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EFEITO DE ALUMÍNIO E DE FÓSFORO EM PARÂMETROS MORFOFISIOLÓGICOS E BIOQUÍMICOS DE TRIGO

DISSERTAÇÃO DE MESTRADO

Gabriel Schaich

Santa Maria, RS, Brasil
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EFEITO DE ALUMÍNIO E DE FÓSFORO EM PARÂMETROS MORFOFISIOLÓGICOS E BIOQUÍMICOS DE TRIGO

Gabriel Schaich

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Orientador: Fernando Teixeira Nicoloso

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EFEITO DE ALUMÍNIO E DE FÓSFORO EM PARÂMETROS MORFOFISIOLÓGICOS E BIOQUÍMICOS DE TRIGO

elaborada por
Gabriel Schaich

Como requisito parcial para obtenção do grau de
Mestre em Agronomia

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Santa Maria, 28 de fevereiro de 2014.

“Aos meus pais Gilson e Maria
e ao meu amigo Bruno Kräulich (in memoriam)”.

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“Non ducor, duco.”

RESUMO GERAL

Dissertação de Mestrado
Programa de Pós-Graduação em Agronomia
Universidade Federal de Santa Maria

EFEITO DO ALUMÍNIO E FÓSFORO EM PARÂMETROS MORFOFISIOLÓGICOS E BIOQUÍMICOS DE TRIGO

Autora: Gabriel Schaich
Orientador: Fernando Teixeira Nicoloso
Data e Local da Defesa: Santa Maria, 28 de fevereiro de 2014.

A cultura do trigo (*Triticum aestivum L.*) é uma importante alternativa utilizada na rotação de culturas do sistema de plantio direto, servindo como opção de cultivo no inverno e contribuindo para a manutenção da cobertura do solo com resíduos culturais. Em virtude de aplicações superficiais de fertilizantes e corretivos adotadas neste sistema, espécies reativas de alumínio podem coexistir com a baixa disponibilidade de fósforo, principalmente em camadas mais profundas do solo, limitando o crescimento das raízes em profundidade e o pleno desenvolvimento da cultura. O presente trabalho busca identificar respostas de cultivares de trigo à exposição aos elementos alumínio (Al) e fósforo (P), classificando os genótipos quanto sua sensibilidade e tolerância ao Al por meio de avaliações da morfologia, anatomia e alterações cromossômicas (Capítulo I), o efeito no dimensionamento amostral em estudos desta natureza (Capítulo II), bem como sua eficiência de uso e resposta ao P considerando parâmetros de crescimento e bioquímico por meio de análise multivariada de componentes principais (Capítulo III). Foi possível identificar notáveis diferenças anatômicas entre a cultivar tolerante (IAC 5) e sensível (Galego Rapado) ao Al. Dentre as adaptações ao metal, estão o aumento do espaço intercelular das folhas e sua densidade estomática, em condições de maior toxicidade do Al, com a formação de agrupamentos estomáticos na cultivar Galego Rapado. Tais diferenças contribuíram para a superioridade do efeito cultivar quando comparado ao efeito dose na determinação do tamanho de amostra dos materiais. Recomenda-se utilizar pelo menos 9 e 74 plantas para avaliação de altura e Cde cultivares de trigo, em estudos de tolerância ao Al, respectivamente, considerando ainda que estimativas com precisão inferior a 10%, de modo geral, são impraticáveis devido o elevado número de plantas necessário. Por fim, foi encontrado um consistente padrão de resposta da atividade da enzima fosfatase ácida da parte aérea com índices de eficiência de uso e resposta ao P, com uma menor atividade em materiais eficientes.

Palavras-chave: *Triticum aestivum L.*; Adaptações morfológicas; Eficiência de uso e resposta ao fósforo; Fosfatase ácida.

GENERAL ABSTRACT

M. S. Dissertation
Post-Graduate Program in Agronomy
Federal University of Santa Maria

ALUMINUM AND PHOSPHORUS EFFECTS ON MORPHOPHYSIOLOGICAL BIOCHEMICAL TRAITS OF WHEAT

Author: Gabriel Schaich
Advisor: Fernando Teixeira Nicoloso
Place and date of defense: Santa Maria, February 28th, 2014.

Wheat (*Triticum aestivum* L.) is an important crop used in crop rotation on no-tillage system, serving as an option for cultivation in winter and contributing to the maintenance of mulch. Due to surface applications of both fertilizers and lime on no-till soils, reactive aluminum species can coexist with low phosphorus availability, especially in deeper soil layers, limiting root growth in depth and by consequence the development of the culture. This work aimed to identify responses of wheat cultivars to aluminum (Al) and phosphorus (P) exposure, classifying the genotypes according to their sensitivity and tolerance to Al through assessments of morphology, anatomy and chromosomal abnormalities (Chapter I), the effect of such differences in sample size dimension (Chapter II) and the use efficiency of P, considering growth and biochemical parameters by multivariate principal component analysis (Chapter III). It was possible to identify notable anatomical differences between the Al-tolerant (IAC 5) and Al-sensitive (Galego Shave) cultivar. Among the array of adaptations to the metal, an increased intercellular space of leaves and their stomatal density were observed in conditions of greater toxicity of Al, with the formation of stomatal clusters in Galego Rapado. These differences contribute to the superiority of cultivar effect when compared to the dose effect in determining the sample size, which is recommended to be used at least 9 and 74 plants for evaluation of height and root length in wheat cultivars used in Al tolerance studies, respectively, also considering that accuracy estimative lower than 10 %, in general, are impractical due to the great number of plants needed. Finally, we found a consistent response pattern of acid phosphatase activity on shoot tissue with use and response efficiency of P, with a lower activity in efficient genotypes.

Keywords: *Triticum aestivum* L.; Adaptations traits; Phosphorus use and response efficiency; Acid phosphatase.

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SUMÁRIO

INTRODUÇÃO GERAL

O trigo (*Triticum aestivum* L.*)* é originário do sudeste asiático e ocupa o segundo lugar em produção global de alimentos (UNITED STATES DEPARTAMENT OF AGRICULTURE, 2011) No Brasil, a região Sul é responsável por mais de 95% da produção nacional, sendo o Rio Grande do Sul seu maior produtor com aproximadamente 880.203 hectares plantados e 2.416.684 de toneladas colhidas, resultando em uma produtividade média de 2.745 kg ha^{-1} (EMATER, 2012). A planta de trigo é considerada anual, com ciclo variando entre 90 a 180 dias conforme o ambiente e o genótipo (EMBRAPA, 2012). O grão é basicamente constituído por amido e proteína (glúten), sendo sua relação ligada à qualidade e finalidade do produto colhido que, por processos de moagem e separação por peneiras, deriva principalmente na farinha e farelo de trigo, usadas na alimentação humana e animal respectivamente (MENG et al., 2009). Neste sentido, o trigo possui grande importância na ingestão diária de energia da população mundial, sendo que em países como Irã e Turquia, representa a fonte de até 1400 kcal *per capita*, que em uma dieta média de aproximadamente 3400 kcal, representa mais de 40% da energia diária consumida (DIXON et al., 2009), fato que denota sua importância social e econômica.

Quanto a tolerância a acidez do solo, as recomendações de calagem vigentes para a cultura sugerem a elevação do pH do solo a 6,0 em solo com correção e gradagem para um pleno desenvolvimento da planta, variando em função do sistema de manejo do solo e da cultura antecessora (CQFS-RS/SC, 2004). Em solos com pH excessivamente ácido (abaixo de 5,0) ocorre redução de produtividade de trigo, uma vez que pH baixo prejudica o crescimento da planta pela própria ação da acidez (H^+), além de diminuir a disponibilidade de nutrientes e aumentar a concentração de alumínio trocável no solo (CASTRO, 1983). Por sua vez, o alumínio (Al) consiste em um metal de transição abundante na crosta terrestre, sendo um dos principais fatores limitantes à produção agrícola em solos ácidos (pH<5,5), que por sua vez perfazem cerca de 30% das terras aráveis do mundo (von UEXKULL; MUTERT, 1995). No Brasil, se levarmos em consideração não apenas áreas já cultivadas, mas também terras com potencial para a atividade agrícola, a presença

de solos com toxidez de Al ocorre em torno de 60% desses (ABREU JR. et al., 2003).

À medida que os solos se acidificam atingindo valores de pH menores que 5,5, ocorre o aumento da dissolução de formas sólidas de Al, liberando formas iônicas na solução do solo (RITCHIE, 1994) que, na superfície eletronegativa dos coloides, passam a ocupar posições de troca catiônica em substituição aos cátions removidos pela lixiviação, onde concentrações de espécies de Al podem alcançar níveis tóxicos para os organismos (RENGEL; ZHANG, 2003). A forma iônica mais fitotóxica do Al consiste no Al^{3+}, comumente encontrado como $Al(H_2O)_6^{3+}$, é predominante em condições de pH abaixo de 4,5. Dentre as demais formas existentes no solo, os hidróxidos de alumínio $Al(OH)^{2+}$, $Al(OH)_2^{+}$ e $AI(OH)_3$, existentes principalmente entre os pHs 5 e 7 também apresentam fitotoxicidade, mas devido sua relação com a variação do pH do meio, se torna difícil quantificar a forma predominantemente atuante (MIYASAKA et al., 2007). Assim, em virtude da dificuldade do isolamento dos efeitos do Al quando se trabalha com solos, devido às múltiplas interações desse elemento com fatores como pH, composição iônica, matéria orgânica e bases trocáveis do mesmo (ROSSIELLO; NETTO, 2006), tem-se o uso de soluções nutritivas como a melhor forma de estudar seu efeito sobre plantas, permitindo não apenas o pronto acesso do sistema radicular, mas também a manutenção da concentração de elemento para um melhor monitoramento das reações de sensibilidade e tolerância.

A primeira resposta ao estresse causado por Al consiste na inibição do crescimento do sistema radicular, onde os ápices e raízes laterais tendem a engrossarem e adquirirem coloração escura (KERRIDGE et al., 1971). Diferentes mecanismos podem estar ligados a tal resposta, entre eles, interação do Al com as paredes celulares, membranas plasmáticas ou simplasto de células do sistema radicular (HORST, 1995; KOCHIAN, 1995), estimulando principalmente a formação de espécies reativas de oxigênio que, em contraste com o oxigênio molecular, podem causar danos às macromoléculas (lipídeos e proteínas), levando à destruição oxidativa da célula (WANG et al., 2007). Como efeito, tais raízes se tornam ineficientes na absorção de nutrientes e água (FOY et al., 1978).

Os mecanismos de tolerância ao alumínio podem ser diferenciados em internos e externos (KOCHIAN, 1995). Os fatores internos correspondem à

capacidade da planta em tolerar o Al no simplasto celular, principalmente por meio da compartimentação do metal no vacúolo, aumento da atividade de enzimas antioxidantes, quelação no citosol e a formação de complexos entre proteínas e Al (TAYLOR, 1991). Os fatores externos consistem na habilidade da planta em evitar a entrada do metal em seus tecidos, principalmente pela exsudação de ácidos orgânicos pelas raízes (RYAN et al., 1995), imobilização do metal na parede celular, ativação da saída de Al pela membrana plasmática (TAYLOR, 1991), produção de mucilagem no ápice das raízes (HENDERSON; OWNBY, 1991), inativação do Al pela alteração do pH da rizosfera e manutenção da permeabilidade seletiva da membrana plasmática (KOCHIAN, 1995).

Além da ocorrência de alumínio trocável, sob condições de baixo pH o crescimento da cultura é prejudicado pela diminuição da disponibilidade de nutrientes (CASTRO, 1983). No caso do P aplicado como fertilizante, grande parte do elemento é adsorvido ao solo ficando menos disponível às plantas (OLIVEIRA; NOVAIS, 2002), contudo em solos ácidos, parte do P é ainda fixado pelos íons de ferro e Al^{3+}, podendo acarretar sintomas de deficiência na planta mesmo sob aplicação de elevadas quantidades de fertilizantes fosfatados (MARSCHNER, 1995).

O P faz parte da constituição de importantes componentes celulares como açúcares fosfatados, nucleotídeos, ácidos nucléicos, fosfolipídios das biomembranas e coenzimas, atuando ainda em componentes metabólicos móveis armazenadores de energia como o ATP e, consequentemente, nas reações que dependem de tal molécula (TAIZ; ZEIGER, 2004). No solo, o P total (Pt) é composto por distintas frações orgânicas (Po) e inorgânicas (Pi) com diferentes graus de disponibilidade às plantas, entretanto, as principais formas absorvidas constituem em ortofosfatos $H_2PO_4^-$ e HPO_4^{2-}, variando conforme o pH do meio (SANCHEZ, 2007). Assim, o Po existente no solo deve ser previamente hidrolisado para que possa ocorrer a absorção (RAGHOTHAMA, 1999).

Mesmo sob a aplicação de P no solo como fertilizante, a absorção desse nutriente pela planta é dificultada em função de sua reduzida concentração na solução do solo, sendo que cerca de 25% dos solos tropicais e subtropicais intemperizados apresentam deficiência acentuada de fósforo (P) (SANCHES; LOGAN, 1992). Tal comportamento é determinado pela adsorção do P aos coloides do solo, sendo essa a habilidade de formar compostos com elevada energia de

ligação e assim adquirir alta estabilidade na fase sólida. Assim, mesmo com elevados teores de Pt, apenas uma pequena fração deste tem baixa energia de ligação que possibilite sua dessorção e disponibilidade às plantas (GATIBONI, 2003).

Os processos que propiciam o aumento na absorção do P estão principalmente ligados a características do sistema radicular, como o aumento do crescimento radicular associado a mudanças em sua arquitetura (WHITE; HAMMOND, 2008), expansão da superfície radicular via crescimento de pêlos radiculares e associação com fungos micorrízicos (HINSINGER et al., 2009), maior atividade de fosfatases ácidas e exsudação de ácidos orgânicos, a fim de explorar o solo com maior eficiência e/ou auxiliar na liberação do Pi de substratos fosforilados (VINCENT et al., 1992). Por sua vez, para aumentar a eficiência de uso do P, podem ocorrer processos como a redução na taxa de crescimento da planta, remobilização do P interno, maior produção de biomassa por unidade de P absorvido e modificações no metabolismo do carbono (VANCE et al., 2003).

Devido à aplicação localizada de adubos fosfatados e uma maior quantidade de resíduos orgânicos na superfície do solo, o sistema de plantio direto (SPD) contribui com o aumento da variabilidade vertical de P (SCHLINDWEIN; ANGHINONI, 2000), gerando um gradiente de concentração a partir da superfície e determinando diferenças na distribuição radicular, com uma maior quantidade de raízes na camada de solo rico em P (KLEPKER; ANGHINONI, 1993). Assim, em virtude da co-ocorrência de concentrações fitotóxicas de alumínio e baixa disponibilidade de fósforo em solos ou extratos com característica ácida, acredita-se que plantas tolerantes ao alumínio desenvolveram mecanismos para um eficiente uso de fósforo.

Distintos estudos têm demonstrado a variabilidade genética do trigo e outras espécies em resposta a condições variadas de disponibilidade de Al e P (MIRANDA, 1985; ALVES et al., 1988; SOON, 1992; ABICHEQUER; BOHNEN, 1998; GAUME et al., 2001; NIAN et al., 2009) demonstrando principalmente o efeito do Al na absorção de P. Assim, o presente trabalho busca primeiramente criar um banco de dados referentes a respostas de cultivares de trigo a exposição aos elementos Al e P, classificando os materiais quanto sua sensibilidade e tolerância ao Al, bem como sua eficiência de uso e resposta ao P para que, a partir do entendimento

aprofundado das diferentes respostas a exposição dos elementos de forma isolada na solução nutritiva, seja possível entender o comportamento da cultura à interação de Al e P no solo ou em experimentos futuros, permitindo um melhor entendimento dos processos envolvidos na interação Al-P e uma máxima utilização da variabilidade genética dos materiais testados na seleção e melhoramento de plantas de trigo mais produtivas através de avaliações morfofisiológicos e bioquímicos da interação entre alumínio e fósforo em plantas de trigo através do cultivo hidropônico.

CAPÍTULO I

EFEITO DE ALUMÍNIO EM CARACTÉRES MORFOLÓGICOS, ANATÔMICOS E CROMOSSÔMICOS DE TRIGO AVALIADOS SOB MICROSCOPIA DE LUZ E DE FLUORESCÊNCIA

MANUSCRITO I

MORPHOLOGY, ANATOMY AND CHROMOSOMAL CHANGES IN AL SENSITIVE VS. TOLERANT WHEAT UNDER LIGHT AND FLUORESCENCE MICROSCOPY

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Abstract

Aluminium (Al) toxicity represents an important concern in acid soils, affecting in short term nutrients and water uptake. Among several mechanisms of Al tolerance, structural and chromosomal differences between Al-tolerant and Al-sensitive cultivars remain less studied. This work aimed to investigate Al accumulation sites in root tips and leaves of “IAC 5” (Al-tolerant) and “Galego Rapado” (Al-sensitive) wheat cultivars growing under 0, 4 and 20 mgL^{-1} Al, considering leaf and root anatomical modifications, overall growth inhibition and mitotic index analysis classifying chromosome aberrations, identifying response patterns under light and fluorescence microscopy. The anatomical data support that different mechanisms of Al tolerance are found in both shoot and roots. Leaf thickness was not affected, although

intercellular spaces and stomatal density increased, resulting in stomatal clustering in the sensitive cultivar at the higher Al concentration. Root system was affected as well, displaying growth inhibition and cell wall disruption of cortical cells. Fluorescence microscopy also showed Al accumulation on vessel (xylem) and sieve tube (phloem) elements as well as in root cell nuclei of both cultivars, resulting in apoplastic transport towards the transpiration stream and mitotic index reduction respectively.

Key words: *Triticum aestivum* L.; Fluorescence microscopy; Morin stain.

