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AVALIAÇÃO BIOQUÍMICA-FISIOLÓGICA DE CLONES DE BATATA EM RELAÇÃO AO ALUMÍNIO

TESE DE DOUTORADO

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Santa Maria, RS, Brasil
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AVALIAÇÃO BIOQUÍMICA-FISIOLÓGICA DE CLONES DE BATATA EM RELAÇÃO AO ALUMÍNIO

por

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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Agronomia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutor em Agronomia.**

Orientador: Prof. Fernando Teixeira Nicoloso

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Universidade Federal de Santa Maria
Centro de Ciências Rurais
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Santa Maria, 11 de abril de 2008.

Viver e não ter a vergonha de ser feliz.
Cantar e cantar e cantar
a beleza de ser um eterno aprendiz!!!

Gonzaguinha

Aos meus queridos pais
Edenir e Joice,
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compreensão e total incentivo
para realizar este trabalho

OFEREÇO

Ao meu grande amor Márcio,
pelo amor,
incentivo, paciência,
apoio e compreensão

DEDICO

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Programa de Pós-graduação em Agronomia
Universidade Federal de Santa Maria

AVALIAÇÃO BIOQUÍMICA-FISIOLÓGICA DE CLONES DE BATATA EM RELAÇÃO AO ALUMÍNIO

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Data e Local: Santa Maria, 11 de abril de 2008.

O alumínio (Al) é o metal mais abundante na crosta terrestre, afetando o crescimento e desenvolvimento das plantas. O objetivo deste trabalho foi investigar e comparar respostas bioquímicas e fisiológicas de clones de batata, Macaca, SMIC148-A, Dakota Rose e *Solanum microdontum,* expostos a 0, 50, 100, 150 e 200 mg Al L^{-1} em solução nutritiva (pH 4,0). Após sete dias, o conteúdo de Al foi em média 3,9, 2,8, 3,6 e 3,7 vezes maior nas raízes que na parte aérea nos clones Macaca, *S. microdontum*, SMIC148-A e Dakota Rose, respectivamente. Baseado no crescimento relativo da raiz, *S. microdontum* e SMIC148-A foram considerados tolerantes ao Al e Macaca e Dakota Rose sensíveis ao Al. Foi observado inibição no crescimento da parte aérea somente no clone Macaca. Vários parâmetros bioquímicos foram afetados, principalmente nos clones sensíveis ao Al, como o aumento na concentração de H_2O_2, a atividade da catalase (CAT) e a peroxidação lipídica, e a redução no conteúdo de clorofila e carotenóides. A concentração de zinco, manganês, ferro e cobre foi maior nas raízes que na parte aérea em todos os clones. Um aumento na concentração desses micronutrientes foi observado somente no clone *S. microdontum*, enquanto uma redução foi observada nos clones Macaca, SMIC148-A e Dakota Rose com o suprimento de Al. Com o objetivo de analisar o efeito do Al na atividade *in vitro* de fosfatases ácidas (APases), os quatro clones de batata cresceram *in vitro*, em hidroponia ou em casa de vegetação. Em plântulas *in vitro*, APases de raízes foram inibidas por Al em *S. microdontum* e Dakota Rose e ativadas em Macaca em todos os níveis de Al. Em plântulas de hidroponia, APases de raízes aumentaram em Macaca em 50 mg L^{-1}, enquanto diminuíram em *S. microdontum* em todos os níveis de Al. Em plântulas de casa de

vegetação, APases de raízes foram inibidas em 200 mg L^{-1} em *S. microdontum* e SMIC148-A, e em 100, 150 e 200 mg L^{-1} em Dakota Rose. APases de parte aérea foram inibidas em Macaca e SMIC148-A e ativadas em 50 e 100 mg L^{-1} Dakota Rose. Os clones Macaca (sensível ao Al) e SMIC148-A (tolerante ao Al) foram utilizados em um outro experimento com o objetivo de analisar se o estresse oxidativo causado por Al é um sintoma primário que pode desencadear inibição do crescimento da raiz. Em 24, 72, 120 e 168 horas após a adição de Al, foi observado inibição do crescimento da raiz e peroxidação lipídica somente no clone sensível ao Al. No clone tolerante, há sempre pelo menos um componente do sistema antioxidante protegendo as plantas do estresse de Al, o mesmo não acontecendo com o clone sensível. Com o objetivo de checar se o estresse oxidativo provocado pelo Al difere entre os clones, Macaca (sensível ao Al) e SMIC148-A (tolerante ao Al), os quais apresentam distinto grau de escape ao Al, esses clones foram cultivados em sistema de raízes divididas por 10 dias, com cinco tratamentos de variação de concentração e localização de Al. Em 200 mg Al L^{-1}, uma redução na concentração de clorofila e aumento na oxidação de proteínas foi observada somente na Macaca. Na presença de 200 mg L^{-1} em metade do sistema radicular, a concentração de H_2O_2 na parte aérea foi menor que com ambas as metades da raiz tratadas com 100 mg L^{-1}. A peroxidação lipídica na parte aérea aumentou com o aumento do suprimento de Al na Macaca, enquanto foi menor em plantas tratadas com 100 e 200 mg Al L^{-1} em somente metade do sistema radicular em SMIC148-A. Quando ambas as metades da raiz foram tratadas com 100 mg Al L^{-1}, Macaca apresentou resposta de tolerância ineficiente, baseado na atividade da CAT, oxidação protéica, peroxidação lipídica, concentração de H_2O_2 e atividade de APases. Esses resultados mostram que o SMIC148-A, embora apresentou menor reação de escape ao Al que a Macaca, mostrou uma resposta antioxidante local e sistêmica mais eficiente frente ao suprimento de Al. Portanto, os clones Macaca e SMIC148-A diferiram na expressão da quantidade e tipo de antioxidante, sugerindo que o estresse oxidativo pode ser um importante mecanismo para toxicidade de Al, principalmente nos clones sensíveis ao metal. Esta toxicidade depende não somente da disponibilidade de Al, mas também do clone e do sistema de crescimento. Além disso, os efeitos adversos do Al não desaparecem quando parte do sistema radicular não está em contato com o Al, principalmente no clone sensível ao alumínio.

Palavras-chave: *Solanum tuberosum*; alumínio; crescimento; estresse oxidativo.

ABSTRACT

Doctoral Thesis
Post-Graduate Program in Agronomy
Federal University of Santa Maria

BIOCHEMICAL AND PHYSIOLOGICAL EVALUATION OF POTATO CLONES IN RELATION TO ALUMINUM

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Place and date of defense: Santa Maria, April 11, 2008.

Aluminum (Al) is the most abundant metal in the earth's crust, affecting the growth and development of plants. The objective of this work was to investigate and compare biochemical and physiological responses of potato clones, Macaca, SMIC148-A, Dakota Rose and *Solanum microdontum*, exposed to 0, 50, 100, 150 and 200 mg Al L^{-1} in nutrient solution (pH 4.0). After 7 days, Al content in roots was on average 3.9, 2.8, 3.6, and 3.7 fold greater than in shoot, in Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones, respectively. Based on the relative root growth, the *S. microdontum* and SMIC148-A were considered Al-tolerant while Macaca and Dakota Rose were considered Al-sensitive. Inhibition in shoot growth was observed only in Macaca clone. After 7 d of Al exposure, several biochemical parameters were affected, mainly in Al-sensitive clones, such as increased H_2O_2 concentration, catalase (CAT) activity and lipid peroxidation, and decreased chlorophyll and carotenoid content. In addition, zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) concentrations were higher in roots than in shoot of all potato clones tested. An increase in the concentration of most of the micronutrients analyzed was observed only in *S. microdontum*, while a decrease was observed in Macaca, SMIC148-A and Dakota Rose. Macaca, SMIC148-A, Dakota Rose and *S. microdontum* were grown *in vitro*, in hydroponics or in greenhouse to evaluate the effect of Al on the *in vitro* activity of acid phosphatases (APases). In plantlets grown *in vitro*, root APases were inhibited by Al in all clones, while shoot APases were inhibited by Al in *S. microdontum* and Dakota Rose and increased in Macaca at all Al levels. In plantlets grown in hydroponics, root APases increased in

Macaca at 50 mg L^{-1}, but decreased at all Al levels in *S. microdontum*. In greenhouse plantlets, root APases were reduced at 200 mg L^{-1} in *S. microdontum* and SMIC148-A, and at 100, 150 and 200 mg L^{-1} in Dakota Rose. Shoot APases were reduced in Macaca and SMIC148-A. Conversely, in Dakota Rose, APases increased at 50 and 100 mg L^{-1}. Macaca (Al-sensitive) and SMIC148-A (Al-tolerant) clones were utilized in another experiment with the objective of evaluating whether the oxidative stress caused by Al is an early symptom than can trigger root growth inhibition. At 24, 72, 120 and 168 hours after Al addition, root growth inhibition and lipid peroxidation was observed only for the Al-sensitive clone. In the Al-tolerant clone, there was always at least one component of the antioxidant system protecting the plant against Al stress, which did not occur in the Al-sensitive clone. With the objective of checking whether Al oxidative stress differs in potato clones, Macaca (Al-sensitive) and SMIC148-A (Al-tolerant), which present distinct degrees of Al- avoidance, were cultivated in a split-root system for 10 days with five treatments of varying concentrations and locations of Al. At 200 mg Al L^{-1}, a significant decrease in chlorophyll concentration and increase in protein oxidation was observed only for Macaca. At 200 mg L^{-1} supplied to half of the root system, shoot H_2O_2 concentration was lower than that with both root halves treated by 100 mg L^{-1}. Shoot lipid peroxidation in Macaca increased with increasing Al supply. In SMIC148-A, plants treated with 100 and 200 mg Al L^{-1} in only one root half showed lower shoot lipid peroxidation. The 200 half of 0/200 plants presented significantly greater lipid peroxidation than the half untreated by Al, mainly in Macaca. At 100 mg Al L^{-1} supplied to both root halves, Macaca showed an inefficient tolerance response, based on CAT activity, protein oxidation, lipid peroxidation, H_2O_2 concentration and APase activity. These results show that SMIC148-A, even though presenting lower Al-avoidance than Macaca, showed a stronger local and systemic antioxidant response to Al supply. Therefore, potato clones differed in their expression of antioxidant responses in terms of amount and type, suggesting that oxidative stress is an important mechanism for Al toxicity, mainly in Al-sensitive clones. This toxicity depends not only on Al availability but also on the clone and the growth system. In addition, it was observed that the adverse effects of Al do not disappear when part of the root system is not in contact with Al, mainly in the Al-sensitive clone.

Keywords: Potato; aluminum; growth; acid phosphatases; oxidative stress.

INTRODUÇÃO

A acidez do solo é um dos principais fatores limitantes à produção agrícola. Os solos ácidos estão presentes em muitas partes do mundo, os quais provocam a inibição do crescimento das plantas devido a uma combinação de vários fatores, incluindo a toxidez do alumínio (Al) e a de manganês, bem como a deficiência de elementos essenciais, particularmente cálcio, magnésio, fósforo e molibdênio. Portanto, os problemas impostos às plantas pela acidez do solo não dependem de um só fator ou agente, mas de uma série de fatores que afetam o crescimento das mesmas por meio de diferentes mecanismos bioquímicos e fisiológicos. As relações entre a acidez do solo e a solubilidade do Al, assim como os efeitos tóxicos desse metal sobre as plantas, começaram a ser estudados nas primeiras décadas do século passado. Mesmo assim, a compreensão dos mecanismos causais da toxidez e da tolerância ao Al em plantas ainda é bastante limitada.

O Al é o terceiro elemento mais abundante na litosfera, após o oxigênio e o silício, participando em aproximadamente 8% na composição da crosta terrestre. Dessa forma, as raízes das plantas estão quase sempre expostas ao Al de alguma forma. A especiação de Al em solução é complexa, e somente recentemente foi demonstrado que Al^{3+} é a espécie de Al mais rizotóxica. Devido ao fato que o Al^{3+} e outras formas monoméricas de Al são potencialmente reativas com ligantes biológicos, pesquisadores têm especulado que a toxicidade do Al (inibição do crescimento da raiz) pode resultar de interações do Al com vários sítios diferentes dentro da parede celular, membrana plasmática e protoplasma. O excesso de Al, além de inibir a formação normal da raiz, interfere nas reações enzimáticas e na absorção, transporte e uso de nutrientes pelas plantas. Além disso, o Al causa estresse oxidativo, levando a oxidação de biomoléculas como lipídios, proteínas, pigmentos e ácidos nucléicos.

Embora o Al possa produzir alguns efeitos comuns sobre as plantas em geral, como inibição do crescimento da raiz, na maioria dos casos há efeitos específicos sobre diferentes genótipos de plantas. Assim, tanto dentro como entre as espécies vegetais pode haver uma ampla variação genética na tolerância ao Al,

sugerindo que espécies ou cultivares tolerantes possuem vários mecanismos para destoxificar o Al. Mesmo após anos de pesquisa sobre os efeitos do Al no crescimento e no desenvolvimento de plantas, os mecanismos primários de sua toxidez e de tolerância ainda precisam ser esclarecidos. Em vista dessa situação, muitos pesquisadores, em diferentes lugares do mundo, postulam que a seleção de variedades produtivas e tolerantes à toxidez do Al seja considerada um componente de grande importância dentro das estratégias de manejo dos solos ácidos. Para isso, uma forma adequada de avaliação de genótipos para a tolerância ao Al pode ser realizada em sistemas hidropônicos sob condições controladas, oferecendo várias vantagens, como o pronto acesso ao sistema radicular e a possibilidade de monitoramento e controle do pH e das concentrações de Al e de outros íons relevantes à expressão das reações de sensibilidade e tolerância.

Apesar de a batata ser um alimento empregado em todo o mundo como fonte de energia, sendo a quarta cultura mais importante do mundo, depois do arroz, trigo e milho, há uma carência de estudos com relação a alguns aspectos nutricionais e bioquímicos nesta espécie. Neste trabalho, quatro clones de batata foram analisados, três adaptados e tetraplóides, da espécie *Solanum tuberosum* (Macaca, SMIC148-A e Dakota Rose) e um diplóide, da espécie selvagem *Solanum microdontum* (PI595511-5).

Tendo em vista a característica ácida dos solos do Rio Grande do Sul e sendo a batata cultivada em grande escala neste estado, o objetivo do presente trabalho foi analisar o comportamento de diferentes clones de batata em relação ao Al, bem como a interação Al-planta nestes clones, tanto em aspectos nutricionais (disponibilidade de nutrientes e crescimento) quanto bioquímicos (atividade de enzimas envolvidas no metabolismo de nutrientes e no sistema antioxidante das plantas).

OBJETIVOS

Objetivo geral

Avaliar as respostas bioquímicas e fisiológicas de clones de batata em relação ao alumínio.

Objetivos específicos

1) Identificar clones de batata sensíveis e tolerantes ao alumínio.
2) Investigar e comparar respostas fisiológicas e de estresse oxidativo de clones de batata expostos ao alumínio em solução nutritiva.
3) Avaliar se o estresse oxidativo causado por toxicidade de alumínio é um sintoma inicial que pode desencadear inibição do crescimento da raiz em clones de batata.
4) Avaliar o efeito do alumínio na atividade *in vitro* de fosfatases ácidas de quatro clones de batata cultivados em três sistemas de crescimento.
5) Analisar a influência do estresse de alumínio no conteúdo de micronutrientes em clones de batata.
6) Examinar os efeitos locais e/ou sistêmicos do alumínio em parâmetros bioquímicos de clones de batata crescendo em sistema de raízes divididas.

REVISÃO BIBLIOGRÁFICA

1 Os solos ácidos no mundo

Os solos ácidos, os quais apresentam pH menor ou igual a 5,5, promovem inibição do crescimento de plantas, especialmente devido a deficiência de fósforo e ao estresse causado pelo alumínio (Al). Considera-se a toxidez do Al um dos principais fatores limitantes da produtividade agrícola em solos ácidos (FOY et al., 1978). O Al afeta aproximadamente 40% das terras aráveis do mundo que são potencialmente usadas para a produção de biomassa e alimentos (MA et al., 2001). Ainda mais relevante é o fato de que muitas dessas áreas estão localizadas em países em desenvolvimento na América do Sul, África Central e Sudoeste da Ásia. A acidez de solos é uma ocorrência natural em áreas tropicais e subtropicais e pode resultar de desbalanços nos ciclos de nitrogênio, enxofre e carbono (BOLAN; HEDLEY, 2003; TANG; RENGEL, 2003); maior captação de cátions em comparação com ânions (TANG; RENGEL, 2003); e menor fixação de nitrogênio por leguminosas (BOLAN et al., 1991; TANG; RENGEL, 2003). Além disso, em várias partes do mundo, os níveis de acidez dos solos estão aumentando, em decorrência de atividades humanas. Entre os motivos da acidificação antropogênica dos solos estão a liberação atmosférica de poluentes industriais, associada à lixiviação de solos com chuvas ácidas, as atividades de mineração e, no setor agrícola, a nitrificação subseqüente à aplicação de altas doses de fertilizantes amoniacais (RENGEL; ZHANG, 2003).

Dentro da faixa intertropical, 37% dos solos do sudeste asiático, 40% dos solos da África e 55% dos solos da América do Sul apresentam limitações ao seu uso agrícola por excesso de acidez (SANCHEZ; SALINAS, 1981). A maioria dos solos do Rio Grande do Sul e do Brasil são ácidos (VOLKWEISS, 1989). No Brasil, cerca de 500 milhões de hectares são cobertos por solos ácidos, compreendendo em torno de dois terços de seu território total – a maior área de solos ácidos dentro de um único país (VITORELLO et al., 2005). Um estudo abrangendo 26 solos de regiões brasileiras mostrou que 75% dos valores de pH da camada superficial

variaram entre 3,78 e 5,52 e que o Al^{3+} foi o cátion trocável predominante em mais de um terço dos solos com pH inferior a 5,6 (ABREU Jr. et al., 2003).

A produção de alimentos, principalmente de grãos, é afetada negativamente por solos ácidos. Por exemplo, 20% da produção de milho e 13% da produção de arroz do mundo estão em solos ácidos (von UEXKÜLL; MUTERT, 1995). Assim, solos ácidos limitam a produtividade de culturas em muitos países em desenvolvimento onde a produção de alimentos é crítica (KOCHIAN et al., 2004).

2 Alumínio (Al)

O Al é um metal leve que compõe aproximadamente 8% da crosta terrestre, ocorrendo como óxidos e aluminosilicatos inofensivos, precipitando em pH alcalino ou neutro na forma de sais insolúveis, como por exemplo, Al_2SiO_5, $Al(OH)_3$, $AlPO_4$, e formas organicamente complexas de Al. Entretanto, sob certas condições, o Al pode tornar-se solúvel, por exemplo, quando o ambiente torna-se ácido ou quando os níveis de matéria orgânica no solo são altos (JANSEN et al., 2002). Quando o pH do solo torna-se ácido, como é o caso de 30% a 40% das terras aráveis do mundo (von UEXKÜLL; MUTERT, 1995), formas solúveis de Al podem se acumular em concentrações que inibem o crescimento e o funcionamento da raiz. Portanto, à medida que os solos se acidificam, íons Al passam a ocupar as posições de troca catiônica, em superfícies eletronegativas dos colóides, em substituição aos cátions removidos pela lixiviação, onde concentrações de espécies de Al podem alcançar níveis tóxicos para os organismos (RENGEL; ZHANG, 2003). Dessa forma, a toxicidade do Al é fortemente dependente das espécies predominantes, onde a concentração de Al total não pode ser usada como um índice real de toxicidade de Al.

2.1 Química do Al

Evidências atuais e reinterpretação das primeiras literaturas sugerem que Al monomérico tais como Al^{3+}, hidróxidos de Al ($AlOH^{2+}$; $Al(OH)^{+}_2$; $Al(OH)^{-}_4$) e espécies de $AlSO^{+}_4$ são as formas mais tóxicas para as plantas, juntamente com os polímeros

Al_{13} (KINRAIDE, 1991; JANSEN et al., 2002). Como pode ser observado na Figura 1, em pHs menores que 5,0, o Al^{3+} fitotóxico domina (ROSSIELLO; NETTO, 2006). O Al monomérico também forma complexos de baixo peso molecular com vários ligantes, tais como grupos carboxilato, sulfato e fosfato. Assim, o Al^{3+} forma complexos com os ácidos orgânicos, o fosfato inorgânico e o sulfato, e também se ligará a esses grupos em macromoléculas tais como as proteínas e os nucleotídeos. Devido ao fato que o Al^{3+} e outras formas monoméricas de Al são potencialmente reativas com ligantes biológicos, pesquisadores têm especulado que a toxicidade do Al (inibição do crescimento da raiz) pode resultar de interações do Al com vários sítios diferentes dentro da parede celular, membrana plasmática e protoplasma.

