and horizontal heterogeneity, the seasonal and regional abiotic threats, the unpredictable climate change, and the old and emergent plant diseases and pests.

In many ecosystems N is the major limi-ting factor of productivity. The improper use of N fertilizers in agriculture can disrupt the global N cycle, and consequences might be dramatic, as the substancial acidification of soils, internal waters, and oceans, photochemical smog and increase of greenhouse gases (like N_2O) in atmosphere. More than ever, the greatest attention applied to critical reductions of pollutants is needed to reach ecological equilibrium in this unique *round earth* and, also, an effective holistic perception of mankind as an important *part of* Nature (Rosen, 1970).

CONCLUSIONS

The following conclusions arise from the results: (1) the plants submitted to Al stress reached higher root length increment in solutions containing 8:1 and 3:1 (respectively with 0.350 and 0.800 mM NH_4^+, and 3.2 mM total-N available in nutrient solution) than in the solutions with 15:1 and 1:1 NO_3^-/ NH_4^+ ratio; (2) the presence of very toxic Al concentration affected less the biomass production in solutions having 8:1 and 3:1 NO_3^-/ NH_4^+ ratio; (3) the root medium pH was more acidic with enhanced NH_4^+-N nutrition, but under the present conditions, the NH_4^+ ions induced an amelioration effect of Al ions toxicity to this triticale genotype; (4) the presence of Al in solutions reduces significantly the nitrate acquisition per plant; (5) the highest plant N use efficiency also indicates the lowest Al toxicity in solution with 8:1 NO_3^-/ NH_4^+ ratio. Present results show that triticale TTE 9203 evidenced for good Al tolerance and, at early plant stages, it might benefit from mixed N inorganic fertilization in acid soils with Al toxic levels.

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Table 1 – Concentration of macronutrients, expressed in µM, in the basic and the ten treatment solutions, which were supplemented with micronutrients and had 0 µM (A_0, B_0, C_0, D_0, and E_0) or 370 µM Aluminium (A_1, B_1, C_1, D_1, and E_1).

Compound	Basic solution	Treatment solutions (with 0 or 370 µM Al)				
		A0/A1	B0/B1	C0/C1	D0/D1	E0/E1
$Ca(NO_3)_2$	1000	1000	1000	1000	300	800
KNO_3	850	1000	850	400	1000	-
NH_4NO_3	-	-	-	-	800	-
$(NH_4)_2SO_4$	175	100	175	400	-	400
NH_4Cl	-	-	-	-	-	800
$CaCl_2$	-				700	200
KCl	150	-	150	600	-	1000
KH_2PO_4	125	-	-	-	-	-
$MgSO_4.7H_2O$	500	500	500	500	500	500
NO_3^-/NH_4^+	8:1	15:1	8:1	3:1	3:1	1:1

Table 2 – Increment of root growth (mm day^{-1}) and reduction relative to control (RR, %) of triticale TTE 9203 plants, during four days of treatment in solutions with different NO_3^-/NH_4^+ ratios (as for Table 1), which were supplemented with 0 µM (A0, B0, C0, D0, and E0) or 370 µM Aluminium (A1, B1, C1, D1, and E1), and during three days of recovery in the respective control solution. Mean values ± standard error (n=3).

Treatment Solutions		Increment of root length Treatment	Increment of root length Recover	Relative Reduction Treatment	Relative Reduction Recover
15:1	A0	18.4 ± 0.6	15.9 ± 1.7		
	A1	8.7 ± 0.6	11.7 ± 1.2	52.7	26.4
8:1	B0	17.4 ± 1.3	15.7 ± 1.6		
	B1	13.1 ± 0.3	10.7 ± 0.3	24.7	31.8
3:1	C0	16.2 ± 2.0	14.1 ± 1.9		
	C1	11.2 ± 0.5	8.4 ± 0.6	30.9	40.4
3:1	D0	16.1 ± 0.9	11.7 ± 0.5		
	D1	11.1 ± 0.7	7.6 ± 0.4	31.1	35
1:1	E0	17.2 ± 2.8	12.4 ± 1.4		
	E1	9.6 ± 0.8	8.6 ± 0.8	44.2	30.6

Table 3 – Nitrogen net acquisition during the four days of the treatment period (μmoles N plant^{-1} day^{-1}), ratio between NO_3^- and NH_4^+ uptaken by plants, and N use efficiency (N U E, g fresh weight. mmole^{-1} N). Mean values ± standard error (n=3).