Introduction

Aluminium (Al) is the third most abundant element (8%) in the Earth's crust, exceeded by oxygen (47%) and silicon (28%) (BERTHON, 2002). Despite the abundance, in agriculture systems Al becomes a matter of concern only when soil pH turns acid (below 5.5), which increase the concentration of ionic forms of Al in the soil solution (RITCHIE, 1994) and can rapidly reduce root growth (LLUGANY et al., 1995), affecting in short term nutrients uptake mechanisms and water supply (OLIVARES et al. 2009). Among the wide range of cellular components and processes proposed to be affected by Al, cell nuclei, mitosis and cell division appears as some of the most important (SILVA et al., 2000), despite little information of chromosomal behavior is available. Aluminium uptake and accumulation also enhances oxidative biomarkers in roots and leaves (ACHARY et al., 2012), such as antioxidant enzymes and reactive oxygen species (BOSCOLO et al., 2003; JONES et al., 2006), the latter leading to cell damage by lipid peroxidation and protein oxidation (YAMAMOTO et al., 2001). Al relation with human health is still under debate. Nevertheless, it has been linked to Alzheimer's disease (EXLEY, 2007), causing cognitive malfunction by Fe-mediated oxidative stress and cell injury (YOKEL, 2000).

Aluminium sensitiveness varies among plant species and despite the mechanisms used to avoid toxicity, studies normally relate Al tolerance to the

capacity of plants to maintain root apices development against the presence of Al (SAMUELS et al., 1997; ALVIM et al., 2007). Mechanisms of aluminum tolerance can be differentiated into tolerance and exclusion (KOCHIAN, 1995). Tolerance mechanisms correspond to the ability of plants to tolerate Al in the cell symplast, mainly through the sequestration into vacuoles, increase of antioxidants (enzymatic and nonenzymatic) and formation of organic Al-complexes with low toxicity (TAYLOR, 1991). On the other hand, exclusion mechanisms prevent the access of the phytotoxic Al^{3+} to target sites in plant tissues, mainly by root exudation of chelating ligands such as organic acids (RYAN et al., 1995), cell wall binding, mucilage production in the root apex (HENDERSON; OWNBY, 1991), plant inducting pH change in the rhizosphere (TAYLOR, 1991) and lower permeability of plasma membrane or enhanced Al efflux (KOCHIAN, 1995).

Once Al is capable of binding on negative charge of the pectin fraction (SCHMOHL; HORST, 2000), in general, cultivated plants are able to reduce the accumulation and subsequent toxicity of Al in photosynthetic tissues by blocking the metal in the root system. In this way, cytological and histological adaptations involving cell walls and intercellular spaces appear as a complementary exclusion mechanism, acting as a physical barrier that contributes to blocking the entry of Al, promoting Al accumulation in the cortex and endodermis of the root (HODSON; WILKINS, 1991; DELHAIZE et al., 1993a), while reduce metal translocation to the shoot (SILVA et al., 2010), until tolerance and exclusion mechanisms overcome Al availability (HARIDASAN, 2008).

Ultrastructural analyses have demonstrated different behaviors of organelles in the presence of Al in the above-ground tissue, resulting in membrane damage in *Camellia sinensis* (LI et al., 2011), but not affecting photosynthetic process in chloroplasts of a *Vochysiaceae* species (ANDRADE et al., 2011). This suggests that different mechanisms of Al tolerance are also found in the shoot rather than just in the roots. Previous studies have already reported differences in the root region of wheat Al-tolerant and Al-sensitive cultivars when exposed to Al (SILVA et al., 2010), but little information about shoot behavior and Al mobility are available. In order to provide further understanding of Al accumulation sites and physiological processes affected, this work aimed to investigate Al distribution and growth effects in root tips and leaves of wheat cultivars, considering anatomical and morphological traits under

light and fluorescence microscopy, as well as mitotic behavior of meristematic cells were evaluated.

Material and methods

Plant material selection and growth condition

The experiment was developed using nine *Triticum aestivum* L. genotypes (BR 23, BRS 208, BRS Umbu, Frondoso, Galego rapado, IAC 5, Marocco, Ônix and Sumai 3) provided by the Brazilian Agricultural Research Corporation (EMBRAPA),), following a randomized complete block design with four replications. The seeds were surface sterilized with 2% sodium hypochlorite, rinsed and germinated (in darkness at 24±2C°) in Petri dishes with moistened filter paper for 3 d. Uniform seedlings were transferred to a net floated in a plastic container filled with 10 L, continuously aerated, nutrient solution composed of (mg L^{-1}): 85.31 N; 7.54 P; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 173.36 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe during 2 d for preconditioning.

After preconditioning, the nutrient solution was supplemented with 0, 4 and 20 mgL^{-1} Al as $AlCl_3.6H_2O$ and to prevent Al interaction with phosphate group, K was added as K_2SO_4 to provide P concentrations of 0. Plants were kept in a climatized greenhouse at 25±2C° under natural day light and under Al treatment for 4 d. The pH of the nutrient solutions was maintained at 4.0 throughout the assay using 0.1 M HCl or 0.1 M NaOH added drop-wise. Due to the results obtained by Voss and Sousa (2001) comparing the reaction of wheat genotypes to Al toxicity in laboratory conditions and soil acidity in the field, the screening for Al tolerance was based on the root growth inhibition at 4 mgL^{-1} Al. The root growth was measured according to Baier et al. (1995) using a millimeter ruler right before Al exposure and by the end of it. Relative root elongation [(elongation with Al/elongation without Al) * 100%] was than calculated for at least 24 plantlets per genotype. Leaf area and height were measured using pixel counting and millimeter ruler, respectively.

Histological analysis

For structural observations, fresh root tips (5 mm) and mid-area of the youngest, fully expanded leaf were rinsed with distilled water and fixed with 3 % glutaraldehyde (GABRIEL, 1982) with the addition of Tween at 2 mL^{-1} (FREUDENSTEIN et al., 2002) in 0.1 M sodium phosphate buffer, pH 7.2; then washed in 0.1 M sodium phosphate buffer, pH 7.2, and lastly with distilled water. Afterwards, the samples were dehydrated in a graded series of ethanol and embedded in 2-hydroxyethylmethacrylate (GERRITS; SMID, 1983). Thin sections (5 µm) obtained with a Leica RM2245 microtome were stained for general histology using 0.05 % toluidine blue in 1 % sodium borate solution (MERCER, 1963).

Observations and image capture were carried out using a Leica DM2000 light microscope system equipped with a Leica DFC295 digital camera on the primary seminal roots of five randomly selected plantlets of each treatment.

Roots and leaves were stained with 100 µM morin (Sigma-Aldrich) (TICE et al., 1992). Thin cross-sections obtained as described for image capture were made from the root tip of the primary seminal roots of four randomly selected plantlets. Sections were placed on a microscope slide and examined with an Olympus IX80 inverted microscope by bright field and epifluorescence (420 nm excitation and 510 nm emission).

Hematoxylin staining

The hematoxylin dye forms complexes with tissue Al that has been immobilized as $AlPO_4$ by phosphate on or immediately below the root surface (OWNBY, 1993). To evaluate Al accumulation in plant tissue, roots were rinsed in distilled water for 30 min to remove any trace of nutrient solution, and then stained with a solution of 0.1% hematoxylin (Sigma-Aldrich) and 0.01% KI for 30 min (CANAÇADO et al., 1999). The excess of hematoxylin stain was washed off with a 30

min rinse in distilled water. After, roots were examined and photographed with a Leica EZ4D digital stereomicroscope.

Leaf stomatal density

Leaf stomatal density was determined using the impression approach and expressed as the number of stomata per unit leaf area (RADOGLOU; JARVIS, 1990). The adaxial epidermis of a selected leaf was cleaned using a dry cotton ball. Then the mid-area of the leaf was carefully glue to a glass slide using a drop of fast drying glue for approximately 10 minutes. Once the glue dried, the leaf was gently remover from the glass slide, leaving a leaf impression. Impressions were taken from the youngest, fully expanded leaves of three randomly selected plants of each treatment. Numbers of stomata cells for each impression were counted and photographed under a Leica DM2000 light microscope system equipped with a Leica DFC295 digital camera.

Genotoxicity analysis

To analyze cell division and chromosomal aberrations, primary seminal roots of approximately 3 cm were collected from four randomly selected plantlets of each cultivar and treatment. Root tips were hydrolyzed in HCl 1M for 10 minutes at 60ºC and stained with 2% acetic orcein (adapted from GUERRA; SOUZA, 2002). Meristematic region was then squashed and placed on a slide for analysis at 100X magnification. The mitotic index was determined by dividing the number of cells in mitosis (prophase, metaphase, anaphase, and telophase) by the total number of cells observed and multiplying the result by 100. Chromosomal aberrations was determined by dividing the number of cells with abnormal chromosome distribution (sticky chromosomes, anaphasic bridges, chromosomal fragments, and laggard chromosomes) by the total number of cells observed and multiplying the result by

100. Five hundred cells were counted 3 times on 3 different root tips and results were compared with recounting of 150 cells with no differences in the proportion of cell phases among counting (data not shown), thus, analysis were performed using 150 cells per root tip.

Statistics

Statistical analysis was carried out by using SISVAR statistical software (FERREIRA, 2008). Differences among means were detected through Tukey's multiple range test ($p \leq 0,05$). Data were expressed as means.

Results and discussion

Despite intraspecific Al resistance mechanisms, relative root growth ratio (RRGR) decreased in a concentration-dependent manner in all tested cultivars, indicating that there was greater partial root growth inhibition at the higher Al concentration (20 mgL^{-1} Al). Under 4 mgL^{-1} Al, root growth inhibition ranged up to 27%, with Al most resistant cultivar IAC 5 and Al most sensitive cultivar Galego Rapado with a RRGR of 76.7% and 49.2%, respectively (fig. 1). Due to its RRGR behavior, both cultivars were used for further comparative analysis.

Both cultivars presented greater Al accumulation on root tissue (fig. 2B), which acts as the main site for Al accumulation (HEIM et al., 1999). Despite plant tissue, tolerant cultivar IAC 5 showed lower Al accumulation due to Al exposure, with less translocation of Al to the photosynthetic tissue (fig. 2A). Once Al toxicity effects such as decrease on mitotic activity (FRANTZIOS et a., 2001), oxidative stress (ZHENG et al., 2005) and changes in cell wall properties (YAMAMOTO et al., 2001), relates to Al concentration on plant tissue, visible symptoms of Al toxicity such as tissue lesions or growth reduction were less pronounced on shoot tissue.

At 4 mgL^{-1} Al, both cultivars presented height reduction (fig. 3B), but IAC 5 maintained leaf area more efficiently than Galego Rapado (fig. 3A).

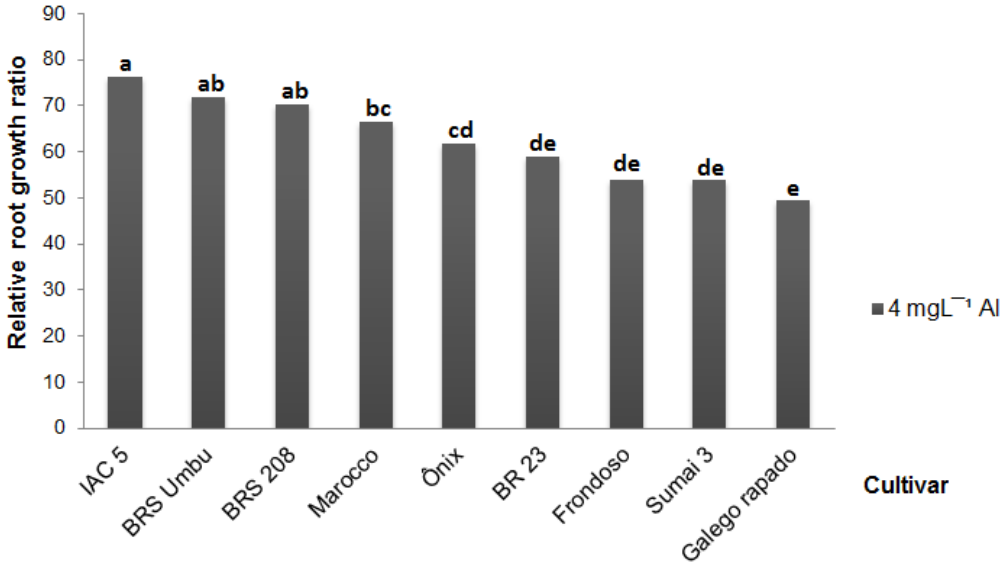

Figure 2: Relative root growth ratio of wheat cultivars exposure to Al-containing hydroponic solution with 4 mgL^{-1} Al, compared to plants in a control solution with 0 mgL^{-1} Al, pH 4.0 for 96 h.

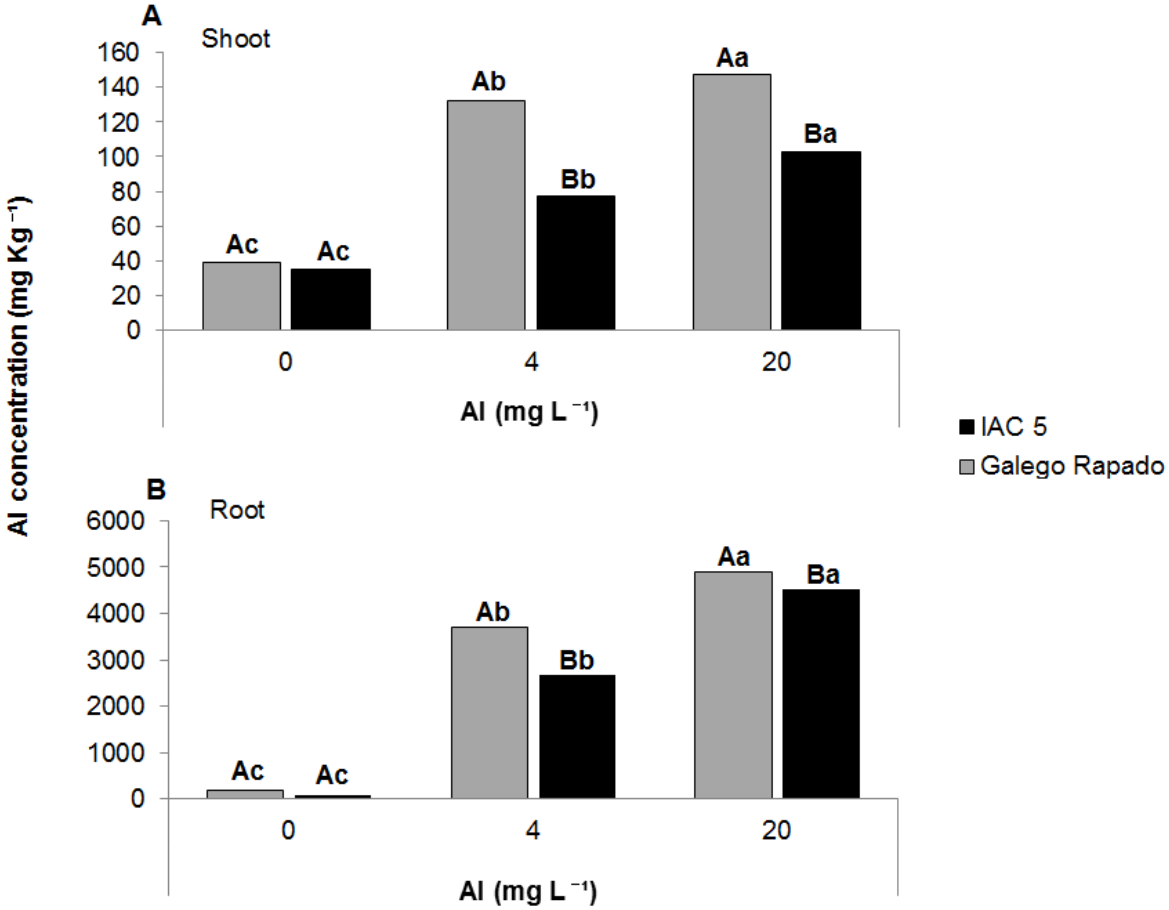

Figure 3: Aluminium concentration on shoot (A) and root (B) tissue of two wheat cultivars exposed to different Al concentrations. Capital letters indicate differences within cultivars while lowercase letters indicate differences within Al levels. Tukey test ($p \leq 0.05$).

Same pattern was observed at 20 mgL^{-1} Al, indicating differences in the way cultivars reduce their transpiration, increasing water use efficiencies while deal with Al toxicity, as found by XU and ZHOU (2005) on *Leymus chinensis* and MONCLUS et al. (2006) on *Populus sp.* when studying water drought stress. Despite Al level in the nutrient solution, both stomatal density and stomata per leaf were affected differently between cultivars. Stomatal density increased 7% in IAC 5 (figs. 3C, 4C) and 28,2% in Galego Rapado (figs. 3C, 4I), while stomata number per leaf decrease 6.6% and 19.1% in IAC 5 and Galego Rapado, respectively (fig. 3D), although not significantly different on the tolerant cultivar. Interestingly, sensitive cultivar displayed stomatal clustering at 20 mgL^{-1} Al (fig. 4I), an abnormal stomatal patterning that attempt to ensure the optimal balance between carbon assimilation and water loss in plants (LARKIN et al., 1997).