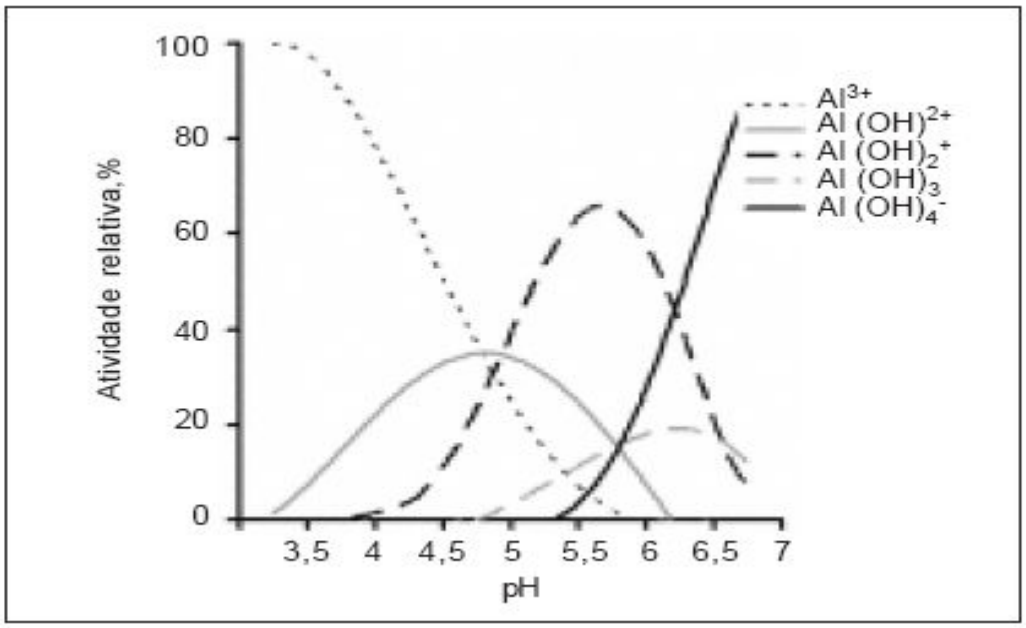

Figura 1. Distribuição das atividades relativas de Al^{3+} e das espécies mononucleares de Al-OH em função do pH. (Fonte: KINRAIDE; PARKER, 1989).

Apesar de sua presença ubíqua, o Al não é essencial para os organismos vivos. Algumas possíveis razões para isso foram resumidas por Williams (1999). Com exceção daqueles cátions que podem sofrer mudanças na valência, tais como o Fe e o Co, os sistemas biológicos são aparentemente incapazes de utilizar efetivamente os cátions trivalentes livres. Os dois fatores que aparentemente determinam isso são o pequeno tamanho desses cátions, os quais determinam

limitações à estequiometria de complexação, e as lentas taxas de troca de ligantes desses metais (WILLIAMS, 1999).

Por ser um cátion trivalente, o Al é retido firmemente e, assim, sua concentração, na solução do solo, é baixa, dentro da faixa de μmol L^{-1} (HAYNES; MOKOLOBATE, 2001). Entretanto, essas baixas concentrações de Al em solução são tóxicas para a maioria das espécies vegetais, primariamente por lesar o funcionamento normal das raízes, inibindo o seu crescimento e bloqueando os mecanismos de aquisição e transporte de água e nutrientes.

2.2 Efeitos bioquímicos e fisiológicos do alumínio

Como as raízes são os primeiros órgãos a entrar em contato com o Al do solo, desde as primeiras observações tem sido registrado que os sintomas de toxidez ao Al expressavam-se de forma mais acentuada no sistema radicular (ROSSIELLO; NETTO, 2006). A inibição do crescimento da raiz é um dos primeiros e mais dramáticos sintomas de toxicidade do Al exibidos pelas plantas (RENGEL; ZHANG, 2003). O excesso de Al além de inibir a formação normal da raiz, interfere nas reações enzimáticas e na absorção, transporte e uso de nutrientes pelas plantas (TAMÁS et al., 2006). Salvador et al. (2000), constataram que doses crescentes de Al reduziram a absorção e o transporte de P, Ca, Mg, S, Fe e Mn para a parte aérea, sugerindo que a redução de Ca e Mg deve-se a uma inibição interiônica desses cátions pelo Al. A inibição do crescimento da raiz foi observada dentro de horas, ou até minutos, de exposição a concentrações micromolares de Al em solução (ZHANG; RENGEL, 1999; MA et al., 2002). Entretanto, com a exposição prolongada ao Al, as plantas exibem vários outros sintomas de toxidez, tanto na raiz como na parte aérea (FOY, 1988; RENGEL, 1996).

A inibição do crescimento da raiz induzida por Al frequentemente precede ou coincide com um declínio nas divisões celulares (HORST, 1995). Entretanto, a rápida inibição do crescimento da raiz induzida por Al é comumente causada pela inibição do alongamento celular mais do que pela divisão celular (RENGEL; ZHANG, 2003).

O confinamento do crescimento radicular ao volume do horizonte superficial tem conseqüências restritivas para o crescimento da parte aérea, assim como para o

pleno crescimento e desenvolvimento da planta, o que resultará em reduções na produtividade das culturas. Essa limitação adquire ainda maior relevância durante períodos de deficiência hídrica (FAGERIA; ZIMMERMANN, 1979), quando a aquisição de água e nutrientes das camadas mais profundas pode ser crucial para a sobrevivência das plantas. Nesse sentido, o estresse hídrico e a toxidez de Al tendem a reforçar os seus efeitos negativos. Entre os relatos sobre os mecanismos envolvidos na tolerância ao Al, Giannakoula et al. (2008) relataram que a tolerância ao Al em milho está correlacionada com maiores níveis de nutrientes minerais. Entretanto, Furlani & Furlani (1991), destacaram que o maior teor de nutrientes verificado nas plantas tolerantes ao Al pode ser devido ao efeito indireto do maior aprofundamento e crescimento das raízes, explorando maior volume de solo. Na parte aérea das plantas, os sintomas resultantes da toxidez de Al não são claramente identificáveis, e as injúrias provocadas pelo Al podem ser confundidas com aquelas decorrentes de desbalanço ou deficiência nutricional, especialmente do fósforo (ROSSIELLO; NETTO, 2006).

A extensão da inibição do crescimento da raiz é comumente usada como uma medida de toxicidade do Al (FOY, 1988). Entretanto, o crescimento da raiz é um processo complexo e dinâmico. Provavelmente, vários processos bioquímicos e fisiológicos podem já ter sido alterados antes da inibição do crescimento da raiz induzida por Al (RENGEL; ZHANG, 2003). A literatura é rica em relatos mostrando que numerosos processos bioquímicos e fisiológicos são afetados em várias espécies dentro de minutos ou horas após a exposição ao Al, entre eles o exacerbado estresse oxidativo (CAKMAK; HORST, 1991; YAMAMOTO et al., 2002). Uma característica comum a vários tipos de estresse, incluindo toxicidade do Al, é a perturbação da homeostase redox celular, e como uma conseqüência, o aumento da produção de espécies reativas de oxigênio (EROs) (CAKMAK; HORST, 1991), incluindo radical superóxido ($O_2^{\bullet -}$), radical hidroxil ($^{\bullet}OH$) e peróxido de hidrogênio (H_2O_2) (CHAOUI; FERJAN, 2005). Essas espécies de oxigênio altamente citotóxicas podem causar dano oxidativo a biomoléculas tais como lipídios, proteínas, pigmentos e ácidos nucléicos, levando a peroxidação de lipídeos de membrana, perda de íons, hidrólise de proteínas, e até mesmo dano ao DNA (GUO et al., 2007). A membrana plasmática, o último obstáculo para o acesso livre de íons Al no simplasto, pode ser o alvo primário do Al rizotóxico (BARCELÓ et al., 1996). Estudos de toxicidade de Al em raízes sugerem que a produção de EROs pode contribuir

significativamente para a inibição induzida por Al do alongamento da raiz (YAMAMOTO et al., 2003; TAMÁS et al., 2004).

Embora os termos “resistência”, “tolerância” e “mecanismos de escape” sejam frequentemente usados na literatura como sinônimos, quando se referem a estresses abióticos, o termo “resistência” se refere a mecanismos que impedem o Al de entrar na planta, enquanto o termo “tolerância” se refere a mecanismos que destoxificam ou seqüestram o Al internamente (DELHAIZE et al., 2007). Além disso, alguns autores relataram também que algumas espécies de plantas desenvolvem um mecanismo chamado de “escape ao Al”. Nesse mecanismo, quando as raízes das plantas crescem em um ambiente heterogêneo com diferentes níveis de Al tóxico, há o desenvolvimento preferencial das raízes nos locais com menor concentração de Al, acompanhado por uma maior inibição do crescimento das raízes em contato com altas concentrações de Al (HAIRIAH et al., 1993).

Há uma ampla variação genética, tanto dentro como entre espécies na tolerância de plantas ao Al (BONA et al, 1993; MA; FURUKAWA, 2003). Algumas plantas são capazes de tolerar até mesmo concentrações fitotóxicas de alumínio (POLAK et al., 2001). A variedade de processos celulares nos quais o Al pode interferir potencialmente sugere que espécies ou cultivares tolerantes ao alumínio possuem vários mecanismos de detoxificação, entre os quais de tolerância internos e externos (TAYLOR, 1988, 1991).

Os mecanismos externos (os quais minimizam a captação de alumínio) incluem a formação de quelados não tóxicos de alumínio com ânions ácidos orgânicos (malato, citrato e oxalato) secretados pelos ápices radiculares ou alcalinização do apoplasto radicular e rizosfera, os quais substituem as espécies tóxicas de alumínio por formas menos tóxicas (WENZL et al., 2001). Há fortes correlações entre tolerância ao Al e liberação de ácidos orgânicos ativada por Al em numerosas espécies de plantas (KOCHIAN et al., 2004).

Recentemente, alguns pesquisadores têm voltado sua atenção para espécies de plantas que podem acumular altos níveis de Al na parte aérea. Essas espécies, consequentemente, possuem mecanismos internos de destoxificação do Al. Dentre os mecanismos para destoxificar o alumínio internamente está a formação de complexos de ácidos orgânicos e alumínio, sendo que essa quelação reduz efetivamente a atividade do alumínio no citosol, prevenindo a formação de complexos entre o alumínio e os componentes celulares (MA et al., 2001).

Dada à natureza do estresse de Al, o meio hidropônico oferece óbvias vantagens aos estudos da interação desse elemento com as plantas, como o pronto acesso ao sistema radicular e a possibilidade de monitoramento e controle do pH e das concentrações de Al e de outros íons relevantes à expressão das reações de sensibilidade e tolerância (ROSSIELLO; NETTO, 2006).

2.3 Estresse oxidativo

O oxigênio molecular (O_2) é necessário para a sobrevivência de todos os organismos aeróbicos. Assim, a obtenção de energia por estes organismos é feita na mitocôndria através da fosforilação oxidativa, onde o O_2 é reduzido por quatro elétrons a H_2O. Quando o oxigênio é parcialmente reduzido, tanto na fosforilação oxidativa quanto em outras reações, há a formação de radicais livres, que constituem moléculas com coexistência independente e que contém um ou mais elétrons não pareados na camada de valência. Esta configuração faz dos radicais livres espécies altamente instáveis, de meia vida relativamente curta e quimicamente muito reativas (SALVADOR; HENRIQUES, 2004).

$$O_2 \xrightarrow{e^-} O_2^{\bullet -} \xrightarrow[2H^+]{e^-} H_2O_2 \xrightarrow[H^+]{e^-} OH^{\bullet} \xrightarrow[H^+]{e^-} H_2O$$

Figura 2. Passos intermediários da redução do oxigênio. A redução por 4 elétrons do oxigênio até a água ocorre em etapas sucessivas de redução por 1 elétron. Neste processo são formados os intermediários: ânion radical superóxido, peróxido de hidrogênio e radical hidroxila, que correspondem à redução por um, dois e três elétrons, respectivamente (SALVADOR; HENRIQUES, 2004).

O estresse oxidativo corresponde a um estado em que há uma elevada produção de espécies reativas de oxigênio, onde os mecanismos celulares pró-oxidantes superam os antioxidantes, como esquematizado na Figura 3.

A terminologia Espécies Reativas de Oxigênio (EROs ou ROS:“reactive oxygen species”) inclui as espécies chamadas de radicais livres e outras que, embora não possuam elétrons desemparelhados, são muito reativas em decorrência de sua instabilidade (MARRONI, 2002). Um dos principais representantes de EROs é o anion radical superóxido ($O_2^{\bullet -}$), o qual é produzido através de uma redução monoeletrônica do oxigênio. Nas células o $O_2^{\bullet -}$ é rapidamente convertido à peróxido

de hidrogênio (H_2O_2) através de sua dismutação espontânea ou enzimática (superóxido dismutase). O H_2O_2 é menos reativo que o $O_2^{\bullet-}$, porém na presença de metais como o ferro (Fe^{2+}) ou o cobre (Cu^{2+}), ele pode gerar radicais hidroxila ($OH^{\bullet}$). O $OH^{\bullet}$ é provavelmente um dos radicais mais reativos dentre os EROs.

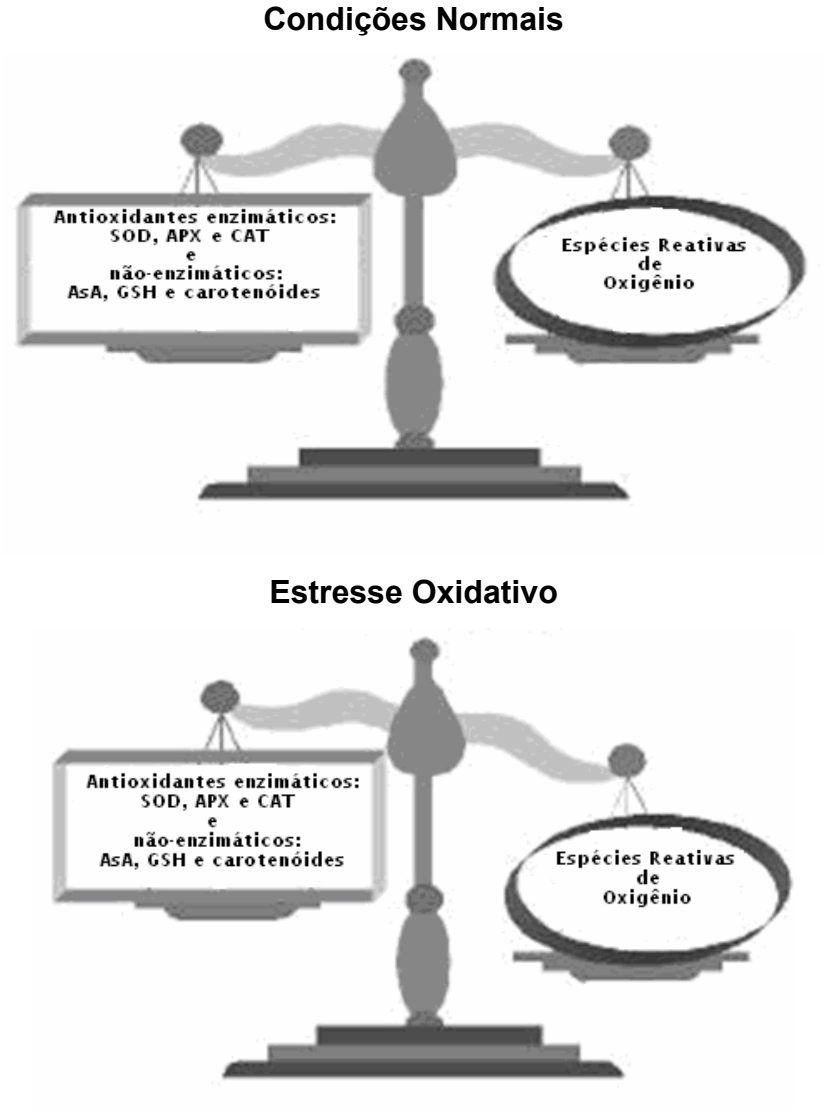

Figura 3. Relação entre mecanismos celulares pró-oxidantes, antioxidantes e estresse oxidativo.

2.4 Sistema antioxidante de plantas

Para atenuar o dano oxidativo iniciado pelas EROs, as plantas desenvolveram um complexo sistema de defesa antioxidante, incluindo antioxidantes de baixo peso molecular, como a glutationa, o ácido ascórbico e os carotenóides, assim como as enzimas antioxidantes, tais como a superóxido dismutase (SOD), a ascorbato

peroxidase (APX) e a catalase (CAT). Essas enzimas reduzem eficientemente as EROs sob circunstâncias normais, mas se a redução completa não ocorrer, como sob condições de alta produção de EROs, o resultado pode ser um estado de estresse oxidativo levando à oxidação de biomoléculas (BOSCOLO et al., 2003).

2.4.1 Sistema antioxidante enzimático

As principais enzimas envolvidas na defesa de plantas contra as EROs incluem as superóxido dismutases (SOD), a ascorbato peroxidase (APX) e a catalase (CAT) (SHAH et al., 2001). As SODs estão localizadas em vários compartimentos celulares e catalisam a conversão de radicais superóxido ($O_2^{\bullet -}$) à H_2O_2, uma espécie de oxigênio menos destrutiva, e O_2. A CAT e APX estão envolvidas na conversão do H_2O_2 à H_2O (Figura 4).

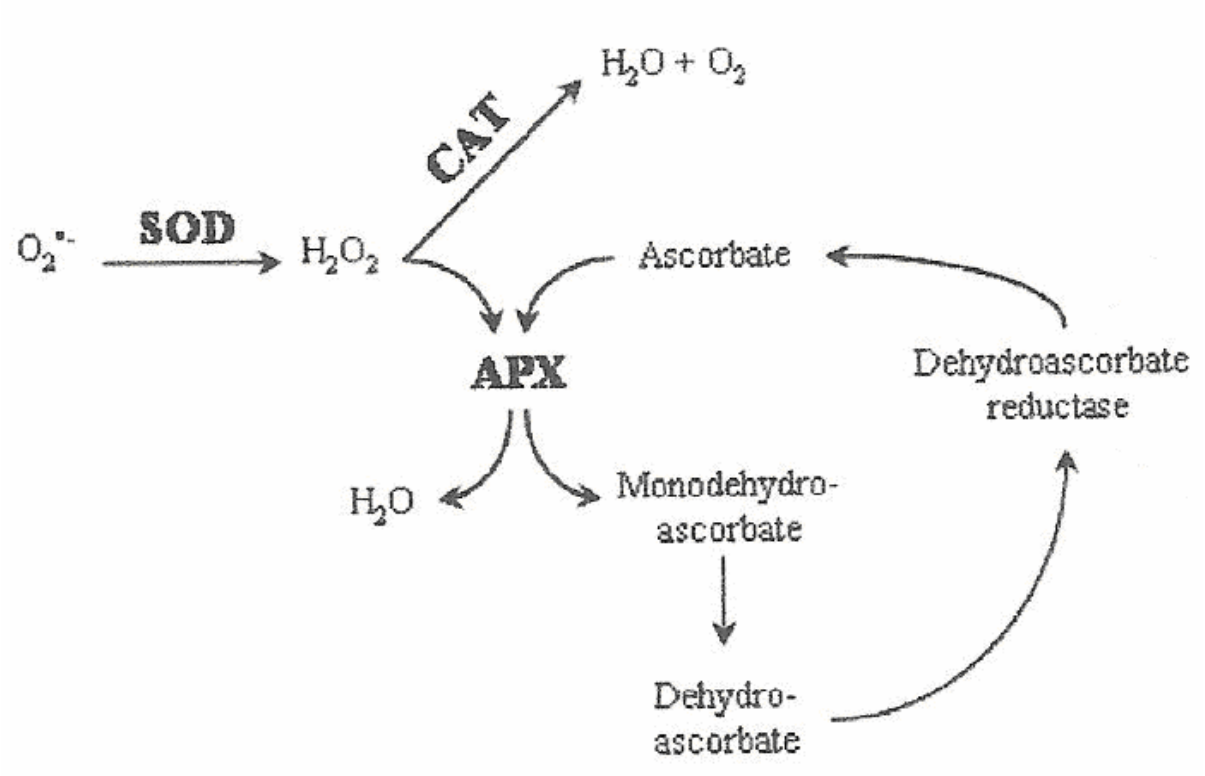

Figura 4. Representação dos mecanismos de defesa usando as enzimas antioxidantes SOD, CAT e APX contra as EROs (FOYER et al., 1994).

As catalases estão localizadas em peroxissomos/glioxissomos e mitocôndrias, enquanto que a APX, a qual utiliza ascorbato como doador de elétrons, está

primariamente localizada em cloroplastos e no citosol (HEGEDÜS et al., 2001). As diferentes afinidades da APX (variação μM) e CAT (variação mM) ao H_2O_2 sugerem que elas pertençam a duas diferentes classes de enzimas removedoras de H_2O_2: APX pode ser responsável pela fina modulação de EROs, enquanto CAT pode ser responsável para a remoção do excesso de EROs durante situações de estresse (MITTER, 2002). O balanço entre as atividades da SOD, CAT e APX é crucial para determinar o estado estável de radicais superóxido e peróxido de hidrogênio nas células. A importância dessas enzimas é baseada no fato de que a atividade de uma ou mais dessas enzimas em geral aumenta em plantas quando as mesmas são expostas a condições de estresse, e esta atividade aumentada está relacionada com um aumento na tolerância ao estresse ou à produção aumentada de EROs.