Treatment Solution		Nitrogen acquisition (μmoles N plant^{-1} day^{-1}) Nitrate	Ammonium	Ratio NO_3^-/ NH_4^+ uptaken	N U E (g Fresh Wt. mmole N^{-1})
15:1	A0	17.53 ± 0.42	1.25 ± 0.00	14.02 ± 0.34	4.51 ± 0.49
	A1	13.65 ± 0.62	1.23 ± 0.02	11.09 ± 0.64	4.36 ± 0.45
8:1	B0	12.04 ± 1.10	2.15 ± 0.02	5.60 ± 0.56	5.10 ± 0.51
	B1	10.37 ± 1.74	2.19 ± 0.06	4.74 ± 0.66	4.79 ± 0.25
3:1	C0	8.99 ± 0.29	4.98 ± 0.02	1.81 ± 0.05	4.42 ± 0.36
	C1	7.13 ± 0.16	4.75 ± 0.16	1.50 ± 0.06	4.19 ± 0.32
3:1	D0	9.00 ± 0.19	4.83 ± 0.24	1.86 ± 0.11	4.54 ± 0.07
	D1	7.19 ± 0.12	4.78 ± 0.12	1.50 ± 0.06	4.43 ± 0.26
1:1	E0	6.87 ± 0.43	8.00 ± 0.12	0.86 ± 0.04	3.68 ± 0.32
	E1	4.89 ± 0.60	6.72 ± 1.73	0.73 ± 0.16	3.18 ± 0.23

Table 4 –Relative Elongation Rate (mm mm^{-1} day^{-1}) of roots of four triticale and one wheat genotypes during four days of treatment in solutions with pH 6.0 or 4.0 with 0 or 185 μM Aluminium (n=3). Between brackets are presented the percentage values relative to the treatment with pH 4 for each genotype.

Genotype	pH 6	pH 4	pH 4 + Al
Arabian	0.249 [126]	0.198 [100]	0.196 [99]
Borba	0.254 [134]	0.190 [100]	0.185 [97]
TTE 9203	0.295 [137]	0.215 [100]	0.207 [96]
Beagle	0.364 [155]	0.235 [100]	0.159 [68]
Anza	0.287 [151]	0.190 [100]	0.009 [5]

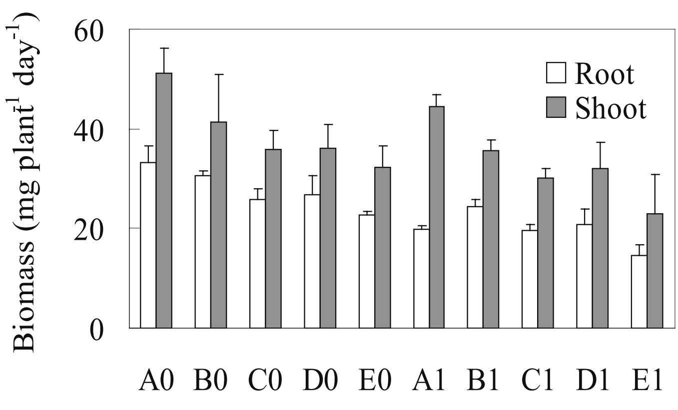

Figure 1 – Daily production of root and shoot fresh weight by triticale plants [mg fr wt (plant day)$^{-1}$] grown in treatment solutions (as for Table 1), during four days. Means (n=3) ± standard error.

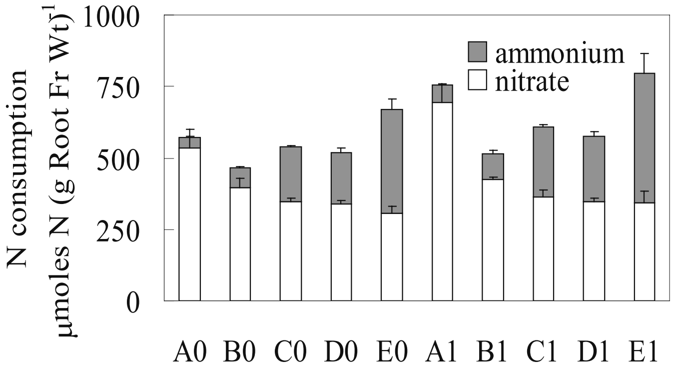

Figure 2 – Nitrate and ammonium net acquisition from different solutions (as for Table 1) per unit of root fresh weight by triticale plants [μmoles N (g root fr wt)$^{-1}$] submitted to treatments during four days. Means (n=3) ± standard error.