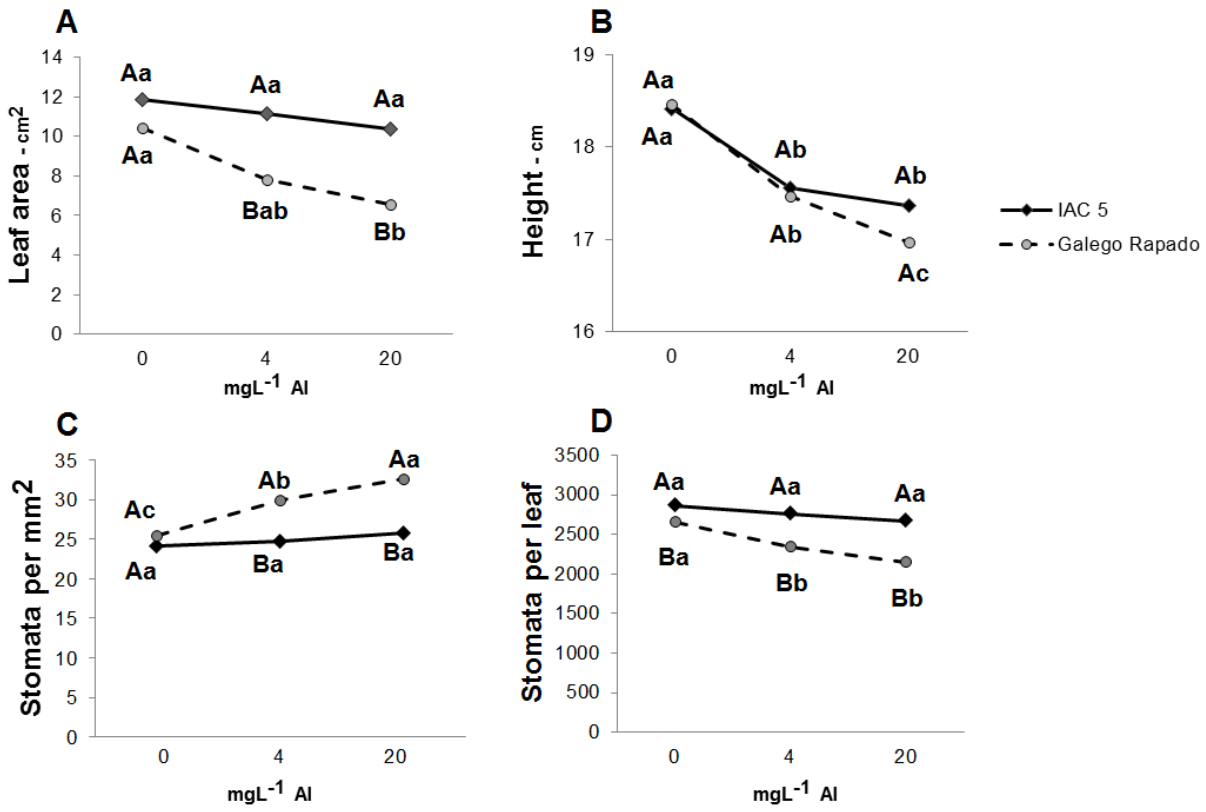

Figure 3: Leaf area (A), height (B), stomatal density (C) and stomata per leaf (D) of two wheat cultivars exposed to different Al concentrations. Capital letters indicate differences within cultivars while lowercase letters indicate differences within Al levels. Tukey test ($p \leq 0.05$).

Aluminium affected overall growth both in IAC 5 and Galego Rapado when compared to control plants, with enhanced changes in the root apex, where several studies have shown consistently that most of the Al is bound to the cell wall (CHANG et al., 1999; MA et al., 1999), blocking Ca^{2+} channels on the root-cell plasma membrane in wheat roots (HUANG et al., 1992). Still, Al interacts with multiple sites of the root cells including plasma membrane and symplast, causing damage as peroxidation of membrane lipids (CAKMAK; HORST, 1991; WAGATSUMA et al., 1995). In this study, it was observed that Al induced disruption on cell wall in cells located in the outer cell layer and cortical cells (fig. 4F, L), being it clear when compared with cross section of control plants (fig. 4E, K).

Leaf thickness was not affected, but both cultivars presented higher intercellular spaces in the mesophyll cells (fig. 4A, D, G, J), similarly as was found by Souza (2010) after exposure of *Genipa americana* to different concentrations of cadmium. Allied with the higher stomatal density, these strategy could improve CO_2 diffusion through the intercellular spaces from the sub-stomatal cavity to the outer surface of the mesophyll cells, enhancing photosynthetic activity, similarly as reported for hydric stress (ZHANG et al., 2006; ENNAJEH et al., 2010), once Al toxicity directly affects nutrients and water uptake as reduce root development (MARIANO; KELTJENS, 2005).

Although symptoms of Al toxicity manifested in the shoots are commonly related as a result of root injury and with the exception of Al-accumulating plants, little Al is transported into the shoot (WATANABE, OSAKI, 2002). The first difference observed between tolerant and sensitive cultivar, related to the increase in the vessel elements diameter, which induces an increase in the vascular bundle diameter. Another difference regards to the greater thickening of the inner tangential cell wall in the mestome sheath, which is due to a greater deposition of cellulose, followed by lignification (KPÉMOUA et al., 1996), similar to “U” thickening in root endodermis (fig. 5C, D). Figure 5 shows an increase of xylem and phloem proportion on both cultivars, with a greater expansion on cv. Galego Rapado (sensitive), probably due to the severe reduction on its root system, indirectly contributing for a higher Al translocation, once xylem represent the pathway for Al-forms to the shoots, translocated by the transpiration stream.

Hematoxylin dark staining showed Al accumulation in the root tips, especially in the sensitive cultivar (fig. 6A, B), reflecting in a “U” shape thickening of the root tip endodermis, more pronounced in cv. Galego Rapado (fig. 6C, D). Al-induced deposition of lignin has been reported by Sazaki et al., 1996, as a result of an increase in the phenylalanine ammonia-lyase activity, key enzyme of lignin synthesis (KPÉMOUA et al., 1996; BACH; SEITZ, 1997), and as callose accumulation, seems to act restricting Al transport to upper plant parts inhibiting intercellular transport through plasmodesmatal connections (SIVAGURU et al., 2000).

Cross sections of root tips and leaves of plants exposed to Al for 96 h were used to identify Al accumulation. A few root border cells detached from the root tip fluoresced brightly. Leaves grown under Al treatment solution with 4 mg Al L^{-1} displayed moderate brightly fluorescing on the vein and some fluorescence can be seen on the epidermal cells, which enhanced at higher Al concentration, despites cultivar tolerance.

Galego Rapado root tips showed Al concentration on stele and cortex (fig. 7E, G), while on IAC 5 most of the Al appears on the cortex cells (fig. 7A, C). As a result, smaller amounts of Al translocate to the shoot, consistent with IAC 5 leaf section lower fluorescence signal (fig. 7B, D) when compared with Galego Rapado (fig. 7F, H). A greater number of root border cells were affected after exposure to high Al concentration and fluorescence was often associated with damaged cells (fig. 7C, G). Outer cortical layers and stele of IAC 5 were affected by Al.

In the vascular region of the leaf, both xylem and phloem had fluorescence detected with similar intensity in between (fig. 7B, D, F, H) suggesting that part of the Al is retrieved from the transpiration stream by lateral export to the phloem tissue. Epidermal cell wall also displayed Al accumulation, although lower than the vascular bundle. Despite above-ground tissues are not sites for high levels of Al take up (JANSEN et al., 2002), Al accumulation in xylem, phloem and both abaxial and adaxial epidermis have been reported on different aluminium-accumulating and nonaccumulating species (HARIDASAN et al., 1986; CARR et al., 2003; TOLRÀ et al., 2011). The mechanisms that allow such behavior vary among species.

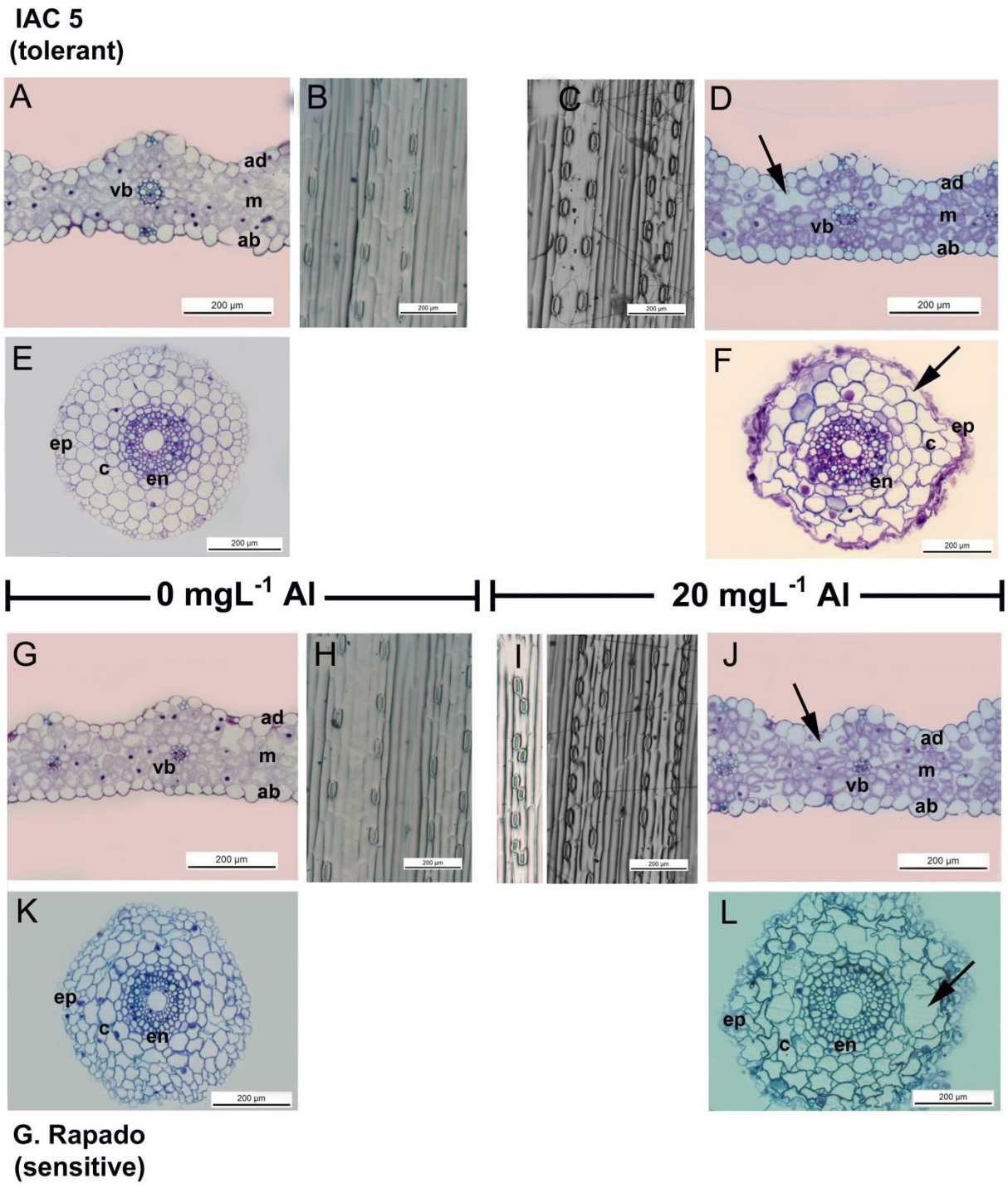

Figure 4: Morphologic and anatomic comparison of Al-tolerant wheat cultivar IAC 5 (A to H) and Al-sensitive wheat cultivar Galego Rapado (I to P) cultivated in 0 and 20 mgL^{-1} Al, pH 4.0 for 96 h. A, D, G, J: Leaf cross section; ad, adaxial epidermis; m, mesophyll cells; ab, abaxial epidermis; vb, vascular bundle. Arrows indicate intercellular space. Scale bar: 200 µm. E, F, K, L: Root tip cross section of the primary seminal roots; ep, epidermis; c, cortex; en, endodermis. Arrows indicate cell wall disruption. Scale bar: 200 µm. (C, D, K, L) Stomata distribution on the adaxial mid-area surface of the youngest, fully expanded leaf, note detail (L) of the stomatal clustering. Scale bar: 200 µm.

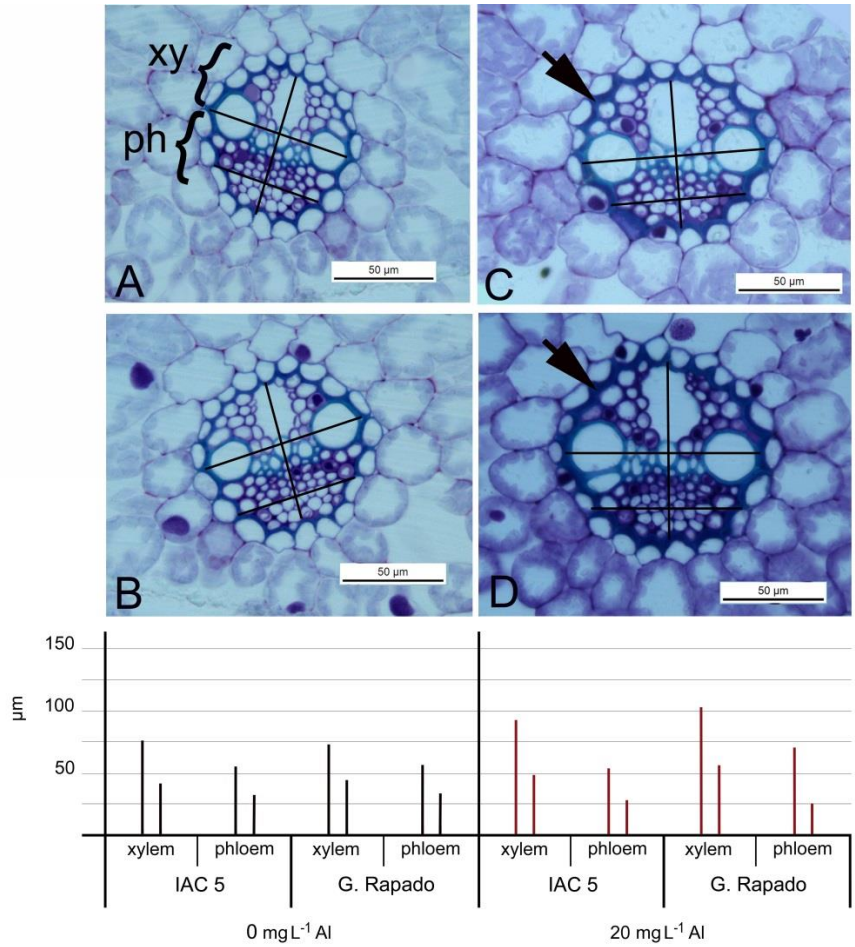

Figure 5: Xylem and phloem size of wheat Al-tolerant cv. IAC 5 (A-C) and Al-sensitive cv. Galego Rapado (B-D) grown under different Al concentration. Arrow indicates endodermis thicking. Scale bar: 50 µm.

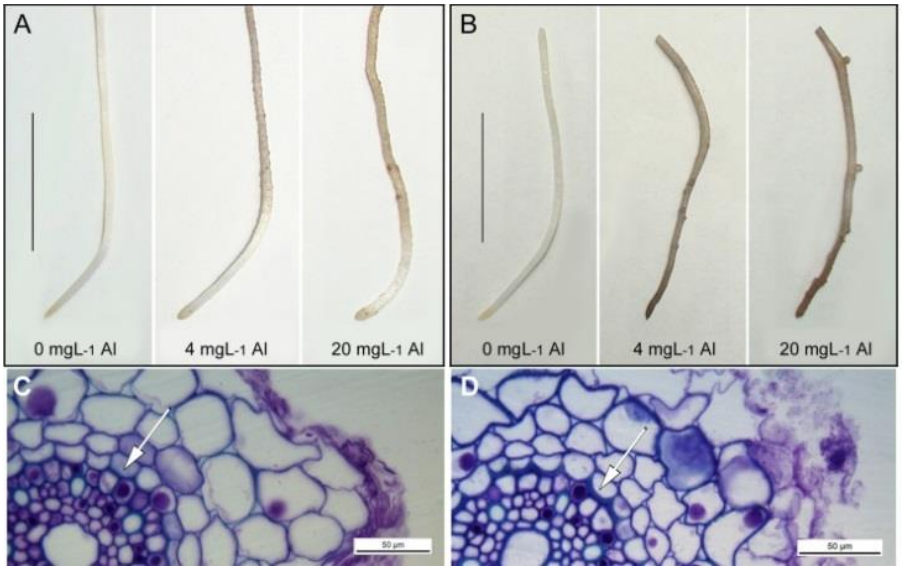

Figure 6: Hematoxylin staining showing Al accumulation on root tips of wheat Al-tolerant cv. IAC 5 (A) and Al-sensitive cv. Galego Rapado (B) under different Al concentration in the nutrient solution. Scale bar: 1 cm. C: Root tip cross section from cv. IAC 5 grown with 20 mgL^{-1} Al. D: Root tip cross section from cv. Galego Rapado grown at 20 mgL^{-1} Al. Arrow indicates endodermis thickening. Scale bar: 50 µm.