2.4.2 Sistema antioxidante não enzimático

Além do sistema de defesa antioxidante enzimático, as defesas antioxidantes não-enzimáticas são de fundamental importância para as células. Os antioxidantes não enzimáticos incluem, entre outros, o ácido ascórbico, a glutationa, o α-tocoferol e os carotenóides. O ácido ascórbico e a glutationa são encontrados em altas concentrações nos cloroplastos e outros compartimentos celulares (5-20 mM de ácido ascórbico e de 1-5 mM de glutationa) e são cruciais para a defesa da planta contra o estresse oxidativo (NOCTOR; FOYER, 1998). Além de seu papel como substratos de enzimas, eles podem reagir quimicamente com quase todas as formas de O_2 ativadas (HALLIWELL; GUTTERIDGE, 1999). O ácido ascórbico é sintetizado nas mitocôndrias e é transportado para todos os compartimentos sub-celulares incluindo o apoplasto, onde é o principal tampão redox modulando respostas fisiológicas e de estresse. Está associado com a remoção do H_2O_2 via ascorbato peroxidase (SAIRAM et al., 1998), além de reagir com radicais superóxido e radicais hidroxil (REDDY et al., 2004). Está também envolvido na regeneração de um outro antioxidante não enzimático, o α-tocoferol (SAIRAM et al., 2005).

Os grupos tióis não protéicos, entre estes a glutationa (GSH), são conhecidos por possuírem um papel central nos mecanismos de resposta aos metais em plantas terrestres (ZENK, 1996; RAUSER, 1999). A GSH é um tripeptídeo contendo enxofre,

e tem sido considerado como um antioxidante muito importante envolvido na defesa celular contra agentes tóxicos (SCOT et al., 1993). Em resposta a estresses, as plantas aumentam a atividade de enzimas biossintéticas de GSH e, consequentemente, as concentrações de GSH (NOCTOR et al., 2002). Além disso, a GSH é precursora na síntese de fitoquelatinas (COBBETT; GOLDSBOROUGH, 2002) e mantém o estado redox celular. Um alto nível de grupos tióis pode proporcionar aos metabólitos funcionarem na detoxificação de EROs e de radicais livres.

Nos últimos anos, uma grande diversidade de resultados, obtidos em estudos fisiológicos e de mapeamento molecular mostraram que a tolerância vegetal ao estresse causado pelo Al é uma característica multigênica complexa, que pode envolver vários mecanismos de tolerância. Além disso, estudos relativos aos mecanismos de resposta vegetal a estresses ambientais comprovaram que os agentes estressantes são percebidos de forma diferenciada pelos sistemas de sinalização das plantas, de acordo com a intensidade da sua ação (PASTORI; FOYER, 2002). No caso do estresse de Al, a situação deve ser similar uma vez que o tempo de exposição e a atividade do Al interagem tanto na manifestação dos sintomas de toxidez quanto na expressão dos mecanismos de tolerância ao estresse (PARKER, 1995; BARCELÓ; POSCHENREIDER, 2002; KOCHIAN et al., 2004).

Embora os mecanismos causais da toxidez do Al possam parecer complicados, não se deve esquecer que eles resultam, na sua essência, da ligação do Al com substâncias situadas na parede celular, na membrana plasmática ou no citoplasma, devido ao fato que o Al possui forte afinidade por compostos doadores de oxigênio (ROSSIELLO; NETTO, 2006). Isso significa um amplo leque de oportunidades de ligação a diversos sítios nos domínios apoplástico e simplástico.

3 Fosfatases ácidas

As enzimas fosfatases ácidas (APases) (E.C.3.1.3.2) catalisam a hidrólise de uma ampla variedade de monoésteres de fosfato, liberando fosfato inorgânico (Pi) de substratos fosforilados em pH abaixo de 7,0 (VINCENT et al., 1992). APases são ubíquas e abundantes em plantas, animais, fungos e bactérias, e exibem baixa especificidade de substratos (VINCENT et al., 1992; DUFF et al., 1994). Estão

presentes em vários órgãos e também em diferentes compartimentos celulares, sugerindo que essas enzimas estão envolvidas em vários compartimentos celulares (YONEYAMA et al., 2007).

O controle da expressão de APases é mediado por uma variedade de fatores ambientais e de desenvolvimento (DUFF et al., 1994). As APases são induzidas sob vários estresses, incluindo deficiência de água, salinidade e ataque de patógenos (BOZZO et al., 2002), assim como na germinação de sementes, florescimento, formação de tubérculos e amadurecimento de frutos (DUFF et al., 1994; GELLATLY et al., 1994; TURNER; PLAXTON, 2001), dificultando a definição de sua função nas células (PENHEITER et al., 1997; BOZZO et al., 2002). Entretanto, a ativação das APases em resposta a deficiência de Pi é bem documentada (DUFF et al., 1994).

As APases existem como isoenzimas específicas de compartimentos celulares ou tecidos, as quais se diferenciam quanto à massa molecular, especificidades quanto a substratos, sensibilidade à inibidores e a presença e número de carboidratos ligados à cadeia polipeptídica (VINCENT et al., 1992; DUFF et al., 1994). Além disso, estão envolvidas na produção, transporte e reciclagem de Pi, o qual é crucial para o metabolismo celular e para processos de transdução de energia (BOZZO et al., 2002). As APases intracelulares normalmente controlam a homeostase interna de Pi enquanto as APases secretadas controlam a aquisição externa de Pi (DUFF et al., 1994). Além disso, estão envolvidas em situações de estresse oxidativo, atuando no metabolismo de espécies reativas de oxigênio (EROs) (del POZZO et al., 1999).

4 Batata

A batata (*Solanum tuberosum* L.) é uma planta dicotiledônea, da família Solanaceae, do gênero *Solanum*. A batata cultivada, com exceção daquela da região dos Andes da América do Sul, pertence à sub-espécie *tuberosum*. É um dos alimentos mais consumidos no mundo como fonte de energia, devido à composição, versatilidade gastronômica e tecnológica e baixo custo de comercialização dos tubérculos (COELHO et al., 1999), sendo a hortaliça de maior importância econômica no Brasil (BISOGNIN, 2006). Os principais estados produtores são Minas

Gerais, São Paulo, Paraná e Rio Grande do Sul, responsáveis por mais de 90% da produção nacional (IBGE, 2004). A produção mundial de batata representa, aproximadamente, a metade da produção mundial de todas as raízes e tubérculos.

Utilizada desde tempos ancestrais pelos povos americanos, o processamento é tão antigo quanto o uso direto na alimentação humana (MELLO, 1997). A batata é plantada em, pelo menos, 125 países e consumida por mais de um bilhão de pessoas em todo o mundo; dentre estes, 500 milhões de consumidores são de países em desenvolvimento e, na sua dieta básica, está incluída a batata (SALLES, 1997). Nenhuma outra cultura pode competir com a batata como alimento energético e em termos de valor alimentar por unidade de área (SIECZA; THORTON, 1993). Possui também uma alta quantidade de vitamina C, niacina e vitamina B6. No Brasil, é a hortaliça mais importante (BISOGNIN, 1996), sendo que o hábito de utilizar batata na alimentação foi trazido pelos imigrantes europeus. O estado do Rio Grande do Sul figura entre os principais estados brasileiros em área cultivada com batata.

A cultura da batata se desenvolve sob uma variedade de altitudes, latitudes, e condições climáticas, desde o nível do mar até 4000 metros de elevação (DAVIES et al., 2005). Tolera uma acidez moderada no solo, produzindo bem na faixa de pH 5,0 a 6,5 (PREZOTTI et al., 1986). Acima desta faixa, pode ocorrer aumento da suscetibilidade dos tubérculos a certos patógenos presentes no solo, como é o caso da sarna. Por outro lado, nos solos excessivamente ácidos (pH abaixo de 5,0) ocorrem decréscimos de produção, uma vez que este pH prejudica o crescimento da planta pela própria ação da acidez, além de diminuir a disponibilidade de nutrientes e aumentar a concentração de alumínio trocável no solo (CASTRO, 1983).

As espécies cultivadas de batata são muito sensíveis a estresses abióticos, enquanto várias espécies primitivas ou selvagens de diferentes níveis de ploidia são bem adaptadas a crescer sob condições desfavoráveis tais como seca, frio, salinidade e alta radiação (LI; FENNEL, 1985; MENDOZA; ESTRADA, 1979). A descoberta que espécies de *Solanum* possuem diferenças genéticas na resistência ao estresse abiótico não é somente interessante para programas de melhoramento da batata, mas também fornece um bom material para se estudar outros aspectos dos mecanismos de resistência ao estresse abiótico.

RESULTADOS E DISCUSSÃO

Os resultados e discussão deste trabalho serão apresentados em cinco artigos científicos, distribuídos em quatro capítulos, como segue:

Capítulo I: Respostas fisiológicas e bioquímicas de quatro clones de batata expostos ao alumínio.

Artigo I: Physiological and oxidative stress responses of four potato clone to aluminum in nutrient solution.

Manuscrito I: Oxidative stress is an early symptom triggered by aluminum in Al-sensitive potato plantlets.

Capítulo II: Efeito do alumínio na atividade *in vitro* de fosfatases ácidas em quatro clones de batata.

Manuscrito II: *In vitro* activity of acid phosphatases of four potato clones cultivated in three growth systems: effect of aluminum.

Capítulo III: Influência do estresse de alumínio no teor de micronutrientes em plântulas de batata.

Manuscrito III: Micronutrient concentration in potato clones with distinct physiological sensitivity to Al stress.

Capítulo IV: Respostas localizadas e sistêmicas de estresse oxidativo induzidas por alumínio em batata (*Solanum tuberosum* L.) cultivadas em sistema de raízes divididas.

Manuscrito IV: Local and systemic oxidative stress responses induced by aluminum in two potato clones (S*olanum tuberosum* L.) that differ in Al-avoidance.

CAPÍTULO I

Respostas fisiológicas e bioquímicas de quarto clones de batata expostos ao alumínio

Artigo I

(Publicado no Periódico "Brazilian Journal of Plant Physiology")

RESEARCH ARTICLE

Physiological and oxidative stress responses of four potato clones to aluminum in nutrient solution

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Physiological and oxidative stress responses of four potato clones to aluminum in nutrient solution

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Aluminum toxicity is a serious problem in Brazilian soils and selecting potato clones is an important strategy to produce this crop on these kinds of soils. Potato clones, Macaca, SMIC148-A, Dakota Rose, and *Solanum microdontum*, were grown in a nutrient solution (pH 4.00±0.1) with 0, 50, 100, 150 and 200 mg Al L^{-1}. After 7 days, Al concentration in both root system and shoot of all clones increased linearly with increasing Al levels. Based on relative root growth, *S. microdontum* and SMIC148-A were considered Al-tolerant clones, whereas Macaca and Dakota Rose were considered Al-sensitive. Shoot growth in Macaca linearly decreased with increasing Al levels. Root H_2O_2 concentration in both Al-sensitive clones increased with increasing Al supply, whereas in Al-tolerant clones it either decreased (SMIC148-A) or demonstrated no alteration (*S. microdontum*). Shoot H_2O_2 concentration increased linearly in Macaca, whereas for Dakota Rose it showed a quadratic relationship with

Al levels. On the other hand, shoot H_2O_2 concentration in the Al-tolerant clones either demonstrated no alteration (*S. microdontum*) or presented lower levels (SMIC148-A). Root catalase (CAT) activity in both Al-sensitive clones increased with increasing Al levels, whereas in Al-tolerant clones it either demonstrated no alteration (SMIC148-A) or presented lower levels (*S. microdontum*). Shoot CAT activity in the *S. microdontum* increased curvilinearly with increasing Al levels. In all potato clones, chlorophyll concentration showed a curvilinear response to Al supply, where in Al-sensitive clones it decreased upon addition of Al exceeding 100 mg L^{-1}, but in SMIC148-A it increased at levels between approximately 100 and 150 mg L^{-1}, and decreased in *S. microdontum* regardless of the Al level. Carotenoid concentrations in the Al-sensitive clones were linearly decreased with increasing Al levels. Al supply caused root lipid peroxidation only in the Al-sensitive clones, whereas in the shoot it increased linearly in the Al-sensitive clones and in *S. microdontum* it only increased at around 50 mg L^{-1}. Most of root protein oxidation was only observed in the Al-sensitive clones. However, shoot protein oxidation was increased with increasing Al levels for all potato clones. These results indicate that oxidative stress caused by Al in potato may harm several components of the cell, mainly in Al-sensitive clones.

Keywords: aluminum toxicity, antioxidative enzymes, growth, oxidative stress, *Solanum tuberosum*

Respostas fisiológicas e de estresse oxidativo de quatro clones de batata ao alumínio em solução nutritiva: A toxicidade do alumínio é um problema sério em solos brasileiros e a seleção de clones de batata é uma estratégia importante para produzir esta cultura em tais solos. Clones de batata, Macaca, SMIC148-A, Dakota Rose e *Solanum microdontum*, foram cultivados em solução nutritiva (pH 4,0±0,1) com 0, 50, 100, 150 e 200 mg Al L^{-1}. Após 7 d, o teor de Al em raízes e parte aérea em todos clones aumentou linearmente com o suprimento de Al.

Baseado no crescimento relativo da raiz, os clones *S. microdontum e* SMIC148-A foram considerados tolerantes ao Al, enquanto os clones Macaca e Dakota Rose foram considerados sensíveis. O crescimento da parte aérea do clone Macaca diminuiu linearmente com o Al. A concentração de H_2O_2 nas raízes de ambos os clones sensíveis ao Al aumentou com o suprimento de Al, enquanto nos clones tolerantes houve declínio (SMIC148-A) ou falta de resposta (*S. microdontum*). A concentração de H_2O_2 na parte aérea aumentou linearmente em Macaca, enquanto em Dakota Rose houve uma relação quadrática com os níveis de Al. Por outro lado, nos clones tolerantes ao Al a concentração de H_2O_2 não foi alterada (*S. microdontum*) ou foi reduzida (SMIC148-A). A atividade da catalase (CAT) nas raízes de ambos os clones sensíveis ao Al aumentou com o suprimento de Al, enquanto nos clones tolerantes não houve alteração (SMIC148-A) ou, então, redução (*S. microdontum*). Na parte aérea, a atividade da CAT em *S. microdontum* aumentou com o suprimento de Al. Em todos os clones de batata, a concentração de clorofila variou curvelinearmente em relação ao suprimento de Al; nos clones sensíveis, a concentração de clorofila diminuiu pela adição de Al em níveis acima de 100 mg L^{-1}, porém em SMIC148-A houve aumento na presença de Al (na faixa próxima a 100 e 150 mg L^{-1}) e diminuição em *S. microdontum,* independentemente do tratamento de Al. A concentração de carotenóides nos clones sensíveis ao alumínio diminuiu linearmente, em resposta ao Al. O Al aumentou a peroxidação lipídica em raízes dos clones sensíveis, enquanto na parte aérea houve aumento linear nesses clones e também em *S. microdontum* (próximo a 50 mg Al L^{-1}). Em raízes, a oxidação protéica foi observada principalmente nos clones sensíveis ao alumínio. Entretanto, na parte aérea, foi observada oxidação protéica em todos os clones de batata em resposta ao Al. Esses resultados indicam que o estresse oxidativo causado por Al em batata pode prejudicar vários componentes celulares, principalmente nos clones sensíveis ao metal.

Palavras-chave: crescimento, enzimas antioxidativas, estresse oxidativo, *Solanum tuberosum*, toxicidade de Al

INTRODUCTION

Most tropical soils present an acid characteristic that decreases nutrient availability and increases aluminum (Al) toxicity, affecting plant growth and development (Marschner, 1991). Aluminum is the most abundant metal and the third most common element in the earth's crust. Aluminum toxicity is considered a major abiotic stress factor in low pH soils. Aluminum stress impairs root growth, decreasing the absorption, transport and use of several nutrients such as P, Ca, Mg, S, Fe and Mn and diminishing biomass production (Brondani and Paiva, 1996).

The initial and most evident symptom of Al-toxicity is a rapid inhibition of root elongation (Dipierro et al., 2005), which can occur within minutes after exposing roots to Al, with less marked effects on shoot development. Severe Al-toxicity reduces and damages the root system, causing plant drought susceptibility and mineral nutrient deficiency. The principal sites of Al-toxicity are the actively dividing and expanding cells of the root apex (Ryan et al., 1993). Aluminum can rapidly enter into the cytoplasm (Lazof et al., 1994), but it is still unknown whether the primary site(s) of toxicity is external (interactions with the cell wall or external face of plasma membrane) or internal (affecting cytoplasmic functions or activities in internal membranes/compartments). After prolonged exposure (e.g. 12 h), Al can affect many physiological processes either directly or indirectly (Kochian, 1995). Genetic variability to Al-tolerance exists among and within plant species.

Many environmental stresses induce the formation of reactive oxygen species (ROS) in plant cells (Schützendübel and Polle, 2002). Al-toxicity in plants is a well-known example of such environmental stress (Kochian, 1995; Ma et al., 2001). Under normal conditions, the production and destruction of these radicals is regulated by cell metabolism. To prevent cellular

compartments from the damaging effects of ROS, organisms have evolved multiple detoxification mechanisms, including synthesis of antioxidant molecules (ascorbic acid, glutathione and carotenoids) and enzyme systems such as superoxide dismutases (SOD, E.C.1.15.1.1), ascorbate peroxidase (APX, E.C.1.11.1.11) and catalase (CAT, E.C.1.11.1.6). These ROS can attack membranes, proteins and nucleic acids causing lipid peroxidation, protein denaturation and DNA mutation (Schützendübel and Polle, 2002). Oxidative stress is probably an important component of plant response to Al-toxicity.

It has been suggested that Al^{3+}, the most toxic of the soluble forms of Al (Parker et al., 1988), induces oxidative stress, since this ion is involved in various process, including an increase in SOD activity and lipid peroxidation in soybeans (Cakmak and Horst, 1991), peas (Yamamoto et al., 2001) and tobacco plants (Ikegawa et al., 2000). Moreover, alterations in the expression of various genes induced by Al in *Arabidopsis* (Richards et al., 1998), tobacco (Ezaki et al., 2000) and wheat (Snowden and Gardner, 1993) have been reported.

Potatos are grown world-wide under a wider range of altitudes, latitudes, and climatic conditions than any other major food crop – from sea level to over 4000 m elevation. No other crop can match potato in its production of food energy and food value per unit area (Sieczka and Thornton, 1993). The widely cultivated potato (*Solanum tuberosum* subsp. *tuberosum*) is very sensitive to abiotic stresses, whereas several wild or primitive cultivated species of different ploidy levels are well adapted to growth under unfavorable conditions such as drought, cold, salinity and high irradiation (Li and Fennell, 1985). The fact that the *Solanum* species possess genetic variation for abiotic stresses is not only interesting for potato breeding but also as a model plant to study other aspects of physiological resistance. An appropriate approach to evaluate the Al stress response is a genotype evaluation in nutrient solution under controlled conditions (Schmohl et al., 2000; Jorge et al., 2001; Boscolo et al., 2003). In spite of the importance of potato, there is no report in the literature on its antioxidant system under Al stress

conditions. The antioxidant system is responsible for scavenging excess free radicals caused by environmental stresses. Studying the major components of the antioxidant system under Al stress, it is possible to ascertain whether Al induces oxidative stress, and whether it is involved in Al-tolerance mechanisms.

The objective of the present study was, therefore, to investigate and compare some physiological and oxidative stress responses of four potato clones, Macaca, SMIC148-A, Dakota Rose (all of *S. tuberosum*) and *Solanum microdontum*, , exposed to Al in nutrient solution.

MATERIAL AND METHODS

Plant materials and growth conditions: Three adapted (2n=4x=48) clones (Macaca, SMIC148-A and Dakota Rose) and one wild species (2n=2x=24) clone (PI595511-5/ *S. microdontum*) were evaluated. The *S. microdontum* clone was identified as highly resistant to *Phytophora infestans* (Bisognin et al., 2005) and has been used in our breeding program. This clone will be referred to as *S. microdontum*. Tissue culture plantlets were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, Brazil. Nodal segments (1.0 cm long) were micropropagated in MS medium (Murashige and Skoog, 1962), supplemented with 30 g L^{-1} of sucrose, 0.1 g L^{-1} of myo-inositol and 6 g L^{-1} of agar. Twenty-day-old plantlets from *in vitro* culture were transferred into plastic boxes (10 L) filled with aerated full nutrient solution of low ionic strength. The nutrient solution had the following composition (in μM): 6090.5 of N; 974.3 of Mg; 5229.5 of Cl; 2679.2 of K; 2436.2 of Ca; 359.9 of S; 0.47 of Cu; 2.00 of Mn; 1.99 of Zn; 0.17 of Ni; 24.97 of B; 0.52 of Mo; 47.99 of Fe (FeSO4/Na-EDTA). Treatments consisted of the addition of 0, 50, 100, 150 or 200 mg L^{-1} of Al as $AlCl_3.6H_2O$. The solution pH was adjusted daily to 4.0 ± 0.1 by titration with HCl or NaOH solutions of 0.1 M. Both *in vitro* and *ex vitro* cultured plants were grown in a growth chamber at 25 ± 2ºC on a 16/8-h light/dark cycle with

35 μmol m^{-2} s^{-1} of irradiance. Aluminum-treated plantlets remained in each treatment for 7 d. At harvest, the plants were divided into shoot and roots. Roots were rinsed twice with distilled water. Subsequently, growth and biochemical parameters were determined. Three replicates with nine seedlings were made for each treatment.