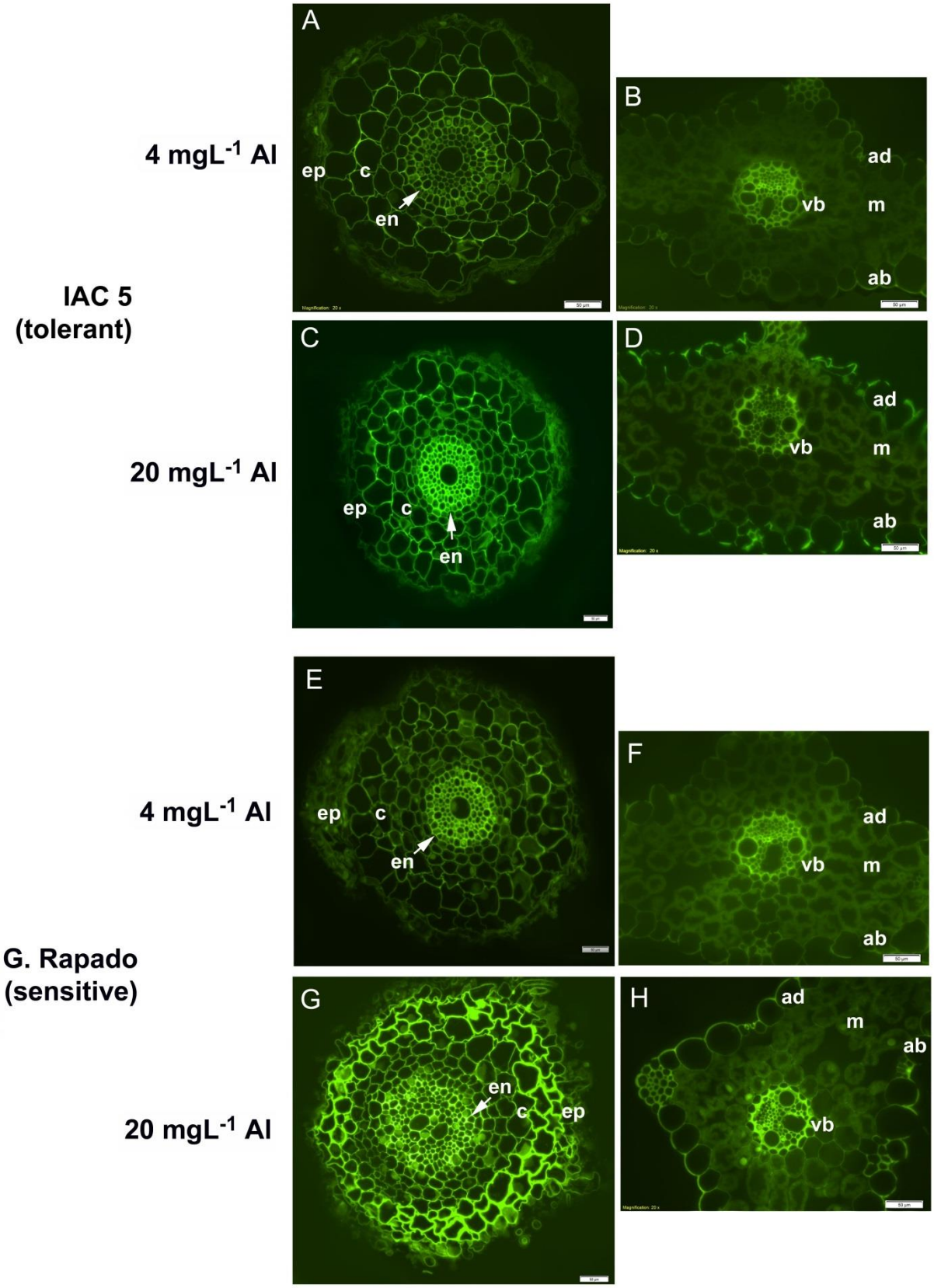

Figure 7: Fluorescence of morin-stained roots and leaves of wheat Al-tolerant cv. IAC 5 and Al-sensitive cv. Galego Rapado grown under different Al concentration. A, C, E, G: Root tip cross section of the primary seminal roots; ep, epidermis; c, cortex; en, endodermis. B, D, F, H: Leaf cross section of the youngest, fully expanded leaf; ad, adaxial epidermis; m, mesophyll cells; ab, abaxial epidermis; vb, vascular bundle. Scale bar: 50 µm.

Aluminium-accumulating species are considered able to detoxify Al by binding to the cell walls, vacuole compartmentalization or formation of Al chelates, exhibiting a high Al tolerance despite the high Al concentration in their above-ground tissues (HARIDASAN, 1988; OSAKI et al., 1997; WATANABE et al., 1997). On the other hand, the majority of plant species avoids Al stress by excluding Al from the roots, mainly by exudation of organic acids (JANSEN et al., 2002; WATANABE; OSAKI, 2002), specially malate and citrate in wheat genotypes (DELHAIZE et al., 1993b; RYAN et al., 2009).

Bundle sheath displayed enhanced signal towards the vein and “U” shape thickening (fig. 8). Apparently acting as an important tolerance mechanism to reduce Al movement towards the mesophyll cells, despite the occurrence of Al accumulation in epidermal cells by apoplastic movement. According to Karley et al. (2000), nonessential or excess ions, as well as ions do not preferentially absorbed by bundle sheath plasma membrane tend to accumulate in epidermal tissues. Such strategy could be linked to the deprivation of photosynthetic mesophyll cells to potential metabolic damage. As found in this study, preliminary report cared by Matsumoto et al. (1976) and Tolrà et al. (2011) relate the accumulation of the metal with apoplastic movement in the leaf (fig. 8). According to the authors, the movement occurs along the water flow generated by the transpiration stream that evaporates when reaches gas exchange sites, causing Al accumulation specially on negatively charged hydroxyl and carboxyl groups of the cell wall (YANG et al., 2008).

Studies have shown that the apoplastic space corresponds the main accumulation site for Al in roots (HEIM et al., 1999), but its presence in the symplast may occur within 30 minutes exposure to a solution containing Al (LAZOF et al., 1994), where can affect membrane and organelles function (Li et al., 2011; Silva et al., 2013). Differences in the nuclei accumulation of Al in Al-tolerant and Al-sensitive soybean cultivars were reported by Silva et al. (2000) indicating not just that substantial Al accumulates in the nuclei but also that the accumulation is higher in the Al-sensitive genotype. Although intracellular concentration of Al was not examined in this study, Al interaction with cell nuclei was found (fig. 9) in root tips of both wheat cultivars despite Al concentration in the nutrient solution. Once the presence of significant amounts of Al in the apoplast raises the possibility of Al movement into the symplasm during tissue dissection (RENGEL, 1996), genotoxic

analysis were performed with intact root tips, showing abnormal cells appearance in a concentration-dependent manner.

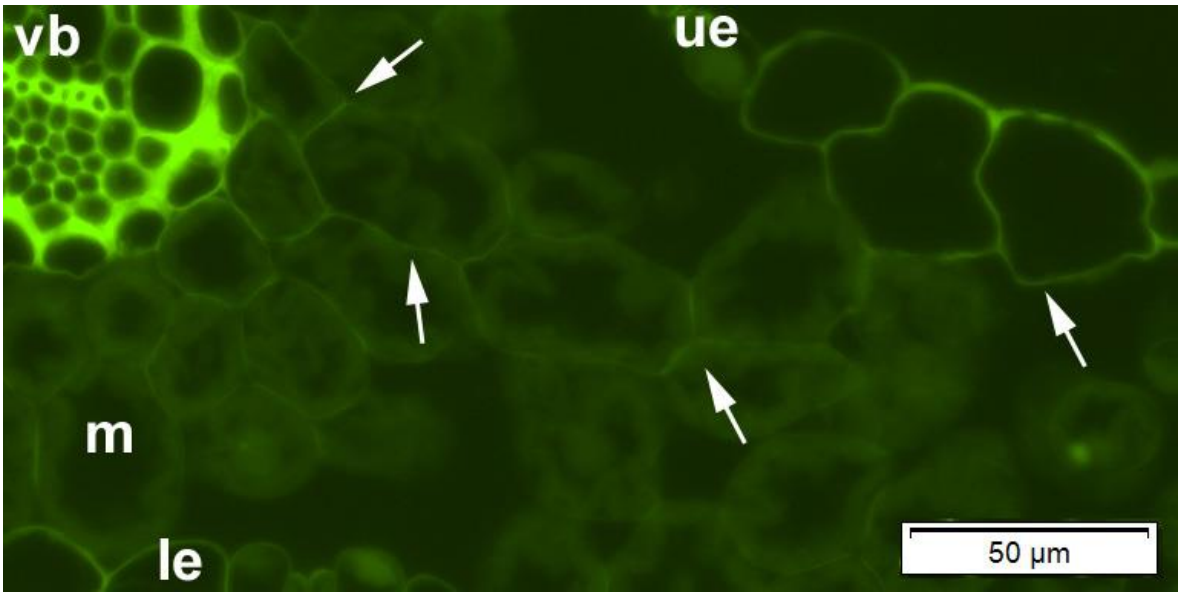

Figure 8: Leaf detail of Al apoplastic movement from vascular bundle to epidermal cells (arrows) on wheat Al-sensitive cv. Galego Rapado grown under 20 mgL^{-1} Al. le, lower epidermis; m, mesophyll cells; ue, upper epidermis; vb, vascular bundle.

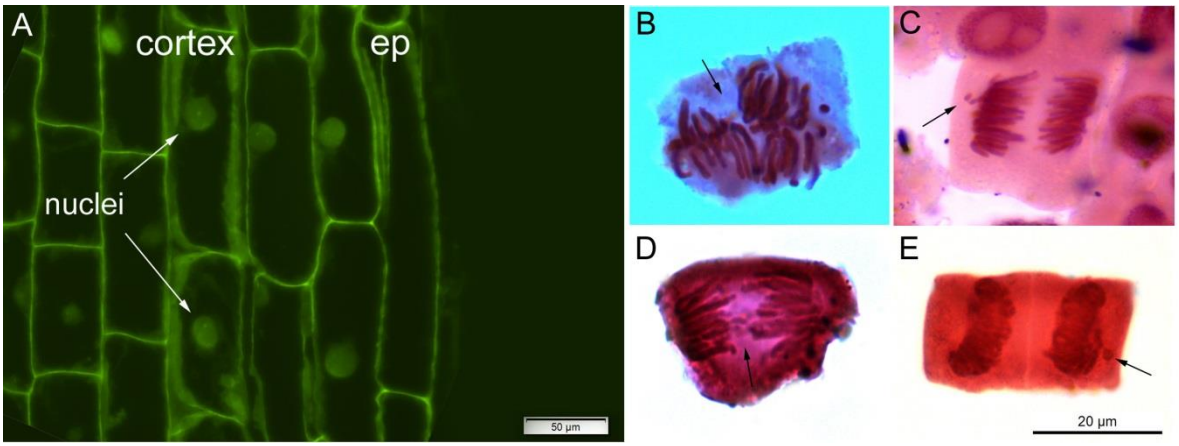

Figura 9: Aluminium accumulation at nuclei of cells of root tip and morphological alterations on meristematic cell due to Al toxicity. A: Root longitudinal section of wheat Al-sensitive cv. Galego Rapado grown under 20 mgL^{-1} Al. Dark arrows indicate: chromosomal disruptions in metaphase (B); laggard chromosome in anaphase (C); formation of an anaphasic bridge (D) and micronuclei formation in telophase (E). ep, epidermis.

In the absence of Al, there were meristematic root-tip cells in all phases of cell division with the highest number of cells in prophase (Table 1). As Al concentration

increased, a consistent reduction in cell division was found, resulting in a lower mitotic index (MI) and consequently root growth (fig. 4F, N). Thus, more cells remained in interphase and a higher number of those that started cell cycle, presented chromosome aberration (CA). Interestingly, MI was differently affected between cultivars, Al-tolerant IAC 5 and Al-sensitive Galego Rapado reduced 50.22% and 48.74%, respectively. Despite higher relative reduction in MI, IAC 5 presented greater values in all tested concentrations, although no difference at 4 mgL^{-1} Al. Furthermore, CA occurred despite Al concentration and cultivar. The alterations observed were micronucleus formation (fig. 9E), sticky-chromosome/laggard (fig. 9C), anaphasic-bridge (fig. 9D) and chromosomal breakage (fig. 9B) with a slightly tendency of greater frequency on the latter one. IAC 5 presented greater CA at 20 mgL^{-1} Al, a pattern that suggests more efficient exclusion mechanisms on the tolerant cultivar, allowing cell division upon high Al concentration.

Tabela 1: Cell cycle profile (interphase, prophase, metaphase, anaphase, and telophase), micronucleus and chromosome aberrations of wheat cultivars exposed to different Al concentration. Data were generated as averages of 4 replications of each treatment.

Cultivar	**IAC 5**			**Galego Rapado**		
Al (mgL^{-1})	**0**	**4**	**20**	**0**	**4**	**20**
Interphase	563	574	581	577	579	588
Prophase	28	17	9	18	10	7
Metaphase	1	1	1	1	3	0
Anaphase	6	1	0	1	0	0
Telophase	2	4	0	3	4	0
Micronucleus	0	0	2	0	0	1
Sticky-chromosome/ laggard	0	1	2	0	0	1
Chromosomal breakage	0	2	3	0	3	2
Anaphasic-bridge	0	0	2	0	1	1
Mitotic index % (MI)	6.57 Aa	4.52 Ab	3.27 Ab	3.98 Ba	3.62 Aab	2.04 Ab
Chromosome aberration % (CA)	0 Ac	0.5 Ab	1.5 Aa	0 Ab	0.66 Aab	0.83 Ba

Capital letters indicate differences within cultivars while lowercase letters indicate differences within Al levels. Tukey test ($p \leq 0.05$).

Conclusion

Comparative data on the Al-tolerant and Al-sensitive cultivar showed leaf thickness was not affected by Al although intercellular spaces increased in both cultivars. Cultivar IAC 5 do not alters leaf area when compared to Galego Rapado. Stomatal density seems to be the most affected trait in above-ground tissue on both cultivars, being stomatal clusters exclusive to Galego Rapado at highest Al concentration. Al-morin fluorescence on leaf showed the bundle sheath cells accumulating most of the Al unloaded from the xylem, which was wider in the sensitive cultivar, allowing more Al to translocate. Furthermore, apoplastic transport act to deposit Al at the end of the transpiration stream, in the epidermal cell wall, despite evidence that some Al also can be retrieved from the transpiration stream and transferred to the phloem.

Al also affected root structure of the sensitive cultivar in greater extent. Both cultivars presented accumulation and translocation of Al in the root elongation zone as high fluorescence signal was obtained on stele and parenchyma cells, especially in Galego Rapado, followed by its lower mitotic index possibly resulting from higher nuclei interaction with Al, causing chromosomal breakage as the main aberration.

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CAPÍTULO II

DIMENSIONAMENTO AMOSTRAL DE CARACTERES DE TRIGO (*Triticum aestivum*, L.) EM ESTUDOS SOBRE O ESTRESSE POR ALUMÍNIO

MANUSCRITO II

DIMENSIONAMENTO AMOSTRAL DE CARACTERES DE TRIGO (*Triticum aestivum*, L.) EM ESTUDOS SOBRE O ESTRESSE POR ALUMÍNIO

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Resumo

O alumínio (Al) encontra-se entre os elementos mais abundantes da crosta terrestre e é considerado um dos principais fatores limitantes a produtividade agrícola em solos ácidos, entretanto, não há um consenso em relação ao número de plântulas de trigo mais indicado para formar uma unidade experimental em estudos de tolerância ao metal em sistema hidropônico. Assim, o objetivo deste trabalho foi determinar o tamanho de amostra (número de plantas) necessário para avaliar a altura e o Cde plântulas de trigo, sob estresse de Al e em sistema hidropônico para avaliação de tolerância ao Al, com o intuito de verificar a variabilidade do tamanho de amostra entre as cultivares Marocco, Ônix, Galego rapado, IAC 5, Sumai 3, Anahuac 75, BR 23, BRS 208 e BRS Umbu) nas doses de 0, 4, 12 e 20 mg L^{-1} Al. Conclui-se que há

variabilidade na estimativa do tamanho de amostra entre cultivares quanto à Hs e comprimento radicular; e da concentração de alumínio, quanto ao comprimento radicular. Apenas a variabilidade devido ao efeito da cultivar deve ser considerada. Deve-se utilizar pelo menos 9 e 74 plantas para avaliação de altura e Cde cultivares de trigo em estudos de tolerância ao Al, respectivamente, considerando ainda que estimativas com precisão inferior a 10%, de modo geral, são impraticáveis.

Palavras-chave: *Triticum aestivum L.*; Alumínio; tamanho de amostra.

Introdução

O alumínio (Al) encontra-se entre os elementos mais abundantes da crosta terrestre e é considerado um dos principais fatores limitantes a produtividade agrícola em solos ácidos (KOCHIAN, 1995; HOUDE e DIALLO, 2008). À medida que os solos se acidificam atingindo valores de pH menores que 5,5, ocorre o aumento da liberação de formas iônicas de Al na solução do solo (RITCHIE, 1994), por sua vez fitotóxicas para a maioria das espécies de plantas mesmo em concentrações micromolares (YE et al., 2011). Dentre os sintomas de toxidez por Al, a inibição do crescimento do sistema radicular consiste na resposta mais sensível (ZHAO et al., 2003; LIN, 2010) e, em virtude de sua precocidade (SILVA, 2012a), representa a variável mais eficiente para avaliar a tolerância de plantas ao metal nos estágios iniciais de desenvolvimento(MAKAU et al., 2011).

Ao afetar a divisão mitótica e elongação celular do ápice radicular (FRANTZIOS et al., 2001; MA et al., 2004), o Al torna as raízes ineficientes na absorção de nutrientes e água, gerando desequilíbrios nutricionais que afetam o desenvolvimento da parte aérea (SILVA, 2012b; LIDON et al., 2000; MENDONÇA et al., 2003). Assim, a seleção de materiais com múltiplos mecanismos de tolerância contribui para a manutenção da segurança e sustentabilidade da agricultura (TANG et al., 2001; TORABI et al., 2012), possibilitando a redução do uso de corretivos agrícolas.

A fim de avaliar um grande número de plantas em uma pequena área experimental, sistemas hidropônicos são comumente utilizados em estudos de nutrição mineral e avaliação de elementos tóxicos, como o Al. Com um elevado controle local e permitindo um fácil acesso ao sistema radicular (NARASIMHAMOORTHYet al., 2007; SHAVRUKOV et al., 2012), tal metodologia é amplamente utilizado na identificação primária de genótipos tolerantes em culturas como trigo (SASAKI et al., 2004), milho (PIÑEROS et al., 2005), batata (TABALDI et al., 2007), cevada, arroz (FAMOSO et al., 2010; NGUYEN et al., 2001), centeio (COLLINS et al., 2008) e soja (OJO e AYUBA, 2012) e permite isolar os efeitos do Al sem a interferência da toxidez de outros elementos como manganês e ferro, também encontrados em solos ácidos (GOEDERT et al., 1997).