Aluminum determination: After Al treatment, samples (roots and shoot) were separated and washed in deionized water twice and dried at 60ºC until reaching a constant weight. The dried tissues were weighed and ground into a fine powder before nitric-perchloric digestion. Aluminum concentrations were determined by atomic absorption spectrometry. A standard calibration curve was prepared for the 0-200 mg L^{-1} Al concentration range.

Growth parameters: To access different responses to Al sensitivity the relative root growth (RRG) of four clones was determined. Before Al treatment, the length of the main root of each plantlet was measured and recorded. Afterwards, the plantlets returned to the nutrient solution. At the end of the experiment (7 d after Al application), the length of the main root was measured again. The RRG was calculated by dividing the root growth of each seedling under a given treatment by the mean root growth of all plantlets grown in the control solution (Jorge et al., 2001). Shoot length and total number of nodal segments per plantlet were also determined.

Determination of hydrogen peroxide: The H_2O_2 concentration was determined according to Loreto and Velikova (2001). Approximately 0.1 g of both roots and shoots was homogenized at 4ºC in 2 mL of 0.1% trichloroacetic acid (TCA) (w/v). The homogenate was centrifuged at 12,000 x *g* for 15 min at 4ºC. Then, 0.5 mL of the supernatant was added to 0.5 mL of 10 mM K-phosphate buffer (pH 7.0) and 1 mL of 1M KI. The H_2O_2 concentration of the supernatant

was evaluated by comparing its absorbance at 390 nm with a standard calibration curve. Hydrogen peroxide concentration was expressed as μmol g^{-1} FW.

Catalase assay: Catalase activity was assayed following the modified Aebi (1984) method. Fresh roots and shoot samples (1 g) were homogenized in 5 mL of 50 mM K-phosphate buffer (pH 7.0), 10 g L^{-1} PVP, 0.2 mM EDTA and 10 mL L^{-1} Triton X-100. The homogenate was centrifuged at 12,000 x *g* for 20 min at 4ºC and the supernatant was used for enzyme assay. Activity of CAT was determined by measuring the decrease in absorbance at 240 nm of a reaction mixture with a final volume of 2 mL containing 15 mM H_2O_2 in K-phosphate buffer (pH 7.0) and 30 μL extract. Activity was expressed as ΔE min^{-1} mg^{-1} protein.

Chlorophyll and carotenoid determination: Chlorophyll and carotenoids were extracted following the method of Hiscox and Israelstam (1979) and estimated with the help of Arnon's formulae (Arnon, 1949). Fresh leaves (0.1 g) were incubated at 65ºC in dimethylsulfoxide (DMSO) until pigments were completely bleached. Absorbance of the solution was then measured at 663 and 645 nm for chlorophyll and 470 nm for carotenoids. Chlorophyll and carotenoid concentrations were expressed as μg g^{-1} FW and mg g^{-1} FW, respectively.

Estimation of lipid peroxides: The degree of lipid peroxidation was estimated following the method of El-Moshaty et al. (1993). Fresh roots and shoot samples of 0.1 g were homogenized in 20 mL of 0.2 M citrate-phosphate buffer (pH 6.5) containing 0.5% Triton X-100, using mortar and pestle. The homogenate was filtered with two paper layers and centrifuged for 15 min at 20,000 x *g*. One milliliter of the supernatant fraction was added to an equal volume of 20% (w/v) TCA containing 0.5% (w/v) of thiobarbituric acid (TBA). The mixture was heated at 95ºC for 40 min and then quickly cooled in an ice bath for 15 min, and centrifuged at 10,000 x *g*

for 15 min. The absorbance of the supernatant at 532 nm was read and corrected for unspecific turbidity by subtracting the value of the absorbance at 600 nm. The lipid peroxides were expressed as nmol MDA mg^{-1} protein, by using an extinction coefficient of 155 L $mmol^{-1}$ cm^{-1}.

Protein oxidation: Samples of roots and shoot (1 g) were homogenized with 25 mM K-phosphate buffer (pH 7.0) containing 10 mL L^{-1} Triton X-100, at a proportion of 1:2 (w/v) (Levine et al., 1990). After the homogenate was centrifuged at 15,000 x *g* for 10 min at 4ºC, the supernatant was used for immediate determination of protein oxidation, which was expressed as nmol carbonyl mg^{-1} protein.

Protein determination: In all the enzyme preparations, protein was determined following Bradford (1976) using BSA for constructing the standard curves.

Statistical analysis: All data were analyzed by ANOVA procedures. The effects of Al on growth and biochemical parameters in potato plantlets were quantified using regression analysis with the SOC statistic package (Software Científico: NTIA/EMBRAPA). Coefficients were included in a regression equation when their values were significant ($P < 0.05$).

RESULTS

Al concentration: Regression analysis showed that the concentration of Al in both the roots and shoot of all clones studied increased linearly with increasing Al levels, and the increase in tissue Al was much steeper for Macaca and SMIC148-A (Figure 1A,B).

Aluminum accumulated more in roots than in shoot (on average of 3.9-, 2.8-, 3.6-, and 3.7-fold greater in roots than in shoot, respectively in Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones). The maximum concentration of Al in roots and shoot was 49,300 and 17,900 mg kg^{-1}, as respectively found in the Dakota Rose clone at 200 mg L^{-1}. In Macaca and SMIC148-A clones, Al concentration was lower at levels above 50 mg L^{-1} when compared with the Dakota Rose and *S. microdontum* clones (Figure 1A, B).

Growth analysis: The response of root growth in the Al-sensitive clones (Macaca and Dakota Rose) to Al levels was linear and negative (Figure 2A), whereas in the Al-tolerant clones there was no alteration. At 200 mg L^{-1} of Al, root growth of Macaca and Dakota Rose clones decreased by about 95 and 70%, respectively, when compared to the control. Therefore, Macaca and Dakota Rose were classified as Al-sensitive clones, the and *S. microdontum* and SMIC148-A as Al-tolerant clones.

Aluminum negatively affected shoot length only in Macaca plantlets (Figure 2B). At 200 mg L^{-1} of Al, shoot length was decreased by 74% when compared to the control. Also, Al treatments linearly reduced the total number of nodal segments in Macaca, SMIC148-A and Dakota Rose clones (Figure 2C).

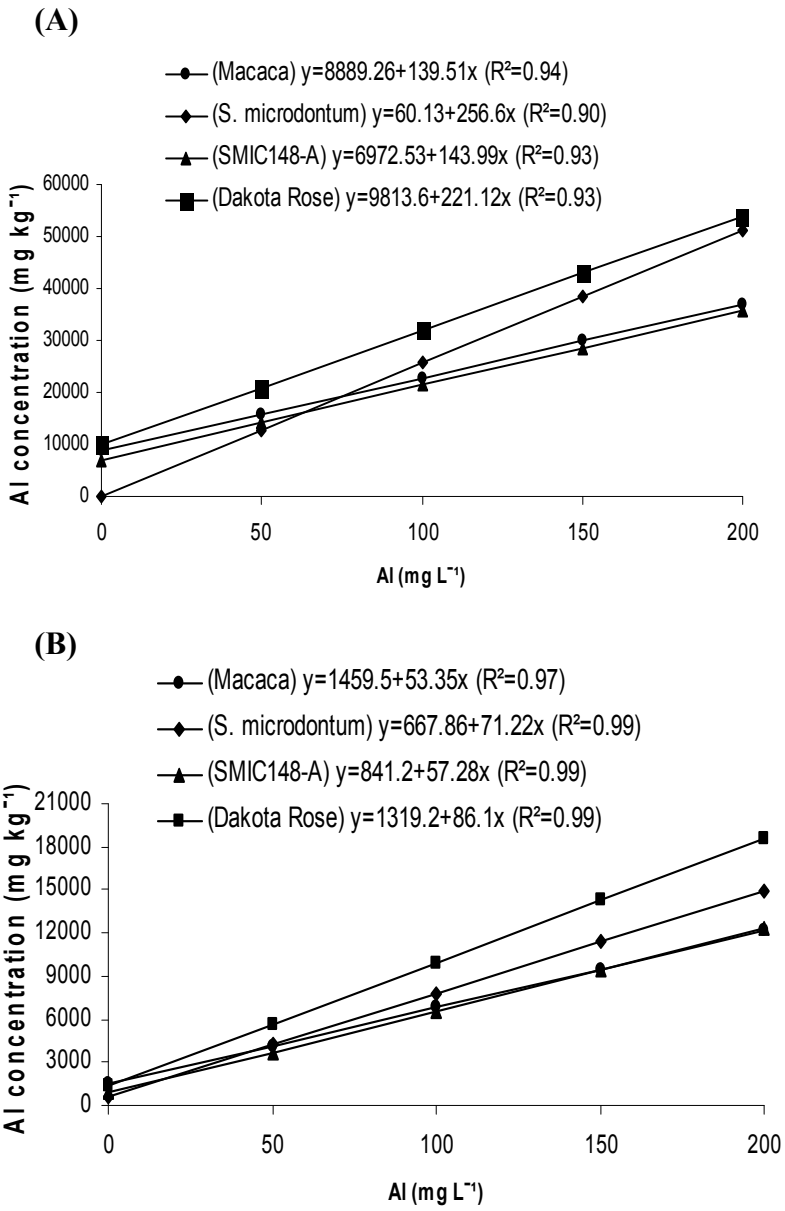

Figure 1. Aluminum concentration in roots (**A**) and shoot (**B**) of potato plants (Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones) submitted to increasing Al levels for 7 d. Each point is the mean of three replicates.

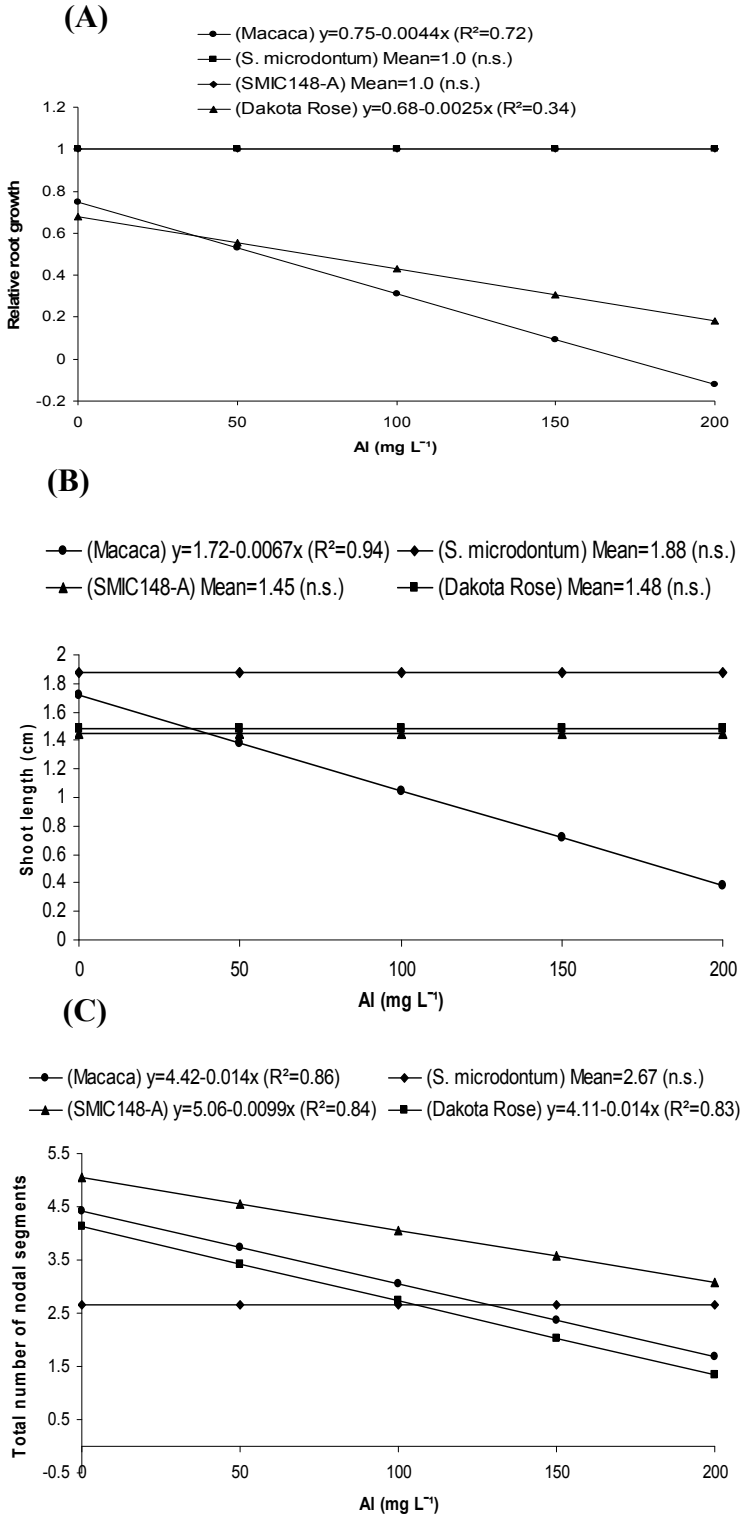

Figure 2. Relative root growth (**A**), shoot length (**B**) and total number of nodal segments (**C**) in potato plants (Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones) submitted to increasing Al levels for 7 d. Each point is the mean of three replicates. n.s. = not significant.

Catalase activity and hydrogen peroxide concentration: Root H_2O_2 concentration in the two Al-sensitive clones increased with increasing Al levels, whereas in the Al-tolerant clones it either decreased (SMIC148-A) or did not demonstrate any alteration (*S. microdontum*) (Figure 3A).

Shoot H_2O_2 concentration increased linearly in Dakota Rose, whereas for Macaca it showed a quadratic relationship with Al levels. On the other hand, H_2O_2 concentration in the Al-tolerant clones either did not demonstrate any alteration (*S. microdontum*) or presented lower levels (SMIC148-A) (Figure 3B).

Root CAT activity increased curvilinearly with increasing Al levels in Macaca, whereas in Dakota Rose it decreased at *ca.* 50 mg L^{-1} and increased at levels exceeding 100 mg L^{-1} (Figure 3C). Root CAT activity in the Al-tolerant clones either did not demonstrate any alteration (SMIC148-A) or presented lower levels (*S. microdontum*). Shoot CAT activity was only altered in *S. microdontum*, where it increased curvilinearly with increasing Al levels exceeding 100 mg L^{-1} (Figure 3D).

Chlorophyll and carotenoids levels: In all potato clones, chlorophyll concentration showed a curvilinear response to Al supply, where it increased at *ca.* 50 mg Al L^{-1}, except for *S. microdontum*, and decreased at levels exceeding 100 mg L^{-1} in the Al-sensitive clones. However, in SMIC148-A, it increased between approximately 100 and 150 mg L^{-1}, and in *S. microdontum* it decreased regardless of the Al level (Figure 4A). Carotenoid concentrations in the Al-sensitive clones linearly decreased with increasing Al levels, whereas in the Al-tolerant clones there was no alteration (Figure 4B).

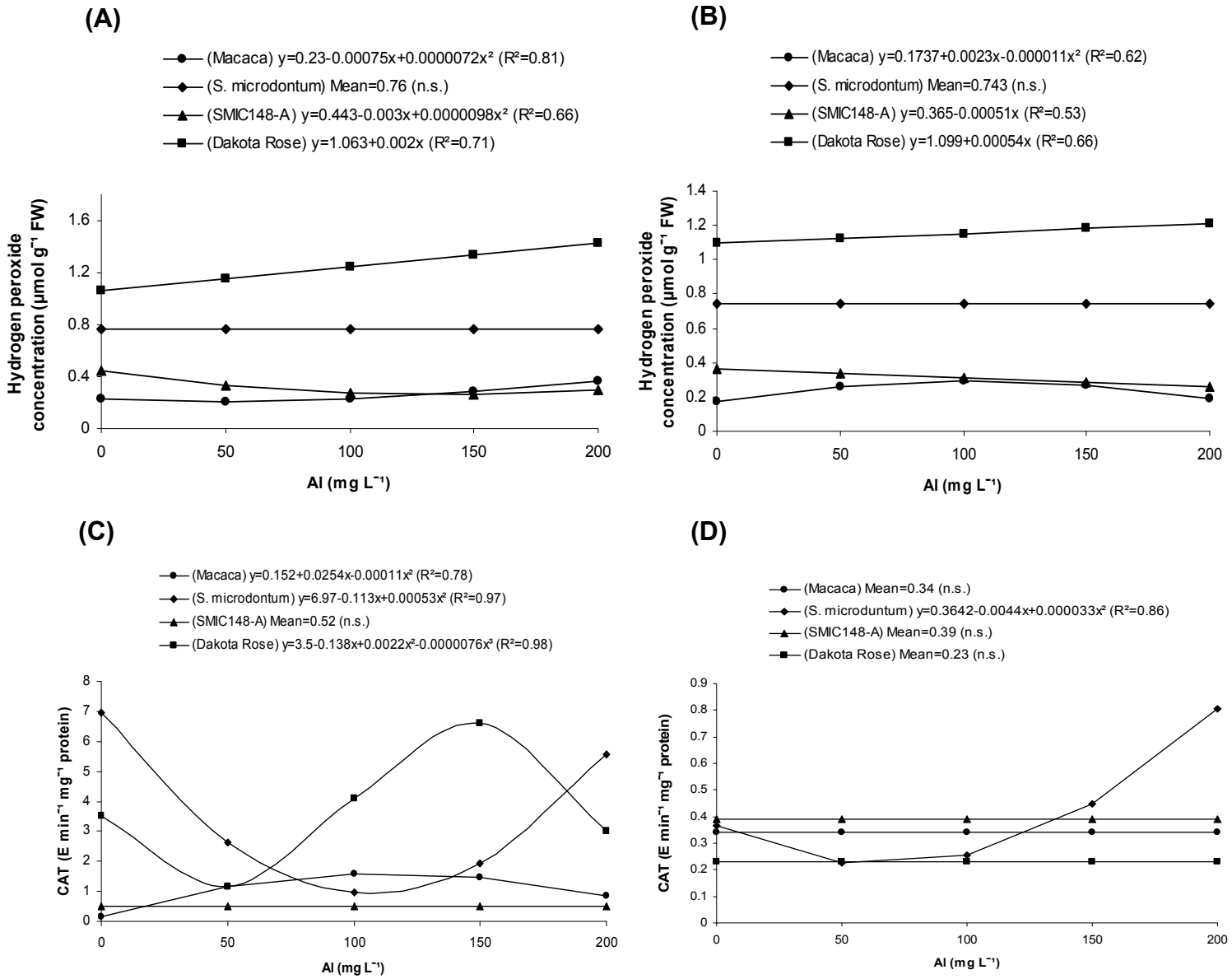

Figure 3. Concentration of H_2O_2 in roots (A) and shoot (B) and catalase activity of roots (C) and shoot (D) in potato plants (Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones) submitted to increasing Al levels for 7 d. Each point is the mean of three replicates. n.s. = not significant.