Em trigo, é possível correlacionar resultados obtidos em experimentos a campo e em hidroponia (BAIER et al., 1995) entretanto, não há um consenso em relação ao número de plântulas mais indicado para estudos desta natureza, que varia de 3 a 26 plântulas por tratamento na maioria dos estudos observados (CAMARGO et al., 2006; DEL GUERCIO et al., 2006; DELHAIZE et al., 1993; DORNELLES et al., 1996; KINRAIDE, 2003; RYAN et al, 2009; YE et al., 2011; ZHOU et al., 2007). Esta variação fato que corrobora com Stuker e Boff (1998), que consideram o tamanho amostral como um dos principais problemas da experimentação agrícola.

Apesar de a precisão experimental caracterizar a qualidade da inferência dos resultados (STORCK et al., 2006), existem poucas referências recentes disponíveis na literatura sobre o planejamento de ensaios e técnicas de controle do erro experimental para a cultura do trigo. Os trabalhos existentes realizam o dimensionamento do tamanho de amostra para caracteres como número de espigas, peso de espigas, produtividade de grãos (LORENTZ et al., 2007), tamanho ótimo de parcela para avaliação de produtividade (HENRIQUE NETO et al., 2004) e populações segregantes de trigo (HARTWIG et al., 2007), em experimentos a campo. Assim, independentemente se o erro experimental tende a diminuir à medida que o tamanho da amostra analisada aumenta (BARBIN, 2003), a escolha no número de plantas utilizadas em experimentos que investigam tolerância ao Al em trigo parece ter sido motivada por aspectos de ordem prática, como limitações no tempo, recursos humanos ou financeiros. Logo a determinação do tamanho de

amostra pode reduzir o custo e aumentar a rapidez na obtenção e análise de dados (BRAGA, 1986), levando em consideração parâmetros como variabilidade dos dados populacionais, grau de confiança e erro permitido (CAMPOS, 1985).

Segundo Heath (1981), dados obtidos pelo uso de amostragem podem conduzir a variações aleatórias, entretanto, tais erros podem ser minimizados realizando as medições de interesse com maior precisão e a partir de amostras dimensionadas para a precisão desejada (CAMPOS, 1985). Uma vez que cultivos hidropônicos possibilitam um elevado controle sobre os efeitos do meio, o presente trabalho tem por objetivo determinar o tamanho de amostra (número de plantas) necessário para avaliar a altura e o C de plântulas de cultivares de trigo (*Triticum aestivum*, L.), cultivadas em sistema hidropônico, em estudos de tolerância ao Al.

Material e métodos

O experimento foi conduzido em casa de vegetação climatizada (25 ± 3° C) pertencente ao Departamento de Biologia da Universidade Federal de Santa Maria, RS. Foram avaliados 36 tratamentos, formados pela combinação de nove cultivares (Marocco, Ônix, Galego rapado, IAC 5, Sumai 3, Anahuac 75, BR 23, BRS 208 e BRS Umbu), oriundas do banco de sementes da EMBRAPA Trigo; e quatro concentrações de alumínio (0, 4, 12 e 20 mg L^{-1} na forma de $AlCl_3.6H_2O$), no delineamento blocos ao acaso, com quatro repetições.

As sementes foram desinfestadas em solução de hipoclorito de sódio (2%) e dispostas sobre papel absorvente umedecido com água destilada para germinação no escuro a 25 ± 2° C. Após três dias do início da germinação, plântulas de tamanho semelhante foram dispostas sobre telas em contato com 10 litros de solução nutritiva contendo (mg L^{-1}): 85,31 N; 7,54 P; 11,54 S; 97,64 Ca; 23,68 Mg; 104,75 K; 173,36 Cl; 0,27 B; 0,05 Mo; 0,01 Ni; 0,13 Zn; 0,03 Cu; 0,11 Mn e 2,68 Fe para aclimatação. Para a adição dos tratamentos, o fósforo foi omitido da solução, evitando a precipitação com o Al. As soluções foram mantidas com pH 4,0, com soluções de HCl e NaOH 1M e aeradas constantemente.

Aos dois e aos sete dias após a semeadura (DAS), foi mensurada a altura (H) e o comprimento radicular (C) a partir da raiz seminal, em centímetros, com régua milimetrada, em um número variado de plantas, tomadas aleatoriamente na área central de cada parcela, conforme método adaptado de Baier et al. (1995). Aos dois dias as avaliações foram realizadas em plantas de dois blocos e aos sete dias em plantas dos quatro blocos. A aplicação das quatro doses de alumínio (0, 4, 12 e 20 mg L^{-1}) foi realizada logo após a primeira avaliação de H e C. Portanto, as avaliações de H e C, aos dois DAS, correspondem ao tratamento controle (0 mg L^{-1} Al). Com os dados de H e C das plantas de cada parcela, em cada época de avaliação, foram calculadas a média (m), a variância (s^2) e o coeficiente de variação (CV). Após, foi verificada a normalidade, por meio do teste de Kolmogorov-Smirnov (SIEGEL; CASTELLAN JÚNIOR, 2006), totalizando 36 testes na primeira avaliação (9 cultivares × 1 dose × 2 blocos × 2 caracteres) e 288 testes na segunda avaliação (9 cultivares × 4 doses × 4 blocos × 2 caracteres). A normalidade foi investigada a fim de verificar a adequação desses dados para o estudo do dimensionamento amostral, com base na distribuição t de *Student*.

Tomando-se por base as plantas amostradas em cada unidade experimental (combinação de cultivar, dose de alumínio e época de avaliação) foi calculado o tamanho de amostra (número de plantas, η) para as semi-amplitudes do intervalo de confiança (erro de estimação) de 2, 4, 6, 8 e 10% da estimativa da média (m), com grau de confiança (1-α) de 95%, por meio da expressão $\eta = \frac{t_{\alpha/2}^2 \; s^2}{(erro\,de\,estimação)^2}$ (BUSSAB; MORETTIN, 2004), na qual $t_{\alpha/2}$ é o valor crítico da distribuição t de *Student*, cuja área à direita é igual a α/2, isto é, o valor de t, tal que $P(t>t_{\alpha/2})=\alpha/2$, com n-1 graus de liberdade, com α=5% de probabilidade de erro, e s^2 é a estimativa de variância amostral. Assim, obtiveram-se 10 variáveis (tamanho de amostra) formadas pela combinação dos caracteres H e C com os níveis de precisão de 2, 4, 6, 8 e 10% da média estimada.

Afim de comparar a variabilidade relacionada com o tamanho de amostra entre cultivares e doses de alumínio, em cada época de avaliação, os dados das 10 variáveis (tamanho de amostra) foram submetidos à análise de variância. Para tal, foi utilizado o modelo matemático do delineamento em blocos ao acaso, definido em Storck et al. (2006). As médias dos efeitos principais de cultivares e de doses de Al

foram comparadas por meio do teste de Scott Knott (RAMALHO et al., 2005) a 5% de probabilidade. As análises estatísticas foram realizadas com o auxílio do programa SISVAR (FERREIRA, 2011) e do aplicativo Office Excel.

Resultados e discussão

Os valores médios do caractere altura (H), avaliado aos dois dias após a semeadura (DAS), oscilaram entre 6,20 e 11,14 cm. Sendo que a cultivar BRS 208, apresentou maior média de H nas duas repetições, e a cultivar Marocco obteve a menor média de H aos dois DAS. Quanto às variações entre blocos, os valores médios de H para o bloco dois foram maiores que os encontrados no bloco um.

Os valores médios do caractere comprimento radicular (C) oscilaram entre 2,43 e 6,43 cm. A cultivar BRS 208 apresentou maior média de C nas duas repetições, e a cultivar Anahuac obteve a menor média de C aos dois DAS. A cultivar BRS 208 obteve as maiores médias nos dois caracteres avaliados nas duas repetições, considerando que o número de plantas avaliadas, foi de 24 para todas as cultivares, em cada uma das duas repetições.

Os valores do coeficiente de variação (CV) para H, avaliado aos dois DAS, oscilaram entre 6,51 e 13,10%. Valores maiores de CV foram relatados para este caractere na cultura da soja em maturação (CARGNELUTTI FILHO et al., 2009). Por sua vez, o CV do C oscilou entre 15,57 e 55,87%, sendo maiores que os encontrados para H (Tabela 1).

Conforme o teste de Kolmogorov-Smirnov, os resultados dos dois caracteres de planta avaliados aos dois DAS, ajustam-se a distribuição normal, dando credibilidade aos dados para o estudo de tamanho de amostra (Tabela 1).

O número de plantas avaliadas (n) por unidade experimental, para determinação da média de H, em cm, e comprimento radicular, avaliados aos sete DAS, oscilou de 11 a 55 plantas (Tabela 2). Os valores médios de H, avaliado aos sete DAS, oscilaram entre 12,94 e 20,90 cm, para as quatro repetições do tratamento sem Al. Na concentração de 4 mg L^{-1} Al, o valor médio de H avaliado aos sete DAS, oscilou entre 11,80 e 19,34 cm. Ao aumentar a dose de Al, para 12 mg L^{-1} de solução nutritiva, a média de H manteve valores semelhantes aos anteriores,

variando de 11,70 a 19,00 cm. Com a solução com 20 mg L^{-1} de Al a oscilação dos valores de H avaliados aos sete DAS ficou de 11,70 a 20,22 cm. De maneira geral as oscilações de H avaliadas foram pequenas (Tabela 2).

Tabela 1: Média (m), coeficiente de variação (CV, em %) e valor-p do teste de normalidade de Kolmogorov-Smirnov, dos caracteres H e comprimento radicular, avaliados aos dois DAS, em nove cultivares de trigo.

Bloco	Cultivar	H, em cm				Comprimento radicular, em cm			
		n	m	CV	Valor-p	n	m	CV	Valor-p[(1)]
1	Marocco	24	6,20	8,61	0,82	24	3,11	32,91	0,62
1	Ônix	24	6,76	6,51	0,97	24	3,63	49,96	0,66
1	Galego Rapado	24	7,47	8,44	0,59	24	3,57	15,57	0,68
1	IAC 5	24	7,92	7,19	0,26	24	3,38	55,87	0,22
1	Sumai 3	24	7,91	9,83	0,62	24	3,09	52,03	0,42
1	Anahuac	24	7,63	8,98	0,66	24	3,75	48,53	0,26
1	BR 23	24	7,95	7,34	0,58	24	3,65	39,66	0,66
1	BRS 208	24	9,40	7,21	0,78	24	4,84	28,67	0,85
1	BRS Umbu	24	7,60	5,24	0,68	24	4,07	25,97	0,53
2	Marocco	21	7,30	9,12	0,30	21	2,98	44,79	0,61
2	Ônix	24	7,26	10,00	0,73	24	4,14	42,27	0,97
2	Galego Rapado	24	9,13	10,51	0,45	24	3,18	42,41	0,60
2	IAC 5	24	9,38	11,16	0,82	24	3,91	52,94	0,14
2	Sumai 3	24	10,73	8,51	0,67	24	5,29	40,99	0,99
2	Anahuac	24	7,88	12,55	0,28	24	2,43	53,13	0,38
2	BR 23	24	8,78	12,83	0,97	24	3,40	50,22	0,70
2	BRS 208	24	11,14	13,10	0,74	24	6,43	22,69	0,28
2	BRS Umbu	24	8,97	10,44	0,87	24	4,85	29,74	0,97

[(1)]Valores do teste de Kolmogorov-Smirnov para normalidade da distribuição dos erros. Valor-p>0,05 Não-significativo (distribuição normal).

Os valores do CV para o caractere H avaliado aos sete DAS, oscilou de 6,55 a 22,54%, para a dose de 0mg L^{-1} de solução nutritiva de alumínio, de 6,63 a 23,01% para a dose de 4 mg L^{-1} de Al, oscilou de 6,91 a 28,71% e de 7,76 a 20,44%, para a dose de 12 e 20 mg L^{-1} de Al, respectivamente, aos sete DAS em nove cultivares de trigo (Tabela 2).

O teste de Kolmogorov-Smirnov, efetuado com os resultados de altura e planta aos sete DAS, na maioria dos casos, os dados ajustam-se a distribuição normal, dando credibilidade ao estudo de tamanho de amostra (Tabela 2).

O valor médio do C avaliado aos sete DAS, oscilou entre 6,72 e 14,02 cm, para as quatro repetições na dose 0 mg L^{-1} de Al. Na dose de 4 mg L^{-1} de Al, o valor médio do C avaliado, oscilou entre 3,47 e 9,73 cm. Ao aumentar a dose, para 12 mg L^{-1} de Al, a média do C manteve valores inferiores aos anteriores, variando de 2,23 a

7,96 cm, e no caso do uso da solução com 20 mg L^{-1} Al a oscilação dos valores de C avaliados aos sete DAS ficou de 0,43 a 6,53 cm (Tabela 3). De maneira geral, o comprimento radicular tende a diminuir à medida que foram expostas a maiores doses de alumínio e consequentemente ao um maior efeito fitotóxico do metal (TICE et al., 1992), atuando prontamente no sistema radicular devido sua alta sensibilidade de resposta (ZHAO et al., 2003; LIN, 2010).

Os valores do CV para C avaliado aos sete DAS, oscilou de 6,84 a 63,87%, para a dose de 0 mg L^{-1} de Al, de 10,26 a 74,41% para a dose de 4 mg L^{-1} de solução nutritiva de alumínio, de 18,56 a 78,83% e de 19,30 a 62,30%, para a dose de 12 e 20 mg L^{-1} Al na solução nutritiva, respectivamente (Tabela 3). O CV para C obteve maiores valores a medida que aumentou-se a concentração de alumínio na solução nutritiva, isso sugere que, para maiores valores de coeficiente de variação, maior tamanho de amostra (número de plantas) são necessários.

O teste de Kolmogorov-Smirnov, para comprimento radicular, avaliada aos sete DAS, na maioria dos casos ajustam-se a distribuição normal, dando credibilidade ao estudo de tamanho de amostra (Tabela 3).

Na tabela 4, o valor-p do teste F das plantas avaliadas aos dois DAS, demonstrou apenas efeito significativo de bloco para H, influenciado pela heterogeneidade de microclimas no interior da estufa plástica (LORENTZET et al., 2004). Os demais valores-p não foram significativos a 5% de probabilidade de erro do teste comparativo de média, principalmente, devido à homogeneidade da solução nutritiva utilizada nos blocos até o momento da avaliação.

Ao avaliarmos os cultivares de trigo expostos as quatro doses de alumínio, aos sete DAS, nota-se a perda de significância para o efeito de bloco para o caractere altura, ao passo que se acentua para efeito de dose no mesmo caractere, haja visto o efeito deletério do Al no desenvolvimento pleno da parte aérea da cultura, gerando variabilidade entre cultivares de diferentes níveis de tolerância (POSCHENRIEDE et al., 2008). Por motivo semelhante, o valor-p do teste F das plantas avaliadas aos sete DAS foi significativo para o efeito de dose e interação entre esse fator e cultivar no caractere C (Tabela 4), uma vez que o sistema radicular atua como primeiro sítio de acúmulo de Al (RYAN et al., 1993).

Tabela 2: Média (m), coeficiente de variação (CV, em %) e valor-p do teste de normalidade de Kolmogorov-Smirnov, da H, em cm, avaliada aos sete DAS, em nove cultivares e quatro doses de alumínio (0, 4, 12 e 20 mg L-1) aleatorizadas em quatro blocos, num determinado número de plantas (n) por unidade experimental.