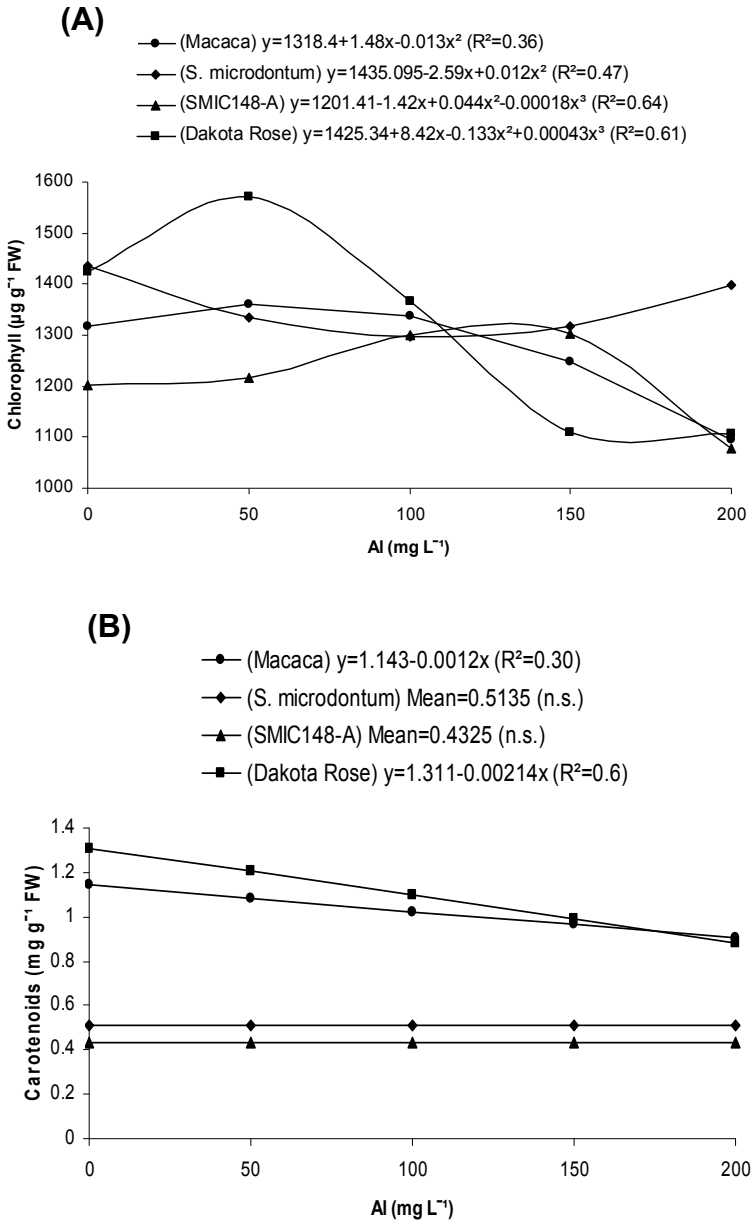

Figure 4. Chlorophyll (**A**) and carotenoid (**B**) concentrations in potato plants (Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones) submitted to increasing Al levels for 7 d. Each point is the mean of three replicates. n.s. = not significant.

Lipid peroxidation and protein oxidation: Concentration of MDA in roots and shoot of both Macaca and Dakota Rose increased linearly with increasing Al levels, indicating enhanced lipid peroxidation for these Al-sensitive clones (Figure 5A, B). In Macaca and Dakota Rose, the increase of lipid peroxidation in roots was of *ca.* 55% and 73%, respectively, and in the shoot it

was of about 72% and 149%, respectively. Interestingly, the basal level of lipid peroxidation both in roots and shoot of *S. microdontum* (Al-tolerant clone) was significantly higher than that of the others. In spite of this, only at lower Al levels did an increase in lipid peroxidation occur (65%) in the shoot of this clone (Figure 5B).

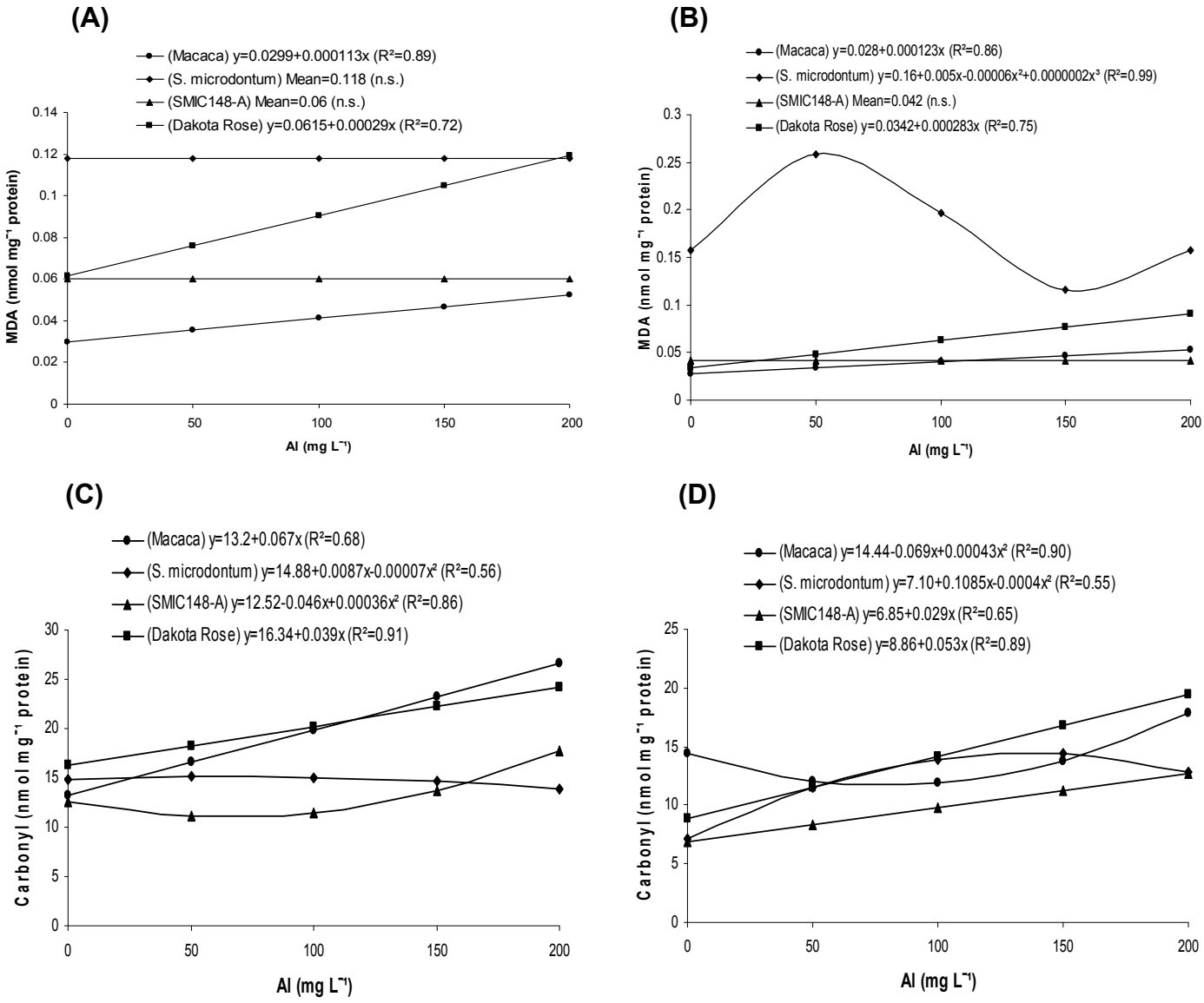

Figure 5. Lipid peroxides in roots (**A**) and shoot (**B**) and protein carbonyl in roots (**C**) and shoot (**D**) in potato plants (Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones) submitted to increasing Al levels for 7 d. Each point is the mean of three replicates. n.s. = not significant.

The carbonyl concentrations in the roots of Al-sensitive clones increased linearly with increasing Al levels, while in the Al-tolerant clones there was a curvilinear response in which SMIC148-A presented increased carbonyl concentrations at levels exceeding 100 mg L^{-1} and, in contrast, *S. microdontum* presented a decline in the same Al range (Figure 5C). In the shoot, an increase in protein oxidation occurred for all clones except in the Macaca clone, where it decreased at levels below 150 mg L^{-1} (Figure 5D). In SMIC148-A and Dakota Rose clones, Al caused a linear increase in carbonyl concentrations, whilst in the *S. microdontum* clones the carbonyl concentrations increased curvelinearly with increasing Al levels.

DISCUSSION

In the present study, the significant, variable reduction of root growth in potato clones exposed to Al suggests a distinct physiological sensitivity to Al stress. The phytotoxic effects of Al to the root system, in turn, can cause susceptibility to drought stress and mineral nutrient deficiencies (Degenhardt et al., 1998). This, consequently, might negatively affect growth and development of Al-sensitive plants. Since Al also induced root damage, and roots are the main site of cytokinin synthesis, the reduction in shoot length may therefore be a consequence of impaired cell division in the root meristem (Meriga et al., 2004). Since Al primarily affects the root tips, effects on shoot development may be expressed only at later stages as a result of altered water and nutrient uptake as well as phytohormone production (Collet and Horst, 2001).

Nutrient solutions used as a substrate contain divalent cations which can compete with Al and influence their availability for plant uptake. Yet, the high Al concentrations of the nutrient solution overcame these limiting factors, since Al concentrations in root tissues showed a significant 3.5-fold increase between Al levels of 0 and 200 mg L^{-1}. Almost all the adsorbed/precipitated Al on the roots' outer surface and in root cortical cells is not removed

after washing with water. Thus, as roots were only washed with deionized water before Al analysis, the values obtained for Al concentrations are related to both absorbed and adsorbed mechanisms. The sharp increase in root Al concentrations was closely related to the level of Al in the nutrient solution, as has been reported elsewhere (Lidon et al., 1999). Moreover, the quite high Al concentration measured in root tissues in the control was related to a direct uptake from the water/tray substrate. Root Al concentrations for the Al treatments were mostly associated with an increase in the level of Al in the nutrient solution. Accumulation of Al was lower in the shoot, which indicates that the absorbed Al was mostly retained in root tissues.

A common feature of several stresses, including Al toxicity, is the perturbation of cell redox homeostasis, enhancing ROS production, which is generally considered harmful to plant cells (Richards et al., 1998). Studies on Al-toxicity in roots suggest that the production of ROS may significantly contribute to Al-induced inhibition of root elongation (Tamás et al., 2004). Furthermore, several reports have shown that Al stress can increase the production of ROS, and activate several oxidative enzymes in plant and animal cells (Cakmak and Horst, 1991). Thus, oxidative stress is possibly an important component of Al-toxicity plant responses.

In the present study, Al stress increased H_2O_2 concentration in roots and shoot of both Al-sensitive clones. Elevated H_2O_2 production due to Al has also been observed in barley (Simonovicová et al., 2004), wheat (Darkó et al., 2004) and pumpkin roots (Dipierro et al., 2005). Tamás et al. (2004) also reported elevated H_2O_2 production in intact germinating barley seeds during Al stress. In relation to roots and shoot of Al-tolerant clones, in the SMIC148-A clone reduced H_2O_2 concentration was observed with increasing Al levels. This decline of H_2O_2 concentration might be due to the scavenging action of the antioxidant system. It can be suggested that Al treatment mainly induced an oxidative burst in both roots and shoot of the Al-sensitive clones, where the antioxidant system was not able to protect these clones from Al toxicity.

Among the enzymatic systems considered to play an important role in the cellular defense strategy against oxidative stress, CAT plays a pivotal role as it decomposes H_2O_2 to water and O_2. Interestingly, the greater CAT activity in roots than in shoot might indicate higher oxidative stress in roots. In fact, the marked increase of CAT activity in the roots with increasing Al levels in Macaca and Dakota Rose clones may indicate enhanced production of ROS under an excess of Al. In contrast, at lower levels of Al exposure, CAT activity was inhibited in roots of *S. microdontum* and Dakota Rose clones. This lower activity in Al-stressed plants is suggestive of a possible delay in removal of H_2O_2 and toxic peroxides mediated by CAT and in turn an enhancement in the free radical mediated lipid peroxidation under Al-toxicity (Shi et al., 2006). Aluminum increased the activity of CAT in the shoot only in the *S. microdontum* clone at higher Al concentrations. This enhanced activity seems to be related to increased oxidative stress tolerance (Allen, 1995).

As a visible symptom, the reduced chlorophyll concentration can be used to monitor Al induced damage in green leaves. In the present study, the reduction in chlorophyll concentration observed for Macaca, *S. microdontum* and Dakota Rose clones indicates oxidative damage induced by Al exposure, possibly due to the inhibition of aminolevulinic acid dehydratase, an important enzyme in chlorophyll biosynthesis (Pereira et al., 2006). Carotenoid concentration decreased in Macaca and Dakota Rose (Al-sensitive clones). Although the principal recognized role of carotenoids is to act as photoreceptive antenna pigment for photosynthesis, collecting wavelengths of light that are not absorbed by chlorophylls, their protective function against oxidative damage has also been recognized for several decades (Larson, 1988). Perhaps the most important function of carotenoid is the dissipation of excess energy of excited chlorophyll and the elimination of ROS (Lawlor, 2001).

Lipid peroxidation is a metabolic process that can occur under normal aerobic conditions and is one of the most investigated ROS effects on membrane structure and function (Blokhina

et al., 2003). It is widely reported that ROS bring about peroxidation of membrane lipids leading to membrane damage. Since cell membranes are the first targets of many plant stresses, the maintenance of their integrity and stability under stress conditions is a major component of Al tolerance in plants. In the present study, MDA concentration in both roots and shoot was significantly increased with increasing Al levels in the two Al-sensitive clones, indicating enhanced lipid peroxidation for these clones and, therefore, the presence of poisoning ROS. In *S. microdontum* (Al-tolerant clone), an increased in shoot lipid peroxidation occurred only at Al levels of approximately 50 mg L^{-1}, indicating that the active stress was lower and growth inhibition was smaller. These results also indicate that the antioxidative system in Al-tolerant clones was more efficient to protect the membrane lipids from Al stress. Several studies have shown increased lipid peroxidation in plants exposed to Al. In wheat, the increase of MDA concentration in the Al-sensitive cultivar was greater than in Al-tolerant cultivar (Dong et al., 2002). Yamamoto et al. (2001) showed the induction of lipid peroxidation in pea plants after 4 h of exposure to Al^{3+}. Cakmak and Horst (1991) also observed an increase in lipid peroxidation of a sensitive soybean cultivar after 24 h of treatment. Basu et al. (2001) found a correlation between decreased lipid peroxidation and increased resistance to Al in *Brassica napus*.

The possible connection between Al stress and oxidative stress had also been previously suggested by Cakmak and Horst (1991) based upon the fact that the Al-induced inhibition of root elongation was correlated with enhanced lipid peroxidation. The interpretation of these findings was that the primary effects of Al could be the induction of free radical generation and related alterations in the membrane structure. Further evidence corroborating the relation between Al stress and oxidative stress in plants has been obtained with transgenic arabidopsis plants (Ezaki et al., 2000).

In the present study, protein damage due to increased stress suggests Al-induced formation of ROS. Halliwell and Gutteridge (1999) suggested that the oxidation of proteins to

form carbonyls occurs via the hydroxyl radical, since neither H_2O_2 nor superoxide is reactive enough to provoke oxidation. The accumulation of carbonyls in the shoot of all potato clones studied indicates that the quantity of radicals generated exceeded the capacity of the antioxidant defensive system, whereas in roots of the Al-tolerant clones, ROS were eliminated by plant defenses more efficiently. Interestingly, Boscolo et al. (2003) found that the onset of protein oxidation in two inbred lines of maize took place after the reduction of RRG observed in the sensitive line, indicating that oxidative stress is not the primary cause of root growth inhibition. In addition, the presence of Al did not induce lipid peroxidation in either line, contrasting with the observations made in other species. In order to characterize four potato clones for their Al sensitivity in hydroponics, using root elongation, Al-induced callose formation and Al concentrations of root tips as parameters, Schmohl et al. (2000) found that the higher genotypic Al sensitivity was related to enhanced Al accumulation in root tips, and that the transgenic potato mutant that overexpressed pectin methylesterase proved to be more Al-sensitive than the wild type. These data clearly demonstrate the importance of apoplast properties for the expression of Al-toxicity.

Although potato is a commercially important plant, there is not much knowledge about its Al stress tolerance and the physiological consequences of this stress. Thus, the finding that *Solanum* species possess genetic differences in abiotic stress resistance shows that it is good plant material for studying other aspects of abiotic stress resistance mechanisms. Based on the present work, it can be suggested that toxic concentrations of Al cause oxidative stress, as evidenced by increased H_2O_2 formation, lipid peroxidation and oxidation of proteins in roots and shoot of plants, mainly in Al-sensitive clones. In this study, a significant reduction in different parameters such as length of shoot and roots, chlorophyll and carotenoid concentrations coupled with lipid peroxidation and protein oxidation indicated that high Al levels in nutrient solution produced toxic effects. It was proposed that the reduced growth in Al-

sensitive clones of potato exposed to toxic levels of Al might be induced by an enhanced production of toxic oxygen species and subsequent lipid peroxidation. Moreover, it was possible to observe that Al-tolerant plants developed some defense mechanisms against oxidative stress. Further studies are required to investigate whether the oxidative stress caused by Al toxic levels is an early symptom that can trigger root growth inhibition.

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REFERENCES

Aebi H (1984) Catalase *in vitro*. Meth. Enzymol. 105:121-126.

Allen RD (1995) Dissection of oxidative stress tolerance using transgenic plants. Plant Physiol. 107:1049-1054.

Arnon DI (1949) Copper enzymes in isolated chloroplasts: polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24:1-15.

Basu U, Good AG, Taylor GJ (2001) Transgenic *Brassica napus* plants overexpressing aluminium-induced mitochondrial manganese superoxide dismutase cDNA are resistant to aluminium. Plant Cell Environ. 24:1269-1278.

Bisognin DA, Douches DS, Buszka L, Bryan G, Wang D (2005) Mapping late blight resistance in *Solanum microdontum* Bitter. Crop Sci. 45:340-345.

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann. Bot. 91:179-194.

Boscolo PRS, Menossi M, Jorge RA (2003) Aluminum induced oxidative stress in maize. Phytochemistry 62:181-189.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantity of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Brondani C, Paiva E (1996) Análise de RFLP da tolerância à toxidez do alumínio no cromossomo 2 do milho. Pesq. Agropec. Bras. 31:575-579.

Cakmak I, Horst WJ (1991) Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tip of soybean (*Glycine max* L.). Physiol. Plant. 83:463-468.

Collet L, Horst WJ (2001) Characterization of maize cultivars in their adaptation to acid soils on the single plant level. In: Horst WJ, Burket A, Schenk MK (eds), Plant Nutrition – Food Security and Sustainability of Agro-Ecosystems, pp.86-87. Kluwer Academic Publishers, Dordrecht.

Darkó E, Ambrus H, Stefanovits-Bányai E, Fodor J, Bakos F, Barnabás B (2004) Aluminium toxicity, Al tolerance and oxidative stress in an Al-sensitive wheat genotype and in Al-tolerant lines developed by in vitro microspore selection. Plant Sci. 166:583-591.

Degenhardt J, Larsen PB, Stephen H, Kochian LV (1998) Aluminum resistance in the Arabidopsis mutant Alr-104 is caused by an aluminum-induced increase in rhizosphere pH. Plant Physiol. 117:19-27.

Dipierro N, Mondelli D, Paciolla C, Brunetti G, Dipierro S (2005) Changes in the ascorbate system in the response of pumpkin (*Cucurbita pepo* L.) roots to aluminium stress. J. Plant Physiol. 162:529-536.

Dong B, Sang WL, Jiang X, Zhou JM, Kong FX, Hu W, Wang LS (2002) Effects of aluminum on physiological metabolism and antioxidant system of wheat (*Triticum aestivum* L.). Chemosphere 47:87-92.

El-Moshaty FIB, Pike SM, Novacky AJ, Sehgal OP (1993) Lipid peroxidation and superoxide production in cowpea (*Vigna unguiculata*) leaves infected with tobacco ringspot virus or southern bean mosaic virus. Physiol. Mol. Plant Pathol. 43:109-119.

Ezaki B, Gardner RC, Ezaki Y, Matsumoto H (2000) Expression of aluminum-induced genes in transgenic *Arabidopsis* plants can ameliorate aluminum stress and/or oxidative stress. Plant Physiol. 122:657-665.

Halliwell B, Gutteridge JMC (1999) Free Radicals in Biology and Medicine. 3rd ed. Clarendon Press, Oxford.

Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1132-1334.

Ikegawa H, Yamamoto Y, Matsumoto H (2000) Responses to aluminum of suspension-cultured tobacco cells in a simple calcium solution. Soil Sci. Plant Nutr. 46:503-514.

Jorge RA, Menossi M, Arruda P (2001) Probing the role of calmodulin in Al toxicity in maize. Phytochemistry 58:415-422.

Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46:237–260.

Larson RA (1988) The antioxidants of higher plants. Phytochemistry 27:969-978.

Lazof DB, Goldsmith JG, Rufty TW, Linton RW (1994) Rapid uptake of aluminum into cells of soybean root tips. Plant Physiol. 106:1107-1114.

Lawlor DW (2001) Photosynthesis.3rd ed. Bios Scientific Publishers, Oxford.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER (1990) Determination of carbonyl content in oxidatively modified proteins. Meth. Enzymol. 186:464-478.

Li PH, Fennell A (1985) Potato Frost Hardiness. In: Li PH (ed), Potato Physiology, pp.457-479. Academic Press, Orlando.

Lidon FC, Barreiro MG, Ramalho JC, Lauriano JA (1999) Effects of aluminum toxicity on nutrient accumulation in maize shoots: implications on photosynthesis. J. Plant Nutr. 22:397-416.

Loreto F, Velikova V (2001) Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol. 127:1781-1787.

Ma JF, Ryan PR, Delhaize E (2001) Aluminum tolerance in plants and the complexing role of organic acids. Trends Plant Sci. 6:273-278.

Marschner H (1991) Mechanism of adaptation of plants to acid soils. Plant Soil 134:1-20.