Bloco	Cultivar	0 mg L^{-1}				4 mg L^{-1}				12 mg L^{-1}				20 mg L^{-1}			
		n	m	CV	Valor-p	n	m	CV	Valor-p	n	m	CV	Valor-p	n	m	CV	Valor-p[1]
1	Marocco	40	14,71	14,43	0,29	17	17,48	7,90	0,98	18	13,82	14,43	0,99	16	14,83	14,27	0,90
1	Ônix	38	14,82	11,73	0,42	41	13,68	10,04	0,25	38	13,72	11,44	0,63	38	13,46	7,90	0,77
1	Galego Rapado	18	19,54	9,20	0,88	17	18,75	13,76	0,44	19	13,63	28,71	0,87	19	18,75	16,88	0,43
1	IAC 5	19	19,38	16,12	0,95	18	19,14	8,21	0,99	21	17,87	15,85	0,56	24	20,22	10,61	0,96
1	Sumai 3	17	18,15	9,17	0,98	21	14,77	10,87	0,98	19	16,72	10,54	0,99	19	14,12	12,41	0,67
1	Anahuac	21	17,02	12,47	0,37	22	13,98	11,34	0,54	21	12,05	16,02	0,99	21	15,25	11,90	0,76
1	BR 23	55	17,71	11,65	0,05	36	17,32	7,78	0,97	40	16,78	13,74	0,39	35	17,46	7,76	0,92
1	BRS 208	20	17,80	19,39	0,84	19	19,23	7,91	0,97	19	18,26	13,31	0,28	17	18,55	12,06	0,17
1	BRS Umbu	35	18,33	9,78	0,11	21	16,95	15,38	0,68	19	17,94	6,91	0,94	21	17,08	13,63	0,21
2	Marocco	18	16,16	22,53	0,61	17	15,36	9,66	0,77	18	13,14	11,20	0,72	19	14,24	8,30	0,89
2	Ônix	36	14,77	7,97	0,83	41	13,88	9,41	0,53	43	13,12	11,24	0,88	41	13,89	10,25	0,42
2	Galego Rapado	13	20,90	8,43	0,53	21	17,63	14,89	0,95	18	17,48	13,26	0,93	12	18,85	12,97	0,89
2	IAC 5	20	19,34	10,80	0,99	22	18,64	20,42	0,51	21	18,77	14,90	0,93	19	18,76	9,51	0,59
2	Sumai 3	19	18,96	6,55	0,95	21	15,56	9,49	0,84	23	16,76	12,24	0,74	17	13,91	26,27	0,27
2	Anahuac	23	16,55	14,23	0,89	17	15,09	7,86	0,99	19	13,51	13,10	0,99	23	14,22	16,00	0,51
2	BR 23	41	17,73	10,32	0,50	39	16,54	9,92	0,97	36	15,84	10,94	0,77	38	15,99	11,12	0,59
2	BRS 208	17	18,79	14,07	0,99	21	19,34	12,42	0,86	20	17,35	10,46	0,83	18	16,41	13,59	0,81
2	BRS Umbu	21	18,58	8,78	0,75	18	18,02	8,41	0,27	19	16,39	12,00	0,19	22	16,48	11,03	0,89
3	Marocco	19	13,57	13,92	0,92	16	12,56	16,32	0,87	13	12,72	15,01	0,79	12	11,89	20,44	0,98
3	Ônix	39	13,85	11,85	0,94	39	13,16	14,33	0,59	39	11,70	12,81	0,71	35	11,70	13,39	0,50
3	Galego Rapado	21	16,81	22,54	0,94	18	18,01	12,94	0,95	18	15,53	12,44	0,99	16	16,20	19,13	0,69
3	IAC 5	19	17,17	13,28	0,76	20	15,14	15,37	0,97	17	16,08	12,65	0,05	19	15,16	16,57	0,72
3	Sumai 3	21	18,79	10,07	0,37	20	16,08	6,63	0,61	22	14,81	10,00	0,51	18	14,74	14,29	0,97
3	Anahuac	21	15,00	16,98	0,93	16	13,39	20,75	0,15	19	13,48	15,24	0,92	18	11,88	18,89	0,91
3	BR 23	38	16,30	15,84	0,38	37	13,99	15,81	0,82	39	15,68	11,28	0,39	33	15,37	10,96	0,73
3	BRS 208	21	20,20	13,06	0,98	17	18,20	14,24	0,84	19	17,49	10,99	0,91	17	17,49	15,18	0,94
3	BRS Umbu	21	18,44	8,55	0,94	22	15,26	9,65	0,92	21	15,70	13,45	0,55	18	15,68	7,96	0,91
4	Marocco	11	12,94	16,77	0,76	18	12,07	13,95	0,99	19	13,21	11,89	0,80	14	12,95	15,74	0,76
4	Ônix	41	14,24	12,28	0,50	42	11,80	14,59	0,36	26	11,72	14,83	0,93	37	10,38	14,61	0,91
4	Galego Rapado	21	17,68	18,25	0,49	14	15,00	23,01	0,63	18	15,71	17,35	0,99	18	14,56	12,46	0,91
4	IAC 5	21	17,70	12,59	0,82	17	17,73	9,75	0,83	19	18,20	13,19	0,88	16	15,68	11,99	0,99
4	Sumai 3	18	18,73	13,93	0,73	23	16,14	7,85	0,35	30	15,29	16,48	0,79	18	16,39	7,81	0,78
4	Anahuac	18	15,49	12,13	0,99	19	14,88	12,64	0,65	16	12,41	16,01	0,37	19	12,52	12,22	0,59
4	BR 23	26	15,52	15,77	0,35	37	14,45	16,25	0,23	30	15,24	11,79	0,80	27	15,99	11,47	0,89
4	BRS 208	17	17,64	18,37	0,99	19	17,80	11,56	0,84	20	19,00	9,93	0,30	19	17,31	14,91	0,55
4	BRS Umbu	21	16,69	9,16	0,99	21	17,37	9,72	0,55	17	15,00	11,90	0,62	22	16,96	11,25	0,24

[1]Valores do teste de Kolmogorov-Smirnov para normalidade da distribuição dos erros. Valor-p>0,05 Não-significativo (distribuição normal).

Tabela 3: Média (m), coeficiente de variação (CV, em %) e valor-p do teste de normalidade de Kolmogorov-Smirnov, do comprimento radicular, em cm, avaliada aos sete DAS, em nove cultivares e quatro doses de alumínio (0, 4, 12 e 20 mg L-1) aleatorizadas em quatro blocos, num determinado número de plantas (n) por unidade experimental.

Bloco	Cultivar	0 mg L^{-1}				4 mg L^{-1}				12 mg L^{-1}				20 mg L^{-1}			
		n	m	CV	Valor-p	n	m	CV	Valor-p	n	m	CV	Valor-p	n	m	CV	Valor-p
1	Marocco	40	7,53	34,53	0,12	17	4,08	54,51	0,79	18	6,04	30,29	0,54	16	4,01	43,49	0,96
1	Ônix	38	10,83	22,97	0,67	41	8,42	28,10	0,50	38	3,84	74,55	0,06	38	4,79	40,80	0,17
1	Galego Rapado	18	8,34	34,08	0,11	17	4,21	32,90	0,30	19	3,17	37,47	0,67	19	4,20	25,52	0,26
1	IAC 5	19	10,96	29,56	0,85	18	8,72	41,54	0,91	21	5,34	48,62	0,78	24	5,40	41,12	0,63
1	Sumai 3	17	9,95	24,64	0,81	21	6,60	34,49	0,66	19	4,94	38,53	0,96	19	2,24	48,41	0,18
1	Anahuac	21	8,28	41,72	0,96	22	3,47	42,00	0,99	21	2,23	78,83	0,16	21	4,68	31,70	0,83
1	BR 23	55	11,24	25,88	0,18	36	6,68	54,60	0,52	40	6,51	41,14	0,35	35	5,27	48,75	0,84
1	BRS 208	20	9,22	44,16	0,84	19	8,83	33,35	0,27	19	5,58	49,11	0,56	17	4,76	40,29	0,65
1	BRS Umbu	35	10,39	6,84	0,42	21	7,30	10,26	0,86	19	5,89	26,34	0,53	21	5,64	21,80	0,36
2	Marocco	18	9,18	26,41	0,87	17	7,25	18,33	0,92	18	4,73	38,87	0,97	19	3,86	52,21	0,63
2	Ônix	36	10,09	35,58	0,38	41	7,99	29,45	0,24	43	3,12	74,42	0,09	41	5,61	31,96	0,13
2	Galego Rapado	13	9,02	16,31	0,94	21	3,59	34,29	0,97	18	3,51	25,65	0,58	12	3,62	21,38	0,78
2	IAC 5	20	10,14	29,55	0,95	22	9,73	19,72	0,99	21	7,45	21,11	0,88	19	4,22	49,84	0,65
2	Sumai 3	19	9,39	18,88	0,96	21	3,67	54,46	0,49	23	5,69	42,90	0,81	17	2,93	62,30	0,63
2	Anahuac	23	10,27	25,60	0,93	17	4,30	36,08	0,58	19	3,95	41,04	0,91	23	3,47	51,94	0,85
2	BR 23	41	10,59	25,09	0,75	39	8,04	28,48	0,48	36	6,39	40,68	0,22	38	3,87	59,44	0,64
2	BRS 208	17	11,55	15,55	0,78	21	7,49	40,79	0,35	20	5,00	47,76	0,93	18	4,34	37,04	0,69
2	BRS Umbu	21	9,69	14,77	0,93	18	7,47	11,39	0,65	19	6,12	18,56	0,47	22	4,44	25,97	0,54
3	Marocco	19	8,02	34,33	0,63	16	4,60	45,52	0,96	13	4,87	38,34	0,64	12	3,63	36,69	0,86
3	Ônix	39	9,89	45,16	0,29	39	4,63	66,05	0,10	39	4,04	57,56	0,36	35	3,82	26,27	0,98
3	Galego Rapado	21	8,54	35,90	0,73	18	4,72	36,23	0,99	18	3,02	42,83	0,89	16	3,86	19,30	0,93
3	IAC 5	19	7,76	52,90	0,90	20	6,38	38,96	0,88	17	5,67	39,78	0,99	19	6,05	32,59	0,85
3	Sumai 3	21	12,86	20,73	0,53	20	6,68	28,18	0,41	22	4,44	38,71	0,71	18	4,56	58,90	0,33
3	Anahuac	21	6,09	63,87	0,64	16	3,63	65,45	0,44	19	3,91	40,13	0,59	18	2,88	44,07	0,28
3	BR 23	38	10,38	32,99	0,55	37	4,77	71,78	0,16	39	6,99	42,92	0,75	33	5,07	50,79	0,97
3	BRS 208	21	14,02	17,04	0,82	17	9,08	32,06	0,72	19	7,53	32,99	0,90	17	6,44	37,45	0,87
3	BRS Umbu	21	12,05	9,13	0,46	22	5,20	46,17	0,87	21	6,00	39,76	0,36	18	6,21	21,40	0,37
4	Marocco	11	7,22	28,56	0,99	18	5,11	36,80	0,89	19	5,28	37,96	0,89	14	4,16	52,76	0,65
4	Ônix	41	10,66	29,96	0,79	42	4,48	65,59	0,04	26	4,83	54,46	0,53	37	3,11	39,71	0,07
4	Galego Rapado	21	7,40	41,57	0,94	14	3,76	34,96	0,94	18	2,93	34,91	0,80	18	1,61	51,56	0,77
4	IAC 5	21	10,64	30,48	0,64	17	4,86	74,41	0,67	19	6,38	46,93	0,66	16	2,78	61,71	0,34
4	Sumai 3	18	11,28	21,38	0,85	23	6,58	23,12	0,55	30	5,89	35,41	0,74	18	5,73	41,78	0,74
4	Anahuac	18	6,72	39,56	0,73	19	3,89	73,71	0,67	16	2,86	41,01	0,20	19	3,36	33,61	0,47
4	BR 23	26	10,12	30,14	0,31	37	5,64	61,07	0,40	30	5,21	60,71	0,49	27	6,20	39,20	0,32
4	BRS 208	17	12,73	17,63	0,98	19	8,18	41,08	0,52	20	7,96	38,10	0,56	19	0,43	39,42	0,16
4	BRS Umbu	21	7,61	33,93	0,14	21	8,81	19,14	0,30	17	5,02	39,26	0,56	22	6,53	34,36	0,17

(1)Valores do teste de Kolmogorov-Smirnov para normalidade da distribuição dos erros. Valor-p>0,05 Não-significativo (distribuição normal).

Tabela 4: Número de graus de liberdade (GL), quadrado médio e valor-p do teste F, entre parênteses, das fontes de variação e média, para os tamanhos de amostra de dois caracteres ([1]) de plantas de trigo avaliados em 9 tratamentos, aos dois DAS (nove cultivares) e 36 tratamentos, aos sete DAS (nove cultivares × quatro doses de alumínio).

Fonte de Variação	GL	H	C
		Avaliação aos dois DAS	
Bloco	1	30,175 (0,0043)	321,588 (0,5099)
Cultivar	8	1,212 (0,7419)	2.220,155 (0,1075)
Erro	8	1,951	676,006
Média (número de plantas)	-	3,91	75,90
			Avaliação aos sete DAS
Bloco	3	32,436 (0,2874)	3.838,716 (0,1400)
Cultivar	8	89,737 (0,0012)	9.112,568 (0,0001)
Dose	3	9,675 (0,7676)	14.450,098 (0,0002)
Cultivar×Dose	24	20,695 (0,7140)	3.175,575 (0,0699)
Erro	105	25,476	2.058,072
Média (número de plantas)	-	8,01	72,90

[(1)] H = tamanho de amostra (número de plantas) para a H e C = tamanho de amostra (número de plantas) para o comprimento radicular, com erro de estimação de 10%.

O tamanho de amostra (número de plantas), para estimar a média do caractere H, com semi-amplitude do intervalo de confiança igual a 2% da estimativa da média e grau de confiança de 95 %, oscilou entre 126 (cultivar BRS Umbu), a 317 plantas (cultivar Galego Rapado) (Tabela 5), sendo que as cultivares diferem pelo teste de Scott Knott. Variabilidade do tamanho de amostra para altura também foi encontrado na cultura do algodoeiro herbáceo (FREITAS et al., 2001). Na cultura de soja, o tamanho de amostra de 28 genótipos de soja para o intervalo com 95% de confiança, avaliados em cinco experimentos, oscilou de 32 a 115 (CARGNELUTTI FILHO et al., 2009).

A variabilidade do tamanho de amostra, para a estimação da média do caractere H em quatro doses de alumínio, com semi-amplitude do intervalo de confiança igual a 2% da estimativa da média e grau de confiança de 95 %, oscilou de 182 plantas para a dose 4 mg L^{-1} de Al a 211 plantas para a dose 20 mg L^{-1} de Al, porem não houve diferença significativa pelo teste de Scott Knott quanto a altura, a medida que foi alterada a concentração de Al.

Tabela 5: Tamanho de amostra (número de plantas) para avaliação dos caracteres altura de planta e comprimento radicular, aos sete dias após a semeadura, para as semi-amplitudes do intervalo de confiança de 95% (erro de estimação) iguais a 2, 4, 6, 8 e 10% da estimativa da média, para nove cultivares de trigo e quatro doses de alumínio (0, 4, 12 e 20 mg L^{-1}).

Cultivar/Dose	2%	4%	6%	8%	10%
			Altura de planta		
Marocco	246	62	28	16	10 a
Ônix	148	37	17	10	6 b
Galego Rapado	317	80	36	20	13 a
IAC 5	203	51	23	13	9 b
Sumai 3	171	43	19	11	7 b
Anahuac	235	59	27	15	10 a
BR 23	157	40	18	10	7 b
BRS 208	203	51	23	13	9 b
BRS Umbu	126	32	14	8	6 b
0 mg L^{-1}	205	52	23	13	9 a
4mg L^{-1}	182	46	21	12	8 a
12 mg L^{-1}	205	52	23	13	9 a
20 mg L^{-1}	211	53	24	14	9 a
			Comprimento radicular		
Marocco	1.748	437	195	110	70 b
Ônix	2.398	600	267	150	96 a
Galego Rapado	1.288	322	144	81	52 b
IAC 5	2.098	525	234	132	84 a
Sumai 3	1.708	427	190	107	69 b
Anahuac	2.670	668	297	167	107 a
BR 23	2.250	563	250	141	90 a
BRS 208	1.483	371	165	93	60 b
BRS Umbu	764	191	85	48	31 b
0 mg L^{-1}	1.084	271	121	68	44 b
4mg L^{-1}	2.111	528	235	132	85 a
12 mg L^{-1}	2.152	538	240	135	87 a
20 mg L^{-1}	1.946	487	217	122	78 a

* Médias de cultivares e de doses não seguidas de mesma letra diferem pelo teste de Scott Knott em nível de 5% de probabilidade. Nas colunas referentes aos erros de estimação de 2, 4, 6 e 8%, as letras são as mesmas da coluna referente ao erro de estimação de 10%, e, por isso, não foram colocadas.

O tamanho de amostra, para a estimação da média do caractere C nas cultivares, com semi-amplitude do intervalo de confiança igual a 2%, oscilou entre

2.670 (cultivar Anahuac) a 764 plantas (cultivar BRS Umbu), sendo que as cultivares diferem pelo teste de Scott Knott. Por sua vez, a variabilidade do tamanho de amostra, para estimar a média do C em quatro doses de alumínio, na mesma semi-amplitude do intervalo de confiança, oscilou de 1.084 plantas para a dose 0 mg L^{-1} de Al a 2.152 plantas para a dose 12 mg L^{-1} de Al, havendo diferença pelo teste de Scott Knott, sendo que a menor dose proporcionou menor tamanho de amostra (número de plantas).

Conclusões

Conclui-se que há variabilidade na estimativa do tamanho de amostra entre cultivares, quanto a altura e comprimento radicular, e entre doses de alumínio quanto ao comprimento radicular. Considerando que o tamanho de amostra médio contempla, em geral, um maior número de casos, é tecnicamente correto optar pelo maior número médio de tamanho de amostra dentre os caracteres observados. Assim, nas condições testadas, apenas a variabilidade existente devido o efeito cultivar se mostrou necessidade ser levada em consideração na estimativa do tamanho de amostra, uma vez que independente da semi-amplitude do intervalo de confiança da estimativa da média (m) a um grau de confiança de 95 %, a variabilidade do tamanho de amostra (número de plantas) foi maior entre cultivares do que entre doses de alumínio.

Deve-se utilizar pelo menos 9 e 74 plantas para avaliação de altura e comprimento radicular de cultivares de trigo em estudos de tolerância ao Al, respectivamente, considerando ainda que estimativas com precisão inferior a 10%, de modo geral, são impraticáveis.

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CAPÍTULO III

ATIVIDADE DE FOSFATASE ÁCIDA E SUA RELAÇÃO COM EFICIÊNCIA DE USO DE RESPOSTA DE CULTIVARES DE TRIGO AO FÓSFORO

MANUSCRITO III

ACID PHOSPHATASE ACTIVITY AND ITS RELATION TO PHOSPHORUS USE AND RESPONSE EFFICIENCY IN WHEAT CULTIVARS

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Abstract

Soil phosphorus (P) deficiency is an important constraint worldwide, limiting crop productivity of the world's arable land. Phosphorus importance regards on its role on cellular components and enzymatic reactions. To deal with P deficiency, plants have develop an array of adaptive responses such as acid phosphatases production, an enzyme group that functions both intracellular and extracellular hydrolyzing organic-P complexes into inorganic-P, thus proposed to enhance plant P acquisition and use. This study aimed to evaluate the relationship of tissue acid phosphatase activity (APA) and P use and response efficiency of wheat (*Triticum aestivum* L.) cultivars under 10 and 125 µM P, evaluating P use, response, uptake and translocation efficiency, P content and concentration in plant tissue, APA in shoot and root, as well as leaf area, height and dry matter yield, applying principal component analysis on the parameters to identify response patterns. Data showed that P deficiency mainly

repressed shoot growth, closely relating shoot dry weight with P responsiveness and use efficiency. In general, growth parameters had better correlation with P uptake efficient and responsive cultivars rather than with P use efficient, presenting low APA in shoot tissue at high P supply. High P use efficiency (PUE) in shoot tissue enhanced cultivars response. Through APA pattern, became clear that P use efficiency is not fully explained by root APA but instead presented a consistent pattern with shoot APA. This way, it is not recommended using root APA as a physiological marker for evaluation of wheat seedlings in relation to P response and use efficiency under low P conditions, while shoot APA could be used to identify tendencies of those parameters.