Meriga B, Reddy BK, Rao KR, Reddy LA, Kishor PBK (2004) Aluminum-induced production of oxygen radicals, lipid peroxidation and DNA damage in seedlings of rice (*Oryza sativa*). J. Plant Physiol. 161:63-68.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiol. 15:473-497.

Parker DR, Kinraide TB, Zelazny LW (1988) Aluminum speciation and phytotoxicity in dilute hydroxyaluminum solutions. Soil Sci. Soc. Am. J. 52:438-444.

Pereira LB, Tabaldi LA, Gonçalves JF, Jucoski JO, Pauletto MM, Weis SN, Nicoloso FT, Borher D, Rocha JBT, Schetinger MRC (2006) Effect of aluminum on aminolevulinic acid dehydratase (ALA-D) and the development of cucumber (*Cucumis sativus*). Environ. Exp. Bot. 57:106-115.

Richards KD, Schott EJ, Sharma YK, Davies KR, Gardner RC (1998) Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. Plant Physiol. 116:409-418.

Ryan PR, DiTomaso JM, Kochian LV (1993) Aluminum toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. J. Exp. Bot. 44:437-446.

Schmohl N, Pilling J, Fisahn J, Horst WJ (2000) Pectin methylesterase modulates aluminium sensitivity in *Zea mays* and *Solanum tuberosum*. Physiol. Plant. 109:419-427.

Schützendübel A, Polle A (2002) Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53:1351-1365.

Sieczka JB, Thornton RE (1993) Commercial Potato Production in North America. Potato Association of America Handbook. Potato Assoc. of America, Orono.

Shi Q, Zhu Z, Xu M, Qian Q, Yu J (2006) Effect of excess manganese on the antioxidant system in *Cucumis sativus* L. under two light intensities. Environ. Exp. Bot. 58:197-205.

Simonovicová M, Huttová J, Siroká B, Tamás L (2004) Root growth inhibition by aluminum is probably caused by cell death due to peroxidase-mediated hydrogen peroxide production. Protoplasma 224:91-98.

Snowden KC, Gardner RC (1993) Five genes induced by aluminum in wheat (*Triticum aestivum*) roots. Plant Physiol. 103:855-861.

Tamás L, Simonovicová M, Huttová J, Mistrík I (2004) Aluminium stimulated hydrogen peroxide production of germinating barley seeds. Environ. Exp. Bot. 51:281-288.

Yamamoto Y, Kobayashi Y, Matsumoto H (2001) Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. Plant Physiol. 125:199-208.

Manuscrito I

Oxidative stress is an early symptom triggered by aluminum in Al-sensitive potato plantlets

OXIDATIVE STRESS IS AN EARLY SYMPTOM TRIGGERED BY ALUMINUM IN Al-SENSITIVE POTATO PLANTLETS

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ABSTRACT

The objective of this study was to evaluate whether the oxidative stress caused by aluminum (Al) toxicity is an early symptom that can trigger root growth inhibition in Macaca and SMIC148-A potato clones. Potato plantlets were grown in a nutrient solution (pH 4.00±0.1) with 0, 100 and 200 mg Al l^{-1}. At 24, 72, 120 and 168 h after Al addition, growth (root length) and biochemical parameters (lipid peroxidation, catalase (CAT) and ascorbate peroxidase (APX) activity, and ascorbic acid (AsA) and non-protein thiol group (NPSH) concentration) were determined. Regardless of exposure time, root length of the Macaca clone was significantly lower at 200 mg Al l^{-1}. For the SMIC148-A clone, root length did not decrease with any Al treatments. Therefore, potato clones were classified as Al-sensitive (Macaca) and Al-tolerant (SMIC148-A). Al

supply caused lipid peroxidation only in the Al-sensitive clone (Macaca), in both roots (at 24, 72, 120 and 168 h) and shoot (at 120 and 168 h). In roots of the Al-sensitive clone, CAT and APX activity decreased at 72 and 120 h, and at 24, 72 and 120 h, respectively. At 168 h, both activities increased upon addition of Al. In the shoot, CAT activity decreased at 72 and 168 h and increased at 120 h, whereas APX activity increased at 120 and 168 h. In roots of the Al-tolerant clone, CAT activity increased at 72 and 168 h, whereas APX activity decreased at 72 h and increased at 24, 120 and 168 h. In the shoot, CAT activity increased only at 120 h, whereas APX activity decreased at 24 h. However, APX activity increased at 72, 120 and 168 h after Al supply. The Al-sensitive clone showed lower root NPSH concentration at 200 mg Al l^{-1} in all evaluations, but the Al-tolerant clone either did not demonstrate any alterations at 24 and 72 h or presented higher levels at 120 h. This pattern was also observed in root AsA concentration at 24 and 120 h. In the shoot, NPSH concentration in the Al-tolerant clone did not increase with Al supply for up to 120 h of exposure, whereas in the Al-sensitive clone it increased at 24, 120 and 168 h with the addition of 200 mg Al l^{-1}. The cellular redox status of these potato clones seems to be affected by Al. Therefore, oxidative stress may be an important mechanism for Al toxicity, mainly in the Al-sensitive Macaca clone.

Keywords: *Solanum tuberosum*; Aluminum; Growth; Antioxidants.

1. INTRODUCTION

Potatoes are grown world-wide under a wider range of altitudes, latitudes, and climatic conditions than any other major food crop - from sea level to over 4000 m elevation. No other crop can match the potato in its production of food energy and food value per unit area (Sieczka & Thornton, 1993). The widely cultivated species of

potato, *Solanum tuberosum* subsp. *tuberosum*, is very sensitive to abiotic stress (Li & Fennell, 1985). However, further studies indicated that the *Solanum* species possess genetic diversity to abiotic stress (Martinez et al., 2001).

On a global scale, acid soils comprise a surface estimated at 37.8 million Km^2 (Eswaran et al., 1997). Moreover, soil acidification has been increased around the world as a result of human activities, atmospheric liberation of industrial contaminants, and continuous use of ammonia- and amide-containing fertilizers (Rengel & Zhang, 2003). Such soils are a major constraint to agricultural production in Latin America. They tend to contain low levels of essential cations, nitrogen and plant-available phosphate, in combination with high levels of phytotoxic aluminum (Al) (Rao et al., 1993) (in this paper we will refer to Al^{3+} as Al). As soil pH decreases, active Al in the soil shows a great increase, resulting in toxicity to plants. In Brazil, roughly 60% of soils with potential for agricultural activity present Al toxicity (Abreu Jr. et al., 2003).

The Al^{3+} cation is toxic to many plants at micromolar concentrations, affecting primarily the normal functioning of roots, with a rapid inhibition of growth (Ryan et al., 1993), which can occur within minutes or hours of exposure of roots to Al. Severe Al phytotoxicity reduces and damages the root system, which results in poor nutrient and water acquisition and transport, consequently leading to nutrient deficiencies and crop yield reduction (Kochian, 1995).

During evolution, plants have developed numerous mechanisms that allow for survival in acid soils with higher availability of Al (Kochian, 1995). As a result of selection pressure, inter- and intra-species differences in response to Al are widely observed in the plant kingdom. Moreover, great differences in tolerance to Al have been reported among genotypes of the same species. Over the last several years, a great diversity of results obtained in physiologic and molecular mapping studies have

shown that plant tolerance to Al toxicity is a complex multigenic characteristic that can involve several mechanisms of tolerance (Kochian, 1995; Barceló & Poschenraider, 2002; Kochian et al., 2004). These include external (or exclusion) and internal detoxification mechanisms, such as the immobilization of Al by cell wall components (Zhang et al., 1997), the exudation of organic acids for the detoxification of Al in the apoplast (Ryan et al., 2001), Al compartmentation in the vacuole, etc. In this context, selection of varieties that are productive and tolerant to Al toxicity must be considered a very important component of strategies for dealing with acid soils.

A wide range of cellular responses occur when plants are exposed to a variety of environmental stresses such as freezing, drought, salinity and metal toxicity. It has been suggested that Al^{3+}, the most toxic of the soluble forms of Al (Parker et al., 1988), induces oxidative stress, since this ion is related to an increased ascorbate peroxidase activity and an increased level of ascorbic acid and H_2O_2 concentration (Dipierro et al., 2005). Richards et al. (1998) reported that oxidative stress genes, including peroxidase and glutathione-S-transferase, were induced in *Arabidopsis thaliana* in the presence of Al. Further evidence corroborating the relation between Al stress and oxidative stress in plants has been obtained with transgenic *Arabidopsis* plants (Ezaki et al., 2000). Induction of oxidative stress-related genes by Al stress confirmed the important role of reactive oxygen species (ROS) in this stress (Tamás et al., 2003).

Oxidative stress is characterized by the production of ROS and reactive nitrogen species (RNS), including the superoxide radical (O_2•−), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2) and nitric oxide (NO), that are able to initiate a free radical chain reaction. ROS and RNS can cause oxidative damage to the biomolecules such as lipid, protein and nucleic acids, leading to cell membrane peroxidation, loss of ions, protein hydrolysis, and even DNA strand breakage (Schützendübel & Polle, 2002).

Studies of Al toxicity in roots suggest that production of ROS may significantly contribute to Al-induced inhibition of root elongation (Tamás et al., 2004). Both plants and animals possess antioxidant systems that counteract the action of ROS. To mitigate the oxidative damage initiated by ROS, plants have developed a complex defense anti-oxidative system, including low-molecular mass antioxidants (ascorbic acid, glutathione and carotenoids) as well as antioxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (Koca et al., 2006).

There are several indications that oxidative stress is involved in the plant responses to Al stress. Therefore, the objective of this study was to evaluate whether oxidative stress caused by Al toxicity is an early symptom that can trigger root growth inhibition in two potato clones that differ in Al tolerance.

2. MATERIALS AND METHODS

2.1. Plant materials and growth conditions

Two clones (Macaca (Al-sensitive) and SMIC148-A (Al-tolerant)) of *Solanum tuberosum* subsp. *tuberosum* were evaluated. These clones were chosen after preliminary tests revealed that they differed in Al-tolerance. Tissue culture plantlets were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, RS, Brazil. Nodal segments (1.0 cm long) were micropropagated in MS medium (Murashige & Skoog, 1962), supplemented with 30 g l^{-1} of sucrose, 0.1 g l^{-1} of myo-inositol and 6 g l^{-1} of agar.

Twenty-day-old plantlets from *in vitro* culture were transferred into plastic boxes (10 L) filled with aerated full nutrient solution of low ionic strength. The nutrient solution contained the following composition (in μM): 6090.5 of N; 974.3 of Mg; 5229.5 of Cl;

2679.2 of K; 2436.2 of Ca; 359.9 of S; 0.47 of Cu; 2.00 of Mn; 1.99 of Zn; 0.17 of Ni; 24.97 of B; 0.52 of Mo; 47.99 of Fe ($FeSO_4$/Na-EDTA). Al stress was induced by adding 0, 100 or 200 mg l^{-1} of Al as $AlCl_3.6H_2O$. The pH of the solutions was adjusted to 4.00±0.1 daily by titration with HCl or NaOH solutions 0.1 M. Both *in vitro* and *ex vitro* cultured plants were grown in a growth chamber at 25±2°C on a 16/8 h light/dark cycle with 35 μmol m^{-2} s^{-1} of irradiance. Al-treated plantlets remained in the respective solutions for 24, 72, 120 and 168 h.

At harvest (24, 72, 120 and 168 h after Al application), all living plantlets from each container were divided into two sub-samples. Plants were partitioned into shoot and roots. During each sampling, a new set of plantlets was used. Roots were rinsed twice with fresh aliquots of distilled water. Subsequently, growth and biochemical parameters were determined. Three replicates were made for each treatment, with twenty five plantlets per replicate.

2.2. Growth parameters

To assess different responses to Al sensitivity, the root length of two clones was determined before and after the treatments, at different times (24, 72, 120 and 168 h after Al application).

2.3. Estimation of lipid peroxides

The degree of lipid peroxidation was used to calculate membrane integrity and was estimated following the method of El-Moshaty et al. (1993). Fresh root and shoot samples were collected at regular intervals (24, 72, 120 and 168 h after Al application), weighed (0.1 g fresh weight) and ground in 10 ml of 0.2 M citrate-phosphate buffer (pH

6.5) containing 0.5% Triton X-100, using mortar and pestle. The homogenate was filtered with two paper layers and centrifuged for 15 min at 20,000 x g. One milliliter of the supernatant fraction was added to an equal volume of 20% (w/v) trichloroacetic acid (TCA) containing 0.5% (w/v) of thiobarbituric acid (TBA). The mixture was heated at 95°C for 40 min and then quickly cooled in an ice bath for 15 min, and centrifuged at 10,000 x *g* for 15 min. The absorbance of the supernatant at 532 nm was read and corrected for unspecific turbidity by subtracting the value at 600 nm. The lipid peroxides were expressed as nmol MDA mg^{-1} protein.

2.4. Catalase assay

Catalase (CAT) activity was assayed following the modified Aebi (1984) method. Fresh root and shoot samples (1 g) were collected at regular intervals (24, 72, 120 and 168 h after Al application) and homogenized in 3 ml of 50 mM KH_2PO_4/ K_2HPO_4 (pH 7.0), 10 g l^{-1} PVP, 0.2 mM EDTA and 10 ml l^{-1} Triton X-100. The homogenate was centrifuged at 12,000 x g for 20 min at 4°C and supernatant was then used for the enzyme assay. CAT activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture with a final volume of 2 ml containing 15 mM H_2O_2 in KPO_4 buffer (pH 7.0) and 30 µl extract. Activity was expressed as $\Delta E\ min^{-1}\ mg^{-1}$ protein.

2.5. Ascorbate Peroxidase assay

Ascorbate peroxidase (APX) was measured according to Zhu et al. (2004). The reaction mixture, at a total volume of 2 ml, contained 25 mM (pH 7.0) sodium phosphate buffer, 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2 and 100 µl enzyme extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the

absorbance at 290 nm (E= 2.8 l mM^{-1} cm^{-1}) and activity was expressed as μmol oxidized ascorbate min^{-1} mg^{-1} protein.

2.6. Ascorbic acid (AsA) and non-protein thiol group (NPSH) concentrations

Potato plantlets were homogenized in a solution containing 50 mM l^{-1} Tris-HCl and 10 ml l^{-1} Triton X-100 (pH 7.5), centrifuged at 6,800 x g for 10 min. To the supernatant was added 10% TCA at a proportion of 1:1 (v/v) followed by centrifugation (6,800 x g for 10 min) to remove protein. Ascorbic acid determination was performed as described by Jacques-Silva et al. (2001). An aliquot of the sample (300 µl) was incubated at 37°C in a medium containing 100 µl TCA 13.3%, 100 µl deionized water and 75 µl 2,4-Dinitrophenylhydrazine (DNPH). The DNPH solution contained 2% DNPH, 0.23% thiourea, 0.27% $CuSO_4$ diluted in 49% H_2SO_4. After 3 h, 500 µl of 65% H_2SO_4 was added and samples were read at 520 nm. A standard curve was constructed using *L*(+) ascorbic acid. Non-protein thiol concentration in plantlets was spectrophotometrically measured with Ellman's reagent (Ellman, 1959). An aliquot of the sample (400 µl) was added to a medium containing 550 µl 1 M l^{-1} Tris-HCl (pH 7.4). The reaction was read at 412 nm after the addition of 10 mM l^{-1} 5-5-dithio-bis 2-nitrobenzoic acid (DTNB) (0.05 ml). A standard curve using cysteine was used to calculate the thiol groups concentration in the samples.

2.7. Protein extraction

In all the enzyme preparations, protein was determined following the method of Bradford (1976) using bovine serum albumin as standard.

2.8. Statistical analysis

Data were submitted to variance analyses and treatment means were compared by Tukey's range test at 5% of error probability. Treatments were presented as mean ± S.D. of three replicates.

3. RESULTS

3.1. Aluminum effects on growth

The effects of Al on the root growth of potato clones are shown in Fig. 1. Significant differences were observed in root length between the potato clones under Al stress. After 24 h of Al exposure, root length in the Macaca clone was significantly lower (about 15%) at 200 mg Al l^{-1} (Fig. 1A). This data clearly showed that in the Macaca clone, root elongation was inhibited by Al at the first point of time after Al exposure. The same behavior was observed at 72, 120 and 168 h of metal exposure, with inhibitions of 15%, 20% and 17%, respectively, compared to the control. On the other hand, in the SMIC148-A clone Al treatment did not affect root length (Fig. 1B). This difference in root growth indicates a distinct sensitivity to Al between the two clones.

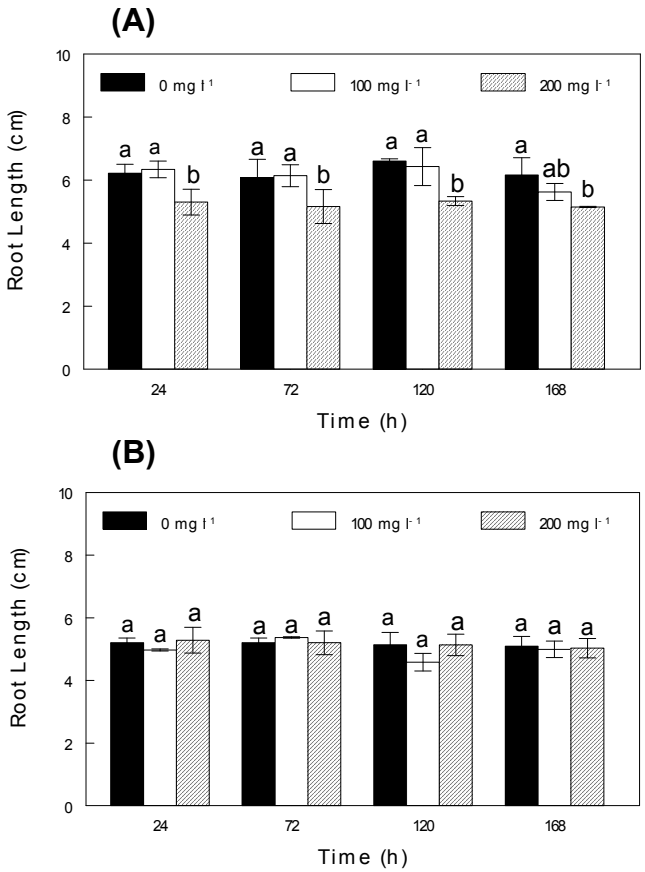

Figure 1- Time-course of Al inhibition of root length (Macaca (A) and SMIC148-A (B) clones) of potato plantlets exposed to different concentrations of Al in nutrient solution. The data are the mean ± S.D. of three different replicates.

3.2. Al effect on lipid peroxidation level

A significant increase in MDA content in both roots and shoot were observed only in the Al-sensitive clone (Macaca) (Fig. 2A and 2B). The highest increase in MDA content was seen in roots under Al treatment. In roots, an increase of 80% in MDA content was observed in the first 24 h at 200 mg Al l^{-1} (Fig. 2A). Such an effect was also observed at later times. At 168 h after Al-treatments, an increase of 45% and 78% in MDA content occurred at 100 and 200 mg Al l^{-1}, respectively, indicating that, after

prolonged incubation at these highly toxic Al levels, the toxicity remained severe. In the shoot (Fig. 2B), lipid peroxidation increased only at 120 and 168 h after Al treatment.

In the SMIC148-A clone, Al treatment did not provoke lipid peroxidation in either roots or shoot at any time of exposure (Fig. 2C and 2D, respectively).