Key-words: *Triticum aestivum* L.; Acid phosphatase activity; Phosphorus; Use efficiency; Response efficiency.

Abbreviations: Acid phosphatase activity (APA), efficient and not responsive (ENR), efficient and responsive (ER), not efficient and not responsive (NENR), nutrient translocation efficiency (S/T), phosphorus (P), phosphorus response efficiency (PRE), phosphorus uptake efficiency (PUtE), phosphorus use efficiency (PUE), responsive and not efficient (RNE), responsive and not efficient (RNE), root dry weight (RDW), shoot dry weight (SDW).

Introduction

Soil phosphorus (P) deficiency is an important constraint worldwide, limiting crop productivity in 40% of the world's arable land (VANCE, 2001), with overall yield reduction of 5 to 15% under sob-optimal levels (SHENOY; KALAGUDI, 2005). Phosphorus importance regards on its role on cellular components such as nucleotides, nucleic acids and membranes, still serving mobile metabolic energy storage components such as ATP and, consequently, acting as a key component for the regulation of many enzymatic reactions and in signal transduction processes that depends on it (MARSCHNER, 1995). Despite the wide deficiency, P starvation can be easily supplied during crops cycle by chemical fertilizer. However, only 15 to 30%

of the applied P fertilizer is taken up by crops in the first year of its application (SYERS et al., 2008) and due to P capacity to form compounds with high binding energy and stability in the soil solid phase, only a small fraction of the nutrient desorb and become available to plants in the soil solution (GATIBONI, 2003), leading to P deficiency on 25% of weathered tropical and subtropical soils even under P application as fertilizer (SANCHES; LOGAN, 1992), which allied to the limited global rock phosphate reserves, becomes a matter of concern specially in developing countries (CORDELL et al., 2009).

Plants achieve P mainly by root uptake of inorganic phosphate (orthophosphate, Pi) (RAGHOTHAMA, 1999), available in a soil pH-dependent matter (SANCHEZ, 2007). Under P starvation, plants have evolved an array of adaptive responses, such as altering root morphology, exudation and P uptake mechanisms to provide higher P acquisition by enabling plants to explore new Pi resources (RAGHOTHAMA, 1999). On the other hand, plants can increase P recycling and scavenging using alternative metabolic pathways to bypass steps requiring P in order to increase its use efficiency, enhancing biomass production per unit of absorbed P (VANCE et al., 2003), providing accelerated growth and greater biomass accumulation in harvestable tissues (VENEKLAAS et al., 2012). Since most of the soil P must be mobilized by root-secreted such as phosphatase enzymes to become available (CORDELL et al., 2009), great attention have been given to the issue, with variable responses reported among plant species (TADANO et al., 1993; LI et al., 1997) and genotypes (GAUME et al., 2001).

Acid phosphatase functions both intracellular (vacuolar) and extracellular (secreted), hydrolyzing organic-P complexes into inorganic-P aiming its efficient transport and remobilization, thus improving plant P acquisition and subsequent use (DUFF et al., 1994). Lower root or leaf acid phosphatase activities (APA) are related to adequate or sufficient P-tissue concentrations (DUFF et al., 1994) as well as with lower P demand, both inhibiting APA by feedback regulation effect (MCLACHLAND; DE MARCO, 1982), thus it could be used as a trait to evaluate plant P efficiency. However, literature data have shown different responses regarding APA and P supply. Tadano et al. (1993), studying nine plant species, observed different magnitudes in root APA increase in response to P deficiency. Positive correlations between leaf and root enzyme activity and P content have been found in bean and

cowpea (FERNANDEZ; ASCENCIO, 1994), while negative correlations were demonstrated in maize (ELLIOT; LÄUCHLI, 1986), proposing that APA could be useful in confirming visual diagnosis rather than to detect P deficiency. Furlani et al. (1984), investigating sorghum and Helal (1990) working with common beans, observed large differences among tolerant and susceptible cultivars to low external P concentrations and evidences have shown that not necessary APA confer adaptation to low phosphorus availability (XIAOLONG et al., 2001) or could be used an efficient physiological marker (MACHADO; FURLANI, 2004) despite positive results found at gene modification level (over-expressing phosphatase) an enhancement in Pi acquisition (TIAN et al., 2012).

Regarding wheat genotypes, studies have shown different nutrient requirements and growth capacities (VALIZADEH et al., 2002; LI et al., 2003; QIU et al., 2004). Thus, once variations on APA depend not just on P availability, but also indirectly by the growth rate, consuming P due to its ATP demand (MARSCHNER, 1995), this study aimed to evaluate the relationship of tissue APA to P use and response efficiency of wheat cultivars to provide better understanding of wheat genotypes to different P supply.

Material and methods

Plant growth conditions and growth measurements

The experiment was carried out using nine wheat (*Triticum aestivum* L.) cultivars (Anahuac, BR 23, BRS 208, BRS Umbu, Galego rapado, IAC 5, Marocco, Ônix and Sumai 3) provided by the Brazilian Agricultural Research Corporation (EMBRAPA), following a randomized complete block design with four replications. Seeds were surface sterilized with 2% sodium hypochlorite, rinsed and germinated in darkness at 24±2C°. After 2 d, uniform seedlings were transferred into a 10 L plastic container, containing a nutrient solution composed of (mg L^{-1}): 85.31 N; 7.54 P; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 173.36 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe during 2 d for preconditioning. Treatments consisted on two

P levels (10 µM and 125 µM P as KH_2PO_4) and exposure occurred until plants, under the higher P concentration, began tillering (13 d). Plants were kept in a climatized greenhouse at 25±2C° under natural day light. Nutrient solutions were continuously aerated and renewed every two days. The pH was maintained at 5.5 with daily additions of 0.1 M KOH or HCl drop-wise. At the end of the experiment, shoot and root tissues were separately oven-dried at 45°C until constant weight to determine shoot (SDW) and root (RDW) dry weight. Height was obtained using a mm ruler and leaf area was measured by pixel counting using Adobe Photoshop 8.0.1 image editor software.

Phosphorus efficiency and accumulation

Dried shoot and root tissues were separately grounded in a Wiley grounder with a 1 mm mesh. Samples were digested according to Tedesco et al. (1995) using 0.2 g of dry tissue with 0.7 of digestion mixture (100g Na_2SO_4, 10g $CuSO_4.5H_2O$ and 1g Se) diluted in H_2SO_4 (0.025 M) with H_2O_2 (30%) at 350°C. The digested solution was then set in a colorimeter for P determination according to Murphy and Riley (1962). Using dry weight values and the P concentration in the tissues, measurements of P efficiency were calculated. P use efficiency (PUE) was calculated based on dry matter yield (MS) related to the P concentration in the respective plant part tissues, that is, for the whole plant, PUE = $(\text{total dry weight})^2$/total plant P content (SIDDIQI and GLASS, 1981). P uptake efficiency (PUtE) was evaluated as PUtE = total plant P content/root dry weight (SWIADER et al, 1994) and P response efficiency (PRE) as the yield increase, expressed by the total dry weight, per unit increase in P supply (EPSTEIN; BLOOM, 2005).

Acid phosphatase activity

Plants were harvested, frozen in liquid nitrogen and stored at -20°C. Subsequently, roots and shoots were manually grounded in liquid nitrogen and homogenized in 100 mM Tris-HCl (pH 7.4), ethylenediaminetetraacetic acid (EDTA) 1.0 mM and 0.1% albumin buffer, then centrifuged at 20000 G for 30 min at -4°C. Only supernatant was used for enzymatic assay. Acid phosphatase activity was determined according to Tabaldi et al. (2007) in the reaction medium consisted of 3.5 mM NaN_3, 2.5 mM NaCl and 100 mM citrate buffer (pH 5.5) to a final volume of 200 µL. Except control samples, an aliquot of 20 µL was then added to the reaction mixture and pre-incubated for 10 min at 35°C. Reaction was initiated by substrate (3.0 mM PPi) addition and paralyzed after 10 min by adding 200 µL of TCA 10% to a final concentration of 5 %. Inorganic phosphate (Pi) was measured at 630 nm in a spectrophotometer SF325NM (Bel Engineering, Italy) using malachite green as a colorimetric reagent and KH_2PO_4 as standard for the calibration curve.

Statistical analysis

Cultivars were evaluated regarding height, leaf area dry matter yield, acid phosphatase activity, P concentration and P accumulation and use efficiency. Results were submitted to Tukey Test (P≤0,05) using Sisvar program (FERREIRA, 2008). Principal components analysis (PCA) was performed using Canoco 4.5 program and Microsoft Excel 2010 for clusters formation by Pearson's correlation (>0,7).

Results and discussion

Wheat cultivars differed in relation to PUE and PRE, as well as in APA in root and shoot tissues under low and high P levels. Through P efficiency diagram, four groups were clustered in relation to PUE and PRE (fig. 1). ultivars BRS Umbu, BR 23 and IAC 5 were classified as P use and response efficient (ER); cultivars Marocco and Galego Rapado were classified as responsive and not efficient (RNE); cultivar

Ônix was classified as not efficient and not responsive (NENR) and Anahuac were classified as P efficient and not responsive (ENR). The cultivars BRS 208 and Sumai 3 were intermediaries between ER to ENR and NENR to ENR respectively (fig. 1).

Interestingly, for the group of ER plants, with the exception of cultivar BR 23, root APA did not change as the increment on P level occurred. On the other hand shoot APA decrease under high P level. Conversely, Anahuac cultivar (ENR) did not change shoot APA with P changes, while both NENR and RNE plants showed lower APA under high P in shoot and root tissues (fig. 1).

In order to provide better understanding of the APA response pattern, one cultivar of each group was analyzed regarding P content, concentration and growth parameters, being IAC 5 (ER), Marocco (RNE), Ônix (NENR) and Anahuac (ENR).

Acid phosphatase activity by hydrolyzing organic-P complexes into inorganic-P is largely related with P transport and remobilization, thus improving plant P acquisition and subsequent use (DUFF et al., 1994). Additionally, APA confers adaptation to low phosphorus availability and an enhancement in Pi acquisition (TIAN et al., 2012). In this view ER plants, with no differences between APA under different P levels, suggests another adaptive system.

In relation to plant tissue P concentration, differences were observed only among P treatments despite plant tissue (Table 1), with similar values between shoots and roots and higher values at 125 µM P. Phosphorus accumulation was different for shoot and total plant tissue. Dependent of the dry matter yield, greater accumulation was obtained in the ER cultivar IAC 5 despite of P level, followed by Marocco, Anahuac and Ônix. Freitas et al. (1999), studying lime and P application as mineral fertilizer, found high correlation (0.83) between dry matter yield and grain production in wheat cultivars. Therefore, even though in the present study plants were not grown until the end of its cycle, there is a tendency of greater grain production for P accumulator cultivars, with higher P accumulation.

Although second in P content, P concentration demonstrate that Marocco was less efficient than Anahuac (table 3), using more P to produce its dry weight. Shoot/total P content ratio (S/T), indicting nutrient translocation efficiency (LI et al., 1991), showed higher translocation at 125 µM P in all cultivars. NENR cultivar Ônix presented the lower translocation efficiency at 10 µM P and Anahuac the highest value at 125 µM P.

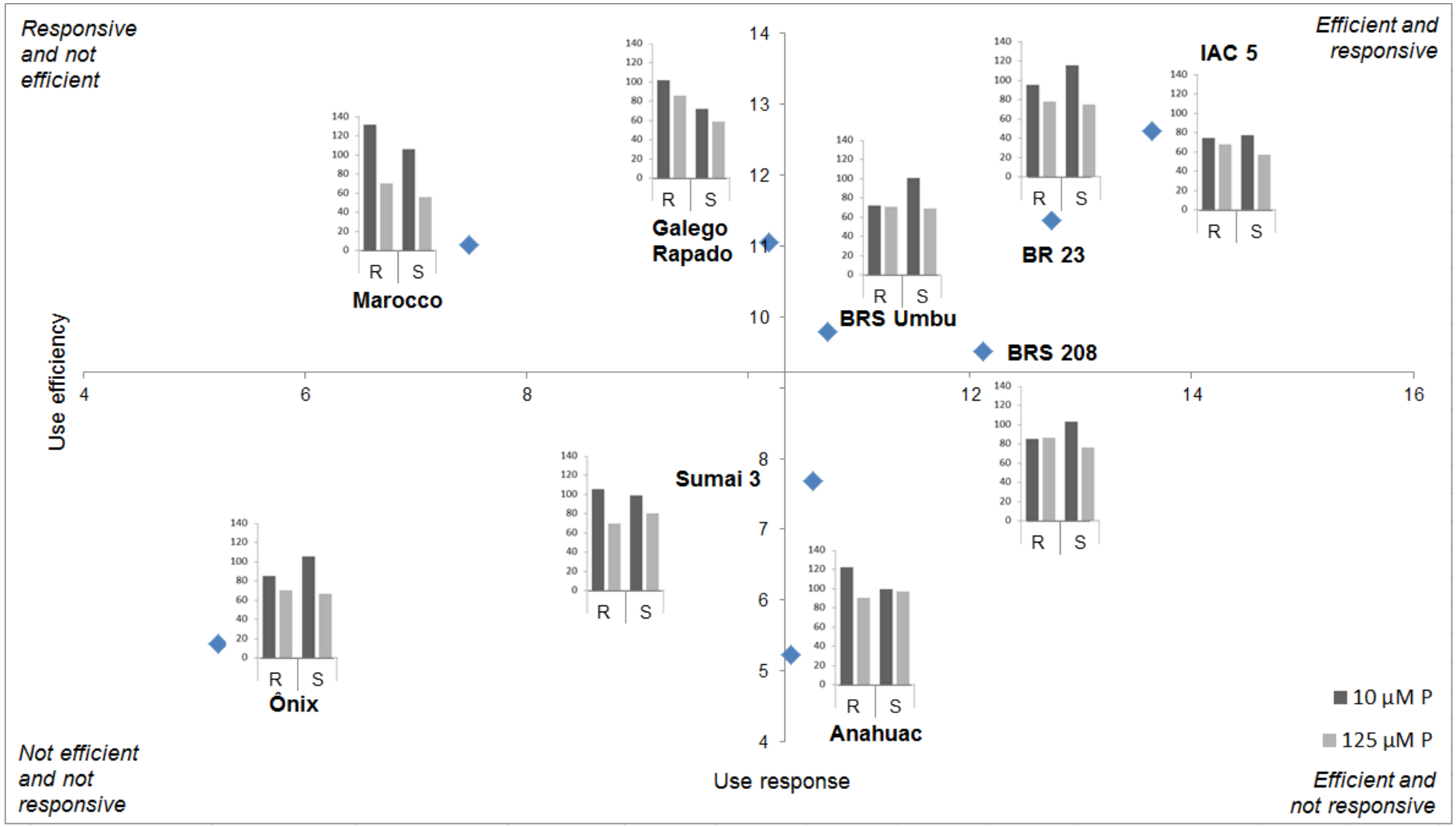

Figure 1: Phosphorus (P) efficiency diagram, displaying nine wheat cultivars based on the P use efficiency for the horizontal axis and P response efficiency for vertical axis. Graphics plotted inside the diagram represent the acid phosphatase activity in root (R) and shoot (S) tissue.

Overall S/T results suggest that this parameter may not be related to PUE, but could somehow be linked to P responsiveness, once both responsive cultivars IAC 5 and Marocco presented higher values at 10 µM P.

Table 1: Phosphorus concentration and content in plant parts(shoot, root, total) and shoot/total P content ratio (S/T), in wheat cultivars exposed to 10 and 125 µM P, differently classified upon P use and response efficiency.

Cultivar	P level	P concentration		P accumulation			
		Shoot	Root	Shoot	Root	Total	S/T
	µM	..... mg P g^{-1} DW		..... mg P plant $tissue^{-1}$.....			
Marocco	10	1.69 *b*	1.81 *b*	0.21 *Ab*	0.09 *b*	0.30 *Ab*	0.70
	125	3.65 *a*	3.59 *a*	0.86 *Ba*	0.23 *a*	1.10 *Ba*	0.78
Ônix	10	1.30 *b*	1.64 *b*	0.11 A*b*	0.08 *b*	0.19 *Bb*	0.58
	125	3.93 *a*	3.12 *a*	0.61 *Ba*	0.18 *a*	0.80 *Ba*	0.76
Anahuac	10	1.42 *b*	1.56 *b*	0.20 *Ab*	0.09 *b*	0.30 *Ab*	0.67
	125	4.98 *a*	3.18 *a*	0.81 *Ba*	0.18 *a*	0.99 *Ba*	0.82
IAC 5	10	1.30 *b*	1.49 *b*	0.22 *Ab*	0.09 *b*	0.31 *Ab*	0.71
	125	4.33 *a*	3.41 *a*	1.20 *Aa*	0.26 a	1.60 *Aa*	0.75

Capital letters indicate mean comparisons among cultivars while lowercase letters indicate mean comparisons between phosphorus (P) levels in the nutrient solution. Tukey's test (p≤0,05).