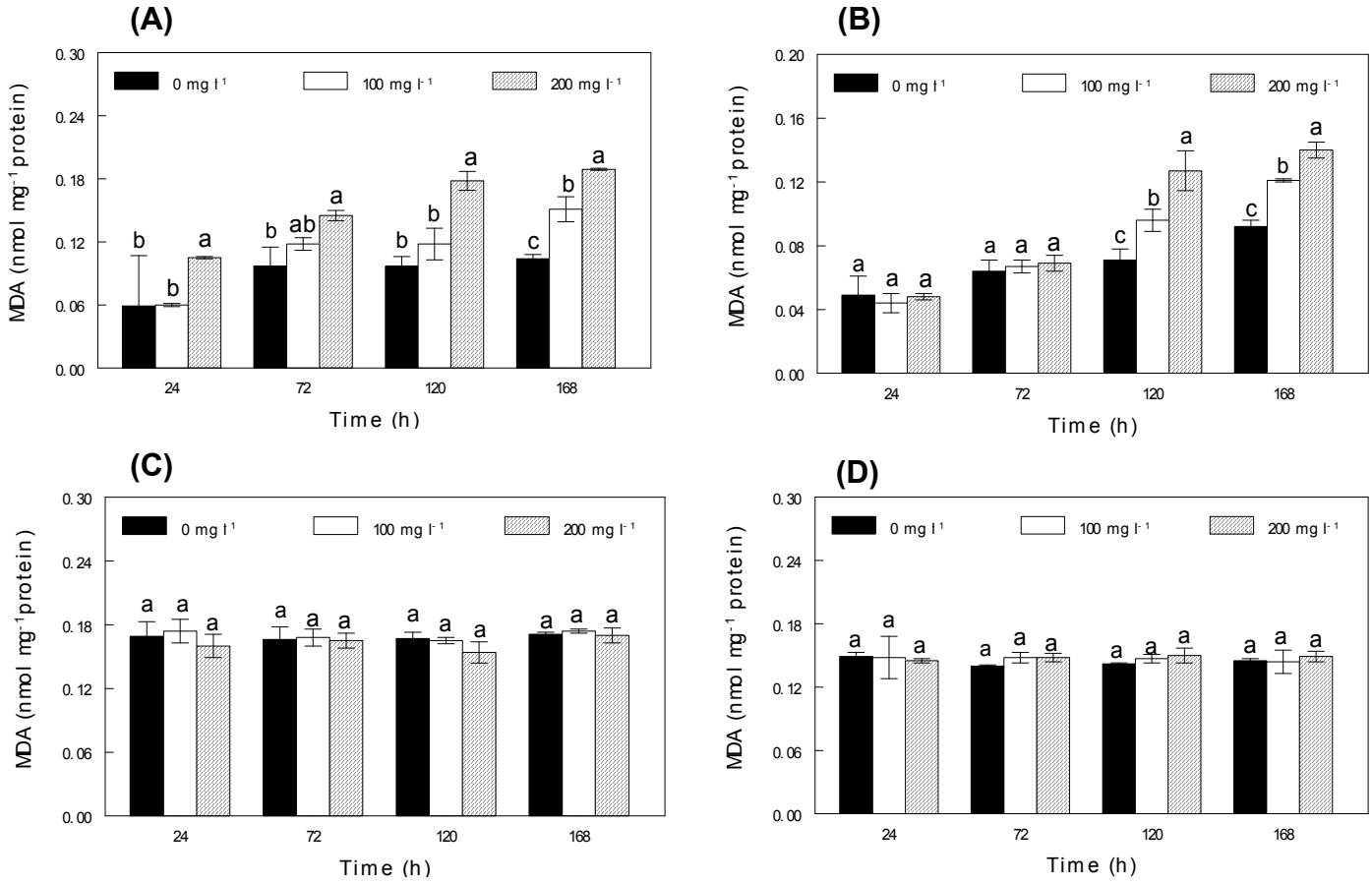

Figure 2- Effect of Al on lipid peroxidation over time in Macaca (Root (A) and Shoot (B)) and SMIC148-A (Root (C) and Shoot (D)) potato clones. The data are the mean ± S.D. of three different replicates.

3.3. Effects on antioxidative systems

3.3.1. Enzymes

Among the enzymatic antioxidants in plants, catalase (CAT) can transform peroxides into non-reactive species. A time-, organ- and clone-dependent response to Al stress was observed (Fig. 3).

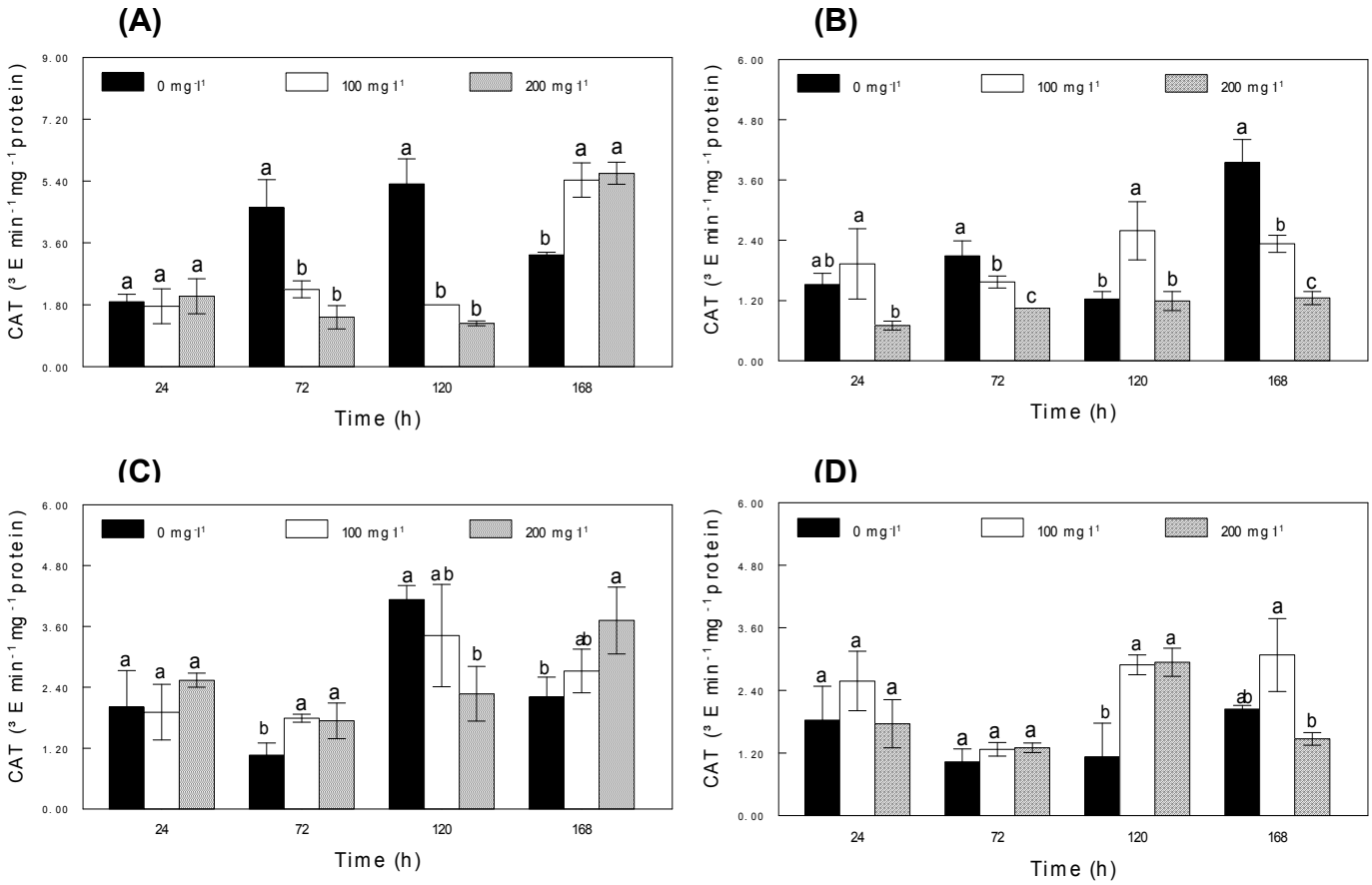

Figure 3- Effect of Al on catalase activity over time in Macaca (Root (A) and Shoot (B)) and SMIC148-A (Root (C) and Shoot (D)) potato clones. The data are the mean ± S.D. of three different replicates.

In general, CAT activity was greater for the Al-sensitive clone (Macaca) than for the Al-tolerant clone (SMIC148-A). Moreover, in both potato clones, root CAT activity was greater than that of the shoot. At 24 h after Al treatment, root and shoot CAT activities remained unchanged, except for the Al-sensitive clone, which showed a significant decrease in shoot tissue at 200 mg Al l^{-1}, in comparison with 100 mg Al l^{-1}. At 72 and 120 h, the presence of Al caused a decrease in root CAT activity for the

Macaca clone. A similar pattern in root CAT activity was observed at 120 h for the SMIC148-A clone (Fig. 3C). Conversely, after prolonged exposure (168 h) to both levels of 100 mg Al l^{-1} and 200 mg Al l^{-1}, root CAT activity was increased for both clones.

In the Al-sensitive clone, shoot CAT activity decreased significantly upon exposition of Al after 72 and 168 h. On the other hand, at 120 h shoot CAT activity increased at 100 mg Al l^{-1}, whereas no significant difference in shoot CAT activity was found between 200 mg Al l^{-1} and the control. In the Al-tolerant clone, shoot CAT activity was altered after prolonged incubation. At 120 h, CAT activity increased at all levels of Al, whereas at 168 h, CAT activity was slightly, but not significantly, increased by 100 mg Al l^{-1}.

Ascorbate peroxidase (APX) activity (Fig. 4) was greater in roots than in shoots for both potato clones. In roots of the Macaca clone, APX activity was inhibited at 24 h (100 and 200 mg l^{-1}), 72 h (100 and 200 mg l^{-1}) and 120 h (200 mg l^{-1}) of Al exposure. On the other hand, at 168 h, APX activity increased by about 80% at 200 mg l^{-1}, when compared to the control (Fig. 4A). In the shoot (Fig. 4B), APX activity was increased upon addition of Al at the later points of time (120 and 168 h). The effect of Al on APX activity in roots and shoot of the SMIC148-A clone is shown in Fig. 4C and 4D, respectively. The presence of 200 mg Al l^{-1} induced the activity of root APX at 120 h (184%) and 168 h (50%). Conversely, at 72 h of Al-exposure, root APX activity was inhibited by about 60% (Fig. 4C). In the shoot, after 24 h Al exposure, at 200 mg l^{-1}, APX activity was inhibited by 46%, when compared to the control. On the other hand, at 72, 120 and 168 h shoot APX activity was induced upon addition of both 100 and 200 mg Al l^{-1}.

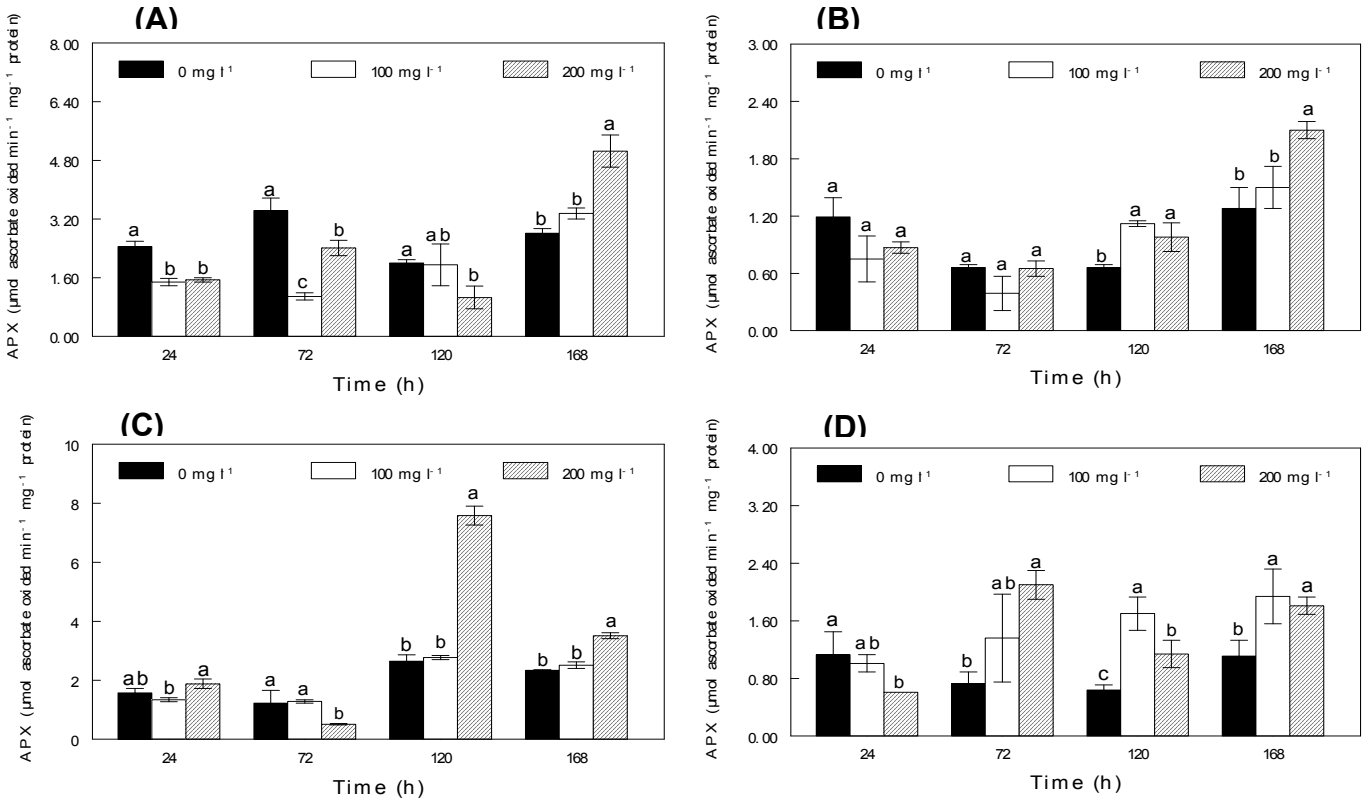

Figure 4- Effect of Al on ascorbate peroxidase (APX) activity over time in Macaca (Roots (A) and Shoot (B)) and SMIC148-A (Roots (C) and Shoot (D)) potato clones. APX activity was expressed as µmol oxidized ascorbate mim^{-1} mg^{-1} protein. The data are the mean ± S.D. of three different replicates.

3.3.2. Non-enzymatic antioxidants

Non-protein thiol group (NPSH) concentration was higher at 72, 120 and 168 h, when compared to 24 h of Al exposure, in both roots and shoot. The content of NPSH was significantly reduced in roots of the Al-sensitive clone at both 24 and 72 h after Al treatment (Fig. 5A). At 120 h of Al-exposure, NPSH in roots of the Al-tolerant clone (Fig. 5C) increased significantly with increasing Al concentration, whereas for the Al-sensitive clone it was slightly, but not significantly, reduced by 200 mg Al l^{-1}, when compared to control.

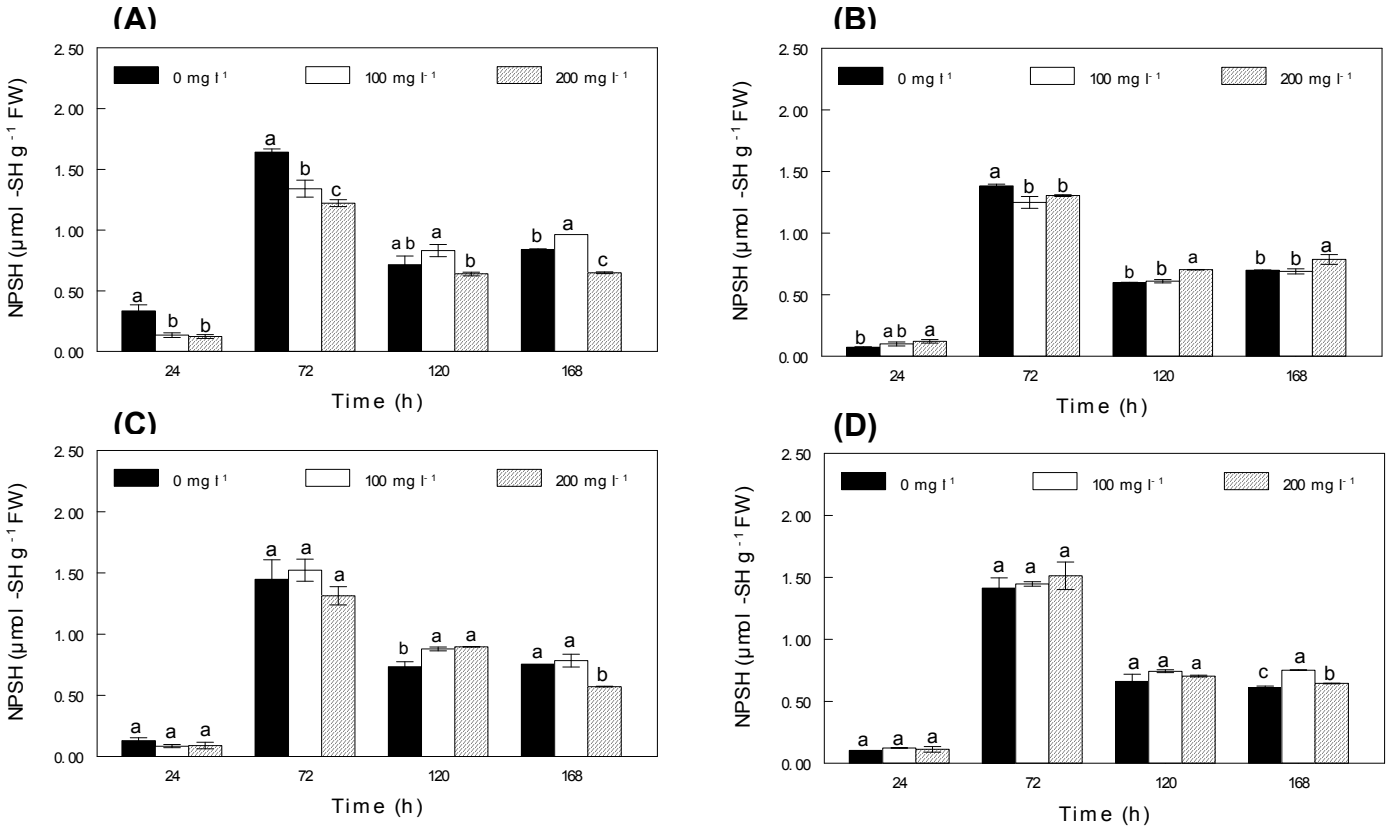

Figure 5- Effect of Al on non-protein thiol group (NPSH) concentration over time in Macaca (Roots (A) and Shoot (B)) and SMIC148-A (Roots (C) and Shoot (D)) potato clones. The data are the mean ± S.D. of three different replicates.

For both potato clones, at 168 h after Al exposure, root NPSH concentration decreased at 200 mg Al l^{-1} when compared to control. Shoot NPSH concentration in the Al-tolerant clone (Fig. 5D) was not affected by Al-treatment for up to 120 h, whereas at 168 h it was significantly increased for all Al levels, when compared to control. By contrast, shoot NPSH concentration in the Al-sensitive clone increased at 200 mg Al l^{-1} at 24 h (65%), 120 h (18%) and 168 h (13%). At 72 h, while the Al-tolerant clone did not demonstrate NPSH concentration alteration, in the Al-sensitive clone, it was significantly decreased at all Al levels.

Regardless of the Al level, the shoot presented more ascorbic acid (AsA) than roots (Fig. 6). The concentration of AsA was significantly reduced (about 20%) in roots of the Al-sensitive clone at 24, 72 and 120 h at 200 mg Al l^{-1}, when compared to control (Fig. 6A). On the other hand, AsA concentration at 24 and 168 h at 100 mg Al l^{-1} was about 41% and 23% higher than that of the control, respectively. In roots of the Al-tolerant clone (Fig. 6C), AsA concentration increased at 24 h upon addition of Al in the substrate. However, at 72 and 168 h, a significant decrease in AsA concentration was observed in the presence of Al. Shoot AsA concentration in the Al-sensitive clone (Fig. 6B) increased significantly at 24, 72 and 168 h upon addition of Al.

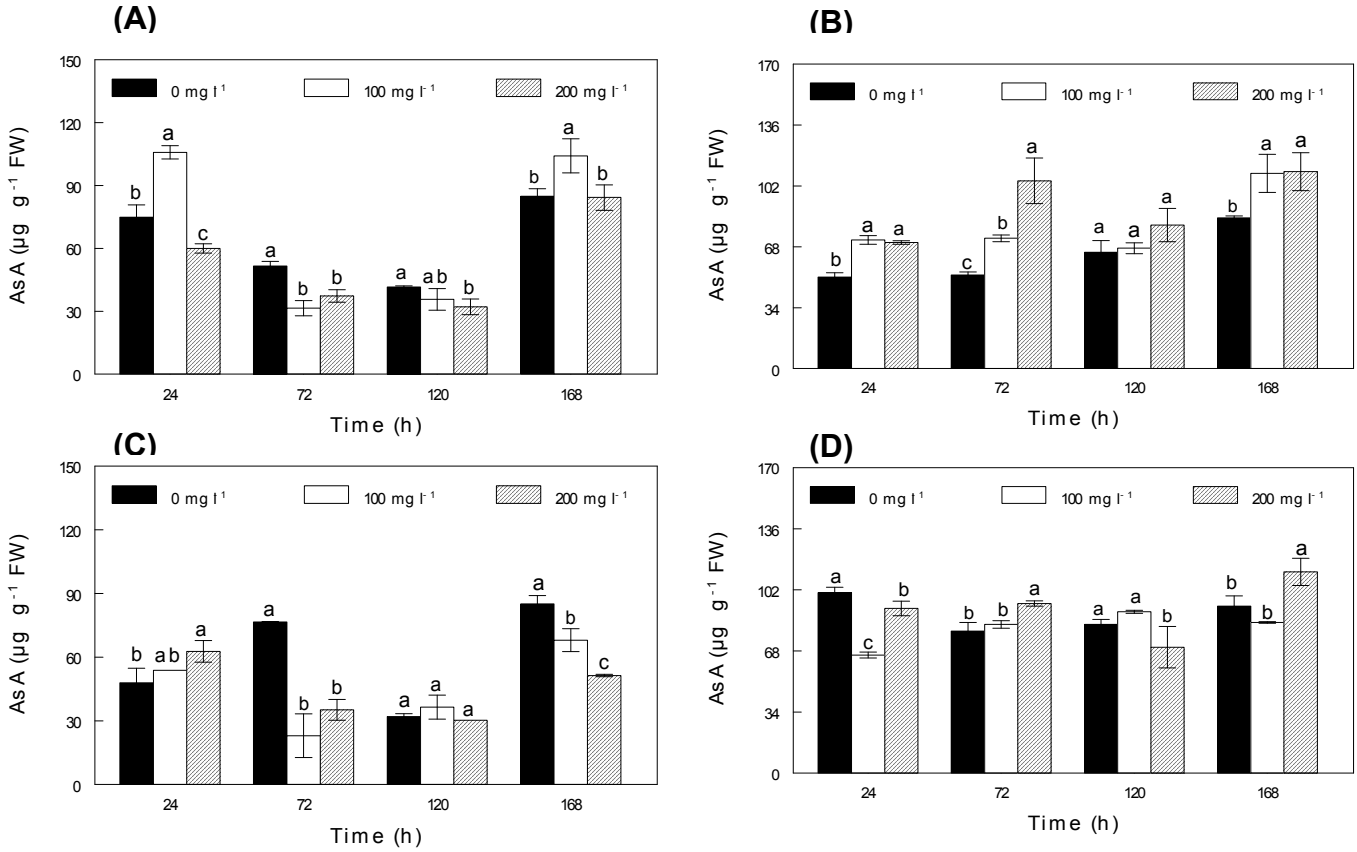

Figure 6- Effect of Al on Ascorbic acid concentration over time in Macaca (Roots (A) and Shoot (B)) and SMIC148-A (Roots (C) and Shoot (D)) potato clones. The data are the mean ± S.D. of three different replicates.