Although second in P content, P concentration demonstrate that Marocco was less efficient than Anahuac (Table 3), using more P to produce its dry weight. Shoot/total P content ratio (S/T), indicating nutrient translocation efficiency (LI et al., 1991), showed higher translocation at 125 µM P in all cultivars. NENR cultivar Ônix presented the lower translocation efficiency at 10 µM P and Anahuac the highest value at 125 µM P. Overall S/T results suggest that this parameter may not be related to PUE, but could somehow be linked to P responsiveness, once both responsive cultivars IAC 5 and Marocco presented higher values at 10 µM P.

Leaf area showed great response to P deficiency, varying 23.9, 39.4, 41.1 and 43.9% for Anahuac, IAC 5, Marocco and Ônix respectively. P deficiency can limit the size of individual leaves by reducing cell division and elongation (JACOB; LAWLOR, 1991), affect hydraulic conductivity in roots and consequently produce a lack of turgor for cell expansion (RADIN; EIDENBOK, 1984) as well as expansion properties of the cell wall (PRITCHARD et al., 1991). Reduced leaf area implicates in limited light interception, this way P depletion affects overall growth by reducing photosynthetic capacity (RODRÍGUEZ et al., 1998), reducing the regeneration of

ribulose 1,5-biphosphate (RAO; TERRY, 1995) and the number of leaves per plant due to the lower emergency of tillers (SATO et al., 1996), and to a slower rate of leaf emergence per stem (RODRÍGUEZ; GOUDRIAAN, 1995). In this study, P deficiency decreased the rate of tiller emergence and increased its duration, resulting in less tillers per plant in low P supply (data not shown).

Height was less affected, varying 5.4, 12.9, 16.1 and 20.4% respectively for Anahuac, Marocco, IAC 5 and Ônix. Despite relative variation within cultivars, highest values were obtained for P ER cultivar IAC 5 while lowest values were obtained by the P NENR cultivar Ônix. In relation to dry matter yield of shoot and root, highest values were observed for IAC 5, although only slightly differences were found among cultivars and P levels for root dry weight (RDW). The cultivar Marocco presented the second higher shoot dry weight (SDW) under 125 µM P, followed by Anahuac and Ônix. At 10 µM P, Anahuac presented higher SDW than Marocco, an expected result due to its enhanced PUE for this parameter (Table 3). Total dry weight reflected SDW pattern and R/S ratio increased at low P level in all cultivars, especially on Ônix at 10 µM P, in response to the carbon partition to enhance root development (PLAXTON; TRAN, 2011), a general adaptive behavior of plants to changes in nutrient availability (LÓPEZ-BUCIO, 2003), triggered by the sugar signaling response to increase P uptake sites under P depletion (HAMMOND; WHITE, 2011). Dry matter yield response pattern showed that P responsiveness and use efficiency are closely related to SDW rather than RDW, possibly due to the short period of P deprivation.

Table 2: Leaf area, height, dry matter yield and root/shoot (R/S) dry matter ratio of wheat cultivars exposed to 10 and 125 µM P, differently classified upon P use and response efficiency.

Cultivar	P level	Leaf Area	Height	Dry matter yield			R/S ratio
				Shoot	Root	Total	
	µM	..... cm		..... g pl^{-1}			
Marocco	10	29.2 *Bb*	31.6 *Bb*	0.128 *Bb*	0.049 *Ab*	0.178 *ABb*	0.38
	125	49.6 *Ba*	36.3 *Ba*	0.236 *Ba*	0.065 *ABa*	0.302 *Aa*	0.27
Ônix	10	20.0 *Cb*	26.0 *Cb*	0.085 *Cb*	0.053 *Aa*	0.138 *Cb*	0.62
	125	35.7 *Ca*	32.7 *Ba*	0.156 *Ca*	0.060 *Ba*	0.217 *Ba*	0.38
Anahuac	10	30.8 *Bb*	31.1 *Ba*	0.145 *Ba*	0.059 *Aa*	0.207 *ABa*	0.42
	125	40.5 *Ca*	32.9 *Ba*	0.163 *Ca*	0.056 *Ba*	0.220 *Ba*	0.34
IAC 5	10	35.0 *Ab*	38.6 *Ab*	0.171 *Ab*	0.060 *Ab*	0.231 *Ab*	0.35
	125	57.8 *Aa*	46.05 *Aa*	0.276 *Aa*	0.078 *Ab*	0.354 *Aa*	0.28

Capital letters indicate mean comparisons among cultivars while lowercase letters indicate mean comparisons between phosphorus (P) levels in the nutrient solution. Tukey's test ($p \leq 0,05$).

Analyzing overall response of P use efficiency, it was possible to notice its decrease under high P exposure (Table 3) despite plant tissue and the higher P concentration in plant tissue (Table 1), suggesting the storage of large amounts of Pi in the vacuoles that does not contribute to metabolism and growth and therefore, has a net negative effect on internal PUE, leading to accumulation of Pi in existing tissues rather than its incorporation in metabolically activity of new tissues (VENEKLAAS et al., 2012). On the other hand, under P deficiency, Pi vacuolar pools are preferentially depleted in order to maintain cytoplasmic Pi levels (RAGHOTHAMA, 1999). Although differences were not significant among cultivars for root tissue, great variation could be seen on shoot. White et al. (2012), studying quantitative trait loci (QTL) mapping associated with PUE, showed that greater focus have been given on traits related with efficient P acquisition like improved root architecture and topsoil foraging rather than efficient internal use of P, fact that could be related to the homogeneity on root response among the cultivars. Under 10 µM P, IAC 5 presented the higher values of PUE in shoot tissue, followed by Anahuac, Marocco and Ônix. At 125 µM P, higher values were obtained by Marocco, followed by IAC 5, Ônix and Anahuac, showing a link between responsiveness and PUE, as high PUE on shoot tissue enhances cultivars response to P supply.

Table 3: Phosphorus (P) use efficiency index and acid phosphatase activity of root and shoot of wheat cultivars exposed to 10 and 125 µM P, and differently classified upon P use and response efficiency.

Cultivar	P level	P use efficiency			APA	
		Shoot	Root	Total	Shoot	Root
	µM	..... mg^2 DW mg^{-1} P			..... µmol g^{-1}FW min^{-1}.....	
Marocco	10	76,01 *Ca*	27,40 *a*	103,31 *Ca*	105,9 *Aa*	131,9 *Aa*
	125	64,75 *Aa*	18,28 *b*	83,03 *Ab*	55,9 *Cb*	70,3 *Bb*
Ônix	10	65,55 *Ca*	32,39 *a*	96,66 *Ca*	105,6 *Aa*	84,8 *Ca*
	125	39,86 *Bb*	19,49 *b*	58,71 *Bb*	66,4 *Bb*	70,0 *Bb*
Anahuac	10	101,87 *Ba*	39,59 *a*	141,22 *Ba*	99,2 *Ba*	122,3 *Ba*
	125	32,83 *Bb*	17,80 *b*	48,73 *Bb*	97,1 *Aa*	90,3 *Ab*
IAC 5	10	131,19 *Aa*	40,39 *a*	170,98 *Aa*	77,45 *Ca*	74,08 *Da*
	125	63,77 *Ab*	22,91 *b*	85,83 *Ab*	57,31 *Cb*	68,08 *Ba*

Capital letters indicate mean comparisons among cultivars while lowercase letters indicate mean comparisons between phosphorus (P) levels in the nutrient solution. Tukey's test (p≤0,05).

Acid phosphatase activities (APA) differed among cultivars and plant parts, increasing under low P supply suchlike PUE. Under 10 µM P, P use efficient cultivars IAC 5 and Anahuac showed lower APA in shoot tissue, but differed greatly for root APA. Otherwise not efficient cultivars Marocco and Ônix presented high shoot APA but also differed on root APA. Relating such response pattern with PUE, it is possible to notice that in the tested wheat cultivars, P use efficiency is not fully explained by root APA but instead presented a consistent pattern with shoot APA, remobilizing organic P from tissues and intracellular compartments (DUFF et al., 1994) as well as bypassing the P-requiring steps in C metabolism (PLAXTON; CARSWELL, 1999) more effective in not efficient genotypes. Under 125 µM P, responsive cultivars IAC 5 and Marocco showed lower shoot APA, while not responsive cultivars Ônix and Anahuac presented higher values. Interestingly, IAC 5 and Marocco were also more efficient at this P level, corroborating results found at low P supply. Once again, root APA was not efficient explaining differences among cultivars, although lower values were obtained under high P supply. The result combination of PUE and shoot APA, confer to acid phosphatase enzyme an important role both on PUE and PRE, depending on P supply.

Principal component analysis (PCA) displayed negative correlation between APA and growth parameters (fig. 2A), showing that under low P supply overall plant growth reduces due to P deprivation, increasing APA activity, especially in shoot tissue. Clustering showed the enhanced growth of P responsive cultivars IAC 5 and Marocco under high P supply. PUtE correlated with leaf area, PRE and SDW, especially with cultivar Marocco, showing that in this cultivar, responsiveness is more affected by its uptake ability than use efficiency. PRE correlated with all growth parameters evaluated and so more closely with P efficient cultivar IAC 5 (fig. 2B). In figure 2C, PUE correlated only with efficient cultivars IAC 5 and Anahuac under 10 µM P, and growth parameters correlate with responsive genotypes.

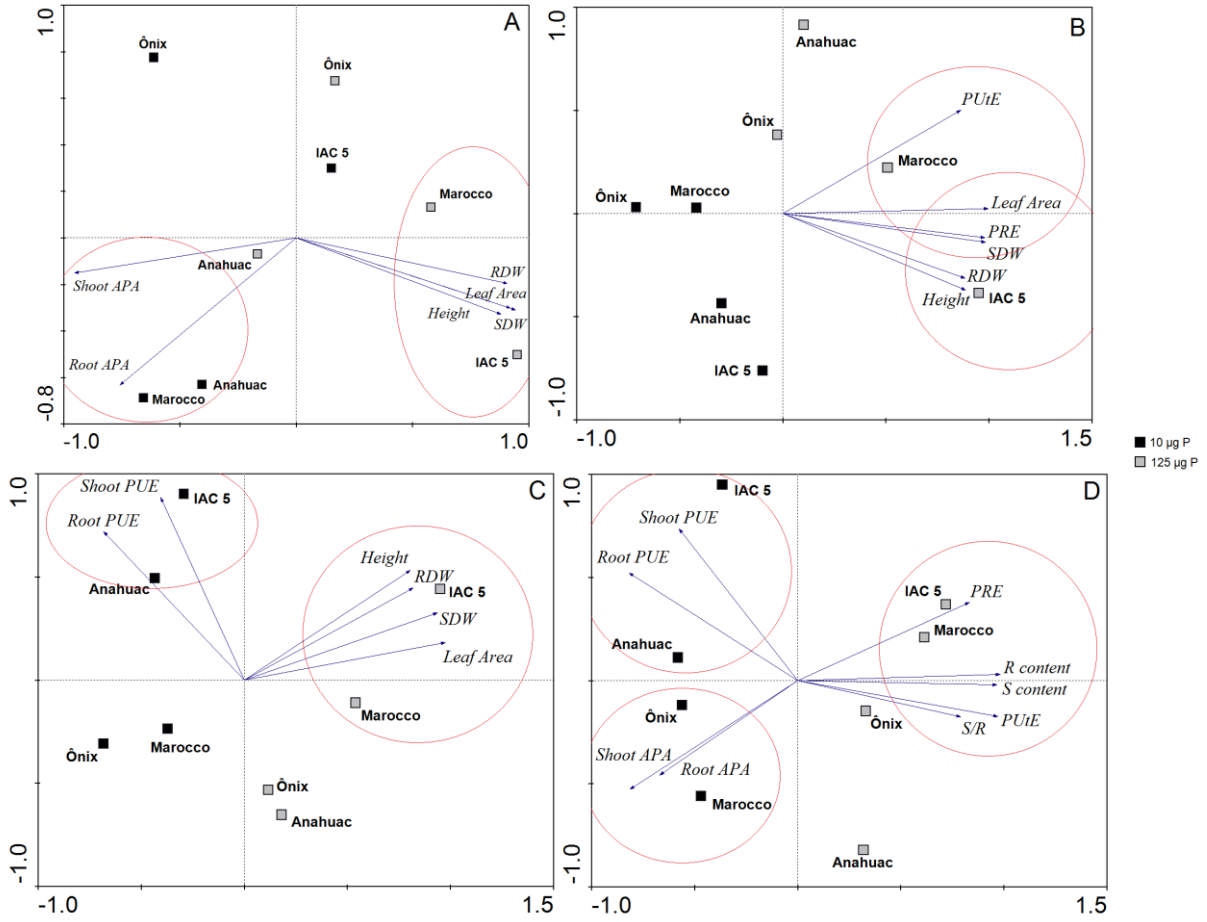

Figure 2: Principal component analysis of wheat cultivars exposed to different P concentrations correlating growth parameters withacid phosphatase activity (A), P uptake efficiency (B) and P use efficiency (C) as well as the relation of these parameters with P tissue content (D). APA: Acid phosphatase activity; PUtE: P uptake efficiency; PUE: P use efficiency; S/T: translocation ratio; PRE: P response efficiency; RDW: root dry weight; SDW: shoot dry weight; R content: P accumulated in root tissue; S content: P accumulated in shoot tissue.

In general, growth parameters had better correlation with cultivars efficient in P uptake (Marocco) and response (IAC 5) rather than use efficiency. Internal PUE is generally lower in plants with high P acquisition efficiency as a result of higher tissue P concentrations (ROSE et al., 2011), hence Marocco and IAC 5 presented low APA in shoot tissue at 125 µM P. In figure 2D, PRE correlated with PUtE index, closely related with P content in shoot and root tissues, as well as with P translocation (S/R). Still, PRE correlated negatively with APA, and due to cultivar position in the PCA, it is possible to notice that under low P supply, not efficient cultivars (Ônix and Marocco) present an increase in the APA, especially in shoot APA (table 3), while responsive cultivars (IAC 5 and Marocco) have low enzyme activity. Once PCA analysis display and overall interaction of cultivars and P levels, PUE did not correlated with APA although and increase in PUE and APA was observed under low P supply.

Conclusion

Phosphorus deficiency mainly repressed shoot growth. Dry matter yield response pattern showed that P responsiveness and use efficiency are closely related to shoot dry weight rather than root dry weight, once high P use efficiency on shoot tissue enhances cultivars response to P supply. In general, growth parameters had better correlation with cultivars efficient in P uptake and response rather than with P use efficient, which presented low acid phosphatase activity in shoot tissue at 125 µM P. Due to acid phosphatase activity response pattern, it is possible to notice that in the tested wheat cultivars, P use efficiency is not fully explained by root acid phosphatase activity but instead presented a consistent pattern with shoot acid phosphatase activity. This way, it is not recommended using root acid phosphatase activity as a physiological marker for evaluation of wheat seedlings in relation to P response and efficiency under low P conditions, while shoot acid phosphatase activity could be used to identify tendencies of those parameters.

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CONSIDERAÇÕES FINAIS

Estudos realizados em meio hidropônico são fundamentais no campo da nutrição avançada de plantas, sendo uma ferramenta imprescindível para isolar fatores e controlar variáveis. No presente estudo, o sistema hidropônico fechado se mostrou eficiente na avaliação dos efeitos deletérios do Al, bem como da eficiência de uso e resposta ao P em plântulas de trigo. Ainda, nota-se como o estudo da fisiologia vegetal pode ser complexo, sendo muitas vezes necessárias múltiplas abordagens para o entendimento de determinados fenômenos, entre os quais podemos observar no Capítulo I, alterações anatômicas e aberrações cromossômicas induzidas pela toxidez de Al que, como resultado, alteram a morfofisiologia das plantas visando sua adaptação ao meio.

Como demonstrado no Capítulo II, a estatística experimental possui grande importância na qualidade dos resultados, sendo que, o uso adequado desta área do conhecimento pode melhorar a eficiência operacional dos experimentos, permitindo como visto no Capítulo III, análises sistêmicas de dados que agregam no entendimento do conjunto de respostas, na maioria das vezes observadas de forma isolada, como para parâmetros de crescimento, atividade enzimática e acúmulo de nutrientes.

Como resultado, com relação à tolerância do Al, os genótipo IAC 5, BRS Umbu e BRS 208 ficam classificados como tolerantes, Marocco, Ônix e BR 23 classificados como de tolerância intermediária, e Frondoso, Sumai 3, Galego Rapado e Anahuac como sensíveis ao Al. Quanto ao comportamento a disponibilidade de P, IAC 5, Frondoso, BR 23, BRS Umbu e BRS 208 se enquadram como eficientes e responsivos, Marocco e Galego Rapado como não eficientes e responsivos, Ônix como não eficiente e não responsivo, e Anahuac e Sumai 3 como eficientes e não responsivos.

Assim, apesar da variabilidade genética encontrada nos genótipos de trigo, não houve uma padrão linear de resposta de coexistência de tolerância ao Al e eficiência no uso de P, como demonstrado para IAC 5, BRS Umbu e BRS 208, ou não eficiente ao uso de P e sensível ao Al como para Galego Rapado. Entretanto, como a interação dos elementos Al e P não foram testados em concomitância,

experimentos futuros devem ser realizados a fim de identificar seus efeitos, preferencialmente em solo, para que as respostas sejam as mais semelhantes possíveis com a realidade encontrada a campo.

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APÊNDICE

APÊNDICE A: Cultivo hidropônico de trigo. (A) Bandeja utilizada como unidade experimental. (B) Detalhe da fixação das sementes em germinação. (C) Bandejas sobre container contendo solução nutritiva representando um bloco experimental. (D, E) Detalhe do desenvolvimento das plântulas de trigo. (F,G) Distribuição do sistema radicular sob a bandeja.