In contrast, shoot AsA concentration in the Al-tolerant clone (Fig. 6D) only increased at 72 and 168 h at 200 mg Al l^{-1}. Moreover, at 24 and 120 h, AsA concentration decreased at 200 mg Al l^{-1}.

DISCUSSION

Our study revealed the importance of the effects of oxidative stress and the antioxidant defense mechanisms after Al exposure in two potato clones. It is clear that Al toxicity depends not only on the concentration and exposure time but also on the clone used.

Inhibition of root elongation is the primary Al toxicity symptom (Dipierro et al., 2005) and has been used as a suitable parameter for assessing genotypic differences in Al tolerance (Collet et al., 2002; Jemo et al., 2007). The presence of 200 mg l^{-1} of Al in solution inhibited significantly root growth only in the Macaca clone (Al-sensitive) (Fig. 1), indicating a distinct genetic sensitivity to Al supply. This effect was observed at the first point of time after Al exposure (24 h) and was observed throughout the experiment. Thus, these two potato clones were classified as Al-sensitive (Macaca) and Al-tolerant (SMIC148-A), confirming our previous results (Tabaldi et al., 2007). Root growth reductions may, therefore, be the result of elevated Al concentrations in the rooting medium, leading to disruption of cell functioning as proposed by Kochian (1995). This is believed to be caused by a number of different mechanisms, including apoplastic lesions, interactions within the cell wall, the plasma membrane, or the root symplasm (for review, see Marschner, 1995), resulting in mineral nutrient and water acquisition deficiency, and, consequently, leading to shoot nutrient deficiencies and poor crop yields.

The imposition of several abiotic stresses, including Al presence, can give rise to excess concentrations of reactive oxygen species (ROS) in plant cells (Noctor & Foyer, 1998; Lin & Kao, 2000), which are potentially harmful since they initiate the peroxidation and destruction of lipids, nucleic acids and proteins (Yamaguchi-Shinozaki & Shinozaki, 2006). The most prominent indicator of plasma membrane damage is Al-stimulated lipid peroxidation (Silva et al., 2002). In the present study, data for root growth (Fig. 1) and lipid peroxidation (Fig. 2) suggest that lipid peroxidation in the Macaca clone (Al-sensitive) might be a direct effect of Al toxicity on root growth, indicating Al induced oxidative stress and, as a result, irreversible damage to tissue development and function. A higher increase in MDA content was seen in roots when compared to the shoots at all times of exposure. In addition, in the shoot, Al provoked lipid peroxidation only after prolonged exposure (120 and 168 h), hence these data show that the active stress in roots was higher. Thus, similar to results obtained with peas (Yamamoto et al., 2001), soybeans (Cakmak & Horst, 1991) and maize (Boscolo et al., 2003), in the present study, lipid peroxidation in the Al-sensitive potato clone seems to be an early symptom induced by Al toxicity, indicating that lipids are the primary cellular target of oxidative stress. In the SMIC148-A clone, Al treatment did not provoke lipid peroxidation in either the roots or shoot at any time of exposure (Fig. 2C and 2D, respectively). This indicates that the antioxidant system in this clone might be more efficient to protect membrane lipids of reactive oxygen species (ROS).

Plants respond to metal stress by physiological and biochemical strategies. Anti-oxidation mechanisms of the cell include the enzymatic ROS-scavenging system and non-enzymatic antioxidants (ascorbic acid (AsA) and non-protein thiol groups (NPSH)), which function to interrupt the cascades of uncontrolled oxidation in each organelle. Among these enzymatic systems, catalase (CAT) and ascorbate peroxidase (APX) can

transform peroxides into non-reactive species, but APX has a very high affinity for H_2O_2 as compared with CAT (Graham & Patterson, 1982). Ascorbate is the primary antioxidant reacting directly with ROS and also acts as secondary antioxidant by reducing the oxidized form of α-tocopherol and preventing membrane damage (Demirevska-Kepova et al., 2004). Glutathione is the predominant NPSH, redox-buffer, phytochelatin precursor and substrate for keeping ascorbate in its reduced form in the ascorbate-glutathione pathway (Noctor & Foyer, 1998).

At the first point of time after Al exposure (24 h), both APX activity and non-enzymatic antioxidant levels (NPSH and AsA) were reduced in roots of the Al-sensitive clone, except at 100 mg Al l^{-1}, at which AsA concentration was increased. These alterations occurred concomitantly with the inhibition of root growth and the increase of lipid peroxidation (Fig. 1 and 2). This might indicate that, at that moment, the reductions in concentration and activity of antioxidants were contributing to enhance damage provoked by Al treatment. The inhibition in root APX activities observed almost exclusively for the Al-sensitive potato clone at times ranging from 24 to 120 h can be attributed to the blockage of essential functional groups, like -SH in the enzymes, or the displacement of essential metal ions from the enzyme, as suggested by Schutzendubel & Polle (2002). On the contrary, in the shoot, an increase in AsA concentration was observed and NPSH concentration increased significantly at 200 mg Al l^{-1} at 24, 72 and 168 h. These antioxidants are present in plant tissue in milimolar concentrations and in stress conditions their levels increase (Noctor & Foyer, 1998) as an attempt to defend the plant from this stress.

Plants can also tolerate Al toxicity by inducing antioxidant defense systems. In roots of the Al-tolerant clone, Al presence for 24 h provoked an increase only in AsA concentration. This increase might have contributed to free radicals and ROS

detoxification and suggests their active participation in Al detoxification, since root growth inhibition and lipid peroxidation were not observed in this clone. As suggested by Wu et al. (2004), AsA can reverse metal toxicity through two possible mechanisms: 1) AsA may bind metals, thereby affecting their movement across biological membranes; or 2) AsA may act as a reducing agent, protecting the oxidation of the mercapto (-SH) group by contributing electron or reducing power for photo-system II. On the other hand, shoot APX activity and AsA concentration decreased at 24 h after addition of Al. Therefore, other antioxidant systems might also be acting, as shoot growth was not affected (data not shown).

At prolonged times of Al exposure (72 h and 120 h), the activity of both enzymatic antioxidant and non-enzymatic antioxidant concentration in roots of the Al-sensitive clone were reduced, except NPSH concentration at 120 h, which remained unaltered. This decrease in CAT and APX activity could be due to the blocking of essential functional groups like –SH in the enzymes or the displacement of essential metal ions from enzymes, as suggested for other metals (Shah & Dubey, 1995; Schutzendubel & Polle, 2002). In the shoot, the same behavior was observed for CAT activity and NPSH concentration at 72 h. On the other hand, at 24 and 72h, AsA concentration was increased. This increase could be explained by the induction of protective mechanisms for detoxifying excess Al, which seems to be efficient in the protection of membrane lipids. At 120 h, CAT and APX activity and NPSH concentration increased. Even so, this activation of the antioxidant system was not enough to avoid cellular damage, since membrane lipids were injured.

In roots of the Al-tolerant clone, the inadequate response of APX activity to Al was compensated by the increased activity of CAT at 72 h. However, at 120 h, a contrary effect was observed. Alscher et al. (2002) reported that lower expression of a

member of one gene family related to antioxidant defense leads to an increase in expression of another member of the family. To that effect, the enzymatic and non-enzymatic antioxidants seem to compensate for each other at 120 h. At 72 h, while the NPSH level remained unaltered, AsA concentration decreased. On the other hand, at 120 h, while NPSH level increased, AsA concentration remained unaltered. Even with these variations, the antioxidant system was efficient, protecting membrane lipids from oxidation.

In the shoot of the Al-tolerant clone at 72 h, while CAT activity remained unaltered, APX activity was activated by the presence of Al. At the same time, AsA concentration increased. On the other hand, enzymatic antioxidants were active, while AsA concentration decreased at 120 h after Al exposure. Therefore, in the Al-tolerant clone, there was always at least one component of the antioxidant system protecting the plant against Al stress. On the other hand, in roots of the Al-sensitive clone, the presence of Al negatively affected the antioxidant system, diminishing activity and concentration of these antioxidants. A decline in both CAT and APX activities and non-enzymatic antioxidant concentrations suggests a possible delay in the removal of ROS, hence an increase of lipid peroxidation and, consequently, root growth inhibition.

At the final point of time (168 h), the presence of antioxidants did not prevent root growth inhibition and lipid peroxidation by Al in the Al-sensitive clone. In this clone, enzymatic activity and the concentration of non-enzymatic antioxidants increased over time, and were not enough to avoid both root and shoot damage. On the other hand, in the Al-tolerant clone, only an increase in enzymatic antioxidants was sufficient to protect the roots from Al stress. In the shoot, both enzymatic (APX) and non-enzymatic (NPSH and AsA) antioxidants increased with Al exposure. Therefore, the Macaca and SMIC148-A clones differed in the expression of the amount and type of antioxidants,

suggesting a varying capacity of these clones to deal with oxidative stress, which resulted in varying sensitivity and tolerance to Al.

These results show that the cellular redox status of potato clones seems to be affected by Al, and oxidative stress may be an important mechanism involved in Al toxicity, mainly in the Al-sensitive Macaca clone. Biochemical (lipid peroxidation) and morphological (root growth) alterations were observed in the first hours of Al exposure. This could indicate that the root elongation impairment observed in the Al-sensitive Macaca clone might be caused by oxidative stress. Unlike redox-active metals (Cu, Fe), Al is not able to induce the production of ROS through a Fenton-like reaction. Al causes oxidative stress probably through indirect mechanisms such as interaction with the antioxidant defense, disruption of the electron transport chain and induction of lipid peroxidation. Further research on the indirect mechanisms of Al-induced oxidative stress is required to reveal the underlying molecular and biochemical events involved.

Acknowledgments

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REFERENCES

Abreu Jr, C.H., Muraoka, T., Lavorante, A., 2003. Relationship between acidity and chemical properties of brazilian soils. Sci. Agric. 60, 337-343.

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121-126.

Alscher, R.G., Ertuk, N., Heath, L.S., 2002. Role of superoxide dismutase (SODs) in controlling oxidative stress in plant. J. Exp. Bot. 372, 1331-1341.

Barceló, J., Poschenrieder, C., 2002. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminum toxicity and resistance: a review. Environ. Exp. Bot. 48, 75-92.

Boscolo, P.R.S., Menossi, M., Jorge, R.A., 2003. Aluminum-induced oxidative stress in maize. Phytochemistry 62, 181-189.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantity of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248-254.

Cakmak, I., Horst, W.J., 1991. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tip of soybean (*Glycine max* L.). Physiologia Plant. 834, 463-468.

Collet, L., de Leon, C., Kollmeier, M., Schmohl, N., Horst, W.J., 2002. Assessment of aluminum sensitivity of maize cultivars using roots of intact plants and excised root tips. J. Plant Nutr. 165, 357-365.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Holzer, R., Feller, U., 2004. Biochemical changes in barely plants after excessive supply of copper and manganese. Environ. Exp. Bot. 52, 253-266.

Dipierro, N., Mondelli, D., Paciolla, C., Brunetti, G., Dipierro, S., 2005. Changes in the ascorbate system in the response of pumpkin (*Cucurbita pepo* L.) roots to aluminum stress. J. Plant Physiol. 162, 529-536.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys.82, 70-77.

El-Moshaty, F.I.B., Pike, S.M., Novacky, A.J., Sehgal, O.P., 1993. Lipid peroxidation and superoxide production in cowpea (*Vigna unguiculata*) leaves infected with tobacco ringspot virus or southern bean mosaic virus. Physiol. Mol. Plant Pathol. 43, 109-119.

Ezaki, B., Gardner, R.C., Ezaki, Y., Matsumoto, H., 2000. Expression of aluminum-induced genes in transgenic Arabidopsis plants can ameliorate aluminum stress and/or oxidative stress. Plant Physiol. 122, 657-665.

Eswaran, H., Reich, P., Beinroth, F., 1997. Global distribution of soils with acidity. In: Moniz, A.C. (Ed.), Plant soil interactions at low pH: sustainable agriculture and forestry production. Brazilian Soil Science Society, Campinas, pp. 159-164.

Graham, D., Patterson, B.D., 1982. Responses of plants to low non-freezing temperatures: proteins, metabolism and acclimation. Annu. Rev. Plant Physiol. 33, 347-372.

Jacques-Silva, M.C., Nogueira, C.W., Broch, L.C., Flores, E.M.M., Rocha, J.B.T., 2001. Diphenyl diselenide and ascorbic acid changes deposition of selenium and ascorbic acid in liver and brain of mice. Pharmacol. Toxicol. 88, 119-125.

Jemo, M., Abaidoo, R.C., Nolte, C., Horst W.J., 2007. Aluminum resistance of cowpea as affected by phosphorus-deficiency stress. J. Plant Physiol. 164, 442-451.

Koca, H., Ozdemir, F., Turkan, I., 2006. Effect of salt stress on lipid peroxidation and superoxide dismutase and peroxidase activities of *Lycopersicon esculentum* and *L. pennelli.* Biol. Plant. 50, 745-748.

Kochian, L.V., 1995. Cellular mechanisms of aluminum toxicity and resistance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46, 237-260.

Kochian, L.V., Hoekenga, O.A., Piñeros, M.A., 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. Annu. Rev. Plant Biol. 55, 459-493.

Li, P.H., Fennell, A., 1985. Potato Frost Hardiness. In: Li, P.H. (Ed.), Potato Physiology. Academic Press, Orlando, pp. 457-479.

Lin, C.C., Kao, C.H., 2000. Effect of NaCl stress on H_2O_2 metabolism in rice leaves. Plant Growth Regul. 30, 151-155.

Martinez, C.A., Loureiro, M.E., Oliva, M.A., Maestri, M., 2001. Differential responses of superoxide dismutase in freezing resistant *Solanum curtilobum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. Plant Sci. 160, 505-515.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiol. 15, 473-497.

Noctor, G., Foyer, C.H., 1998. Ascorbate and glutathione: keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 249-279.

Parker, D.R., Kinraide, T.B., Zelazny, L.W., 1988. Aluminum speciation and phytotoxicity in dilute hydroxy-aluminum solutions. Soil Sci. Soc. America J. 52, 438-444.

Rao, I.M., Zeigler, R.S., Vera, R., Sarkarung, S., 1993. Selection and breeding for acid-soil tolerance in crops: upland rice and tropical forages as case studies. BioScience 43, 454-465.

Rengel, Z., Zhang, W.H., 2003. Role of dynamics of intracellular calcium in aluminium-toxicity syndrome. New Phytol. 159, 295-314.

Richards, K.D., Schott, E.J., Sharma, Y.K., Davis, K.R., Gardner, R.C., 1998. Aluminum induces oxidative stress genes in *Arabidopsis thaliana*. Plant Physiol. 116, 409-418.

Ryan, P.R., DiTomaso, J.M., Kochian, L.V., 1993. Aluminum toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. J. Exp. Bot. 44, 437-446.

Ryan, P.R., Delhaize, E., Jones, D.J., 2001. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. Plant Mol. Biol. 52, 527-560.

Schützendübel, A., Polle, A., 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. J. Exp. Bot. 53, 1351-1365.

Shah, K., Dubey, R.S., 1995. Effect of cadmium on RNA as well as activity and molecular forms of ribonuclease in growing rice seedlings. Plant Physiol. Biochem. 33, 577-584.

Sieczka, J.B., Thornton, R.E., 1993. Commercial Potato Production in North America. Potato Association of America Handbook. Potato Assoc. of America, Orono, Maine.

Silva, I.R., Smyth, T.J., Barros, N.F., Novais, R.F., 2002. Physiological aspects of aluminum toxicity and tolerance in plants. In: Alvarez, V.H., Schaefer, C.E.G.R., Barros, N.F., Mello, J.W.V., Costa, L.M. (Eds.). Tópicos em Ciência do Solo. Viçosa: Sociedade Brasileira de Ciência do Solo, Viçosa, MG, pp. 277-336.

Tabaldi, L.A., Nicoloso, F.T., Y Castro, G., Cargnelutti, D., Gonçalves, J.F., Rauber, R., Skrebsky, E.C., Schetinger, M.R.C., Morsch, V.M., Bisognin, D.A. 2007. Physiological and oxidative stress responses of four potato clones to aluminum in nutrient solution. Braz. J. Plant Physiol. 19, 211-222.

Tamás, L., Huttová, J., Mistrík, I., 2003. Inhibition of Al-induced root elongation and enhancement of Al-induced peroxidase activity in Al-sensitive and Al-resistant barley cultivars are positively correlated. Plant Soil 250, 193-200.

Tamás, L., Simonovicová, M., Huttová, J., Mistrík, I., 2004. Aluminium stimulated hydrogen peroxide production of germinating barley seeds. Environ. Exp. Bot. 51, 281-288.

Wu, F-B., Chen, F., Wei, K., Zhang, G-P., 2004. Effect of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. Chemosphere 57, 447-454.

Yamaguchi-Shinozaki, K., Shinozaki, K., 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu. Rev. Plant Biol. 57, 781-803.

Yamamoto, Y., Kobayashi, Y., Matsumoto, H., 2001. Lipid peroxidation is an early symptom triggered by aluminum, but not the primary cause of elongation inhibition in pea roots. Plant Physiol. 125, 199-208.

Zhang, G., Hoddinott, J., Taylor, G.J., 1994. Characterization of 1,3-beta-D-glucan (callose) synthesis in roots of *Triticum aestivum* in response to aluminum toxicity. J. Plant Physiol. 144, 229-234.

Zhu, Z., Wei, G., Li, J., Qian, Q., Yu, J., 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). Plant Sci. 167, 527–533.

Capítulo II

Efeito do alumínio na atividade *in vitro* de fosfatases ácidas em quatro clones de batata

Manuscrito II

In vitro activity of acid phosphatases of four potato clones cultivated in three growth systems: aluminum effects

Aluminum on the *in vitro* activity of acid phosphatases of four potato clones cultivated in three growth systems

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Abstract - The aim of this study was to evaluate the effect of aluminum (Al) on the *in vitro* activity of acid phosphatases (APases) of four potato clones cultivated in three growth systems. Potato clones Macaca, SMIC148-A, Dakota Rose and *Solanum microdontum* were grown *in vitro*, in hydroponics or in a greenhouse. The enzyme was assayed in the presence of 0, 50, 100, 150 and 200 mg Al L^{-1}. In plantlets grown *in vitro*, root APases were inhibited by Al in all clones, while shoot APases were inhibited by Al in *S. microdontum* and Dakota Rose and increased in Macaca at all Al levels. In plantlets grown in hydroponics, root APases increased in Macaca at 50 mg L^{-1}, whereas decreased at all Al levels in *S. microdontum*. In greenhouse plantlets, root APases were reduced at 200 mg L^{-1} in *S. microdontum* and SMIC148-A, and at 100, 150 and 200 mg L^{-1} in Dakota Rose. Shoot APases were reduced in Macaca and SMIC148-A. Conversely, in Dakota Rose, APases increased at 50 and 100 mg L^{-1}. These results show that

the effect of Al toxicity on *in vitro* APase activity depends not only on Al availability but also on the clone and the growth system evaluated.

Index terms: acid phosphatases, aluminum, phosphorus, *Solanum tuberosum, S. microdontum.*

Atividade *in vitro* de fosfatases ácidas de quatro clones de batata cultivados em três sistemas de cultivo: efeitos do alumínio

Resumo: O objetivo deste trabalho foi avaliar os efeitos do alumínio (Al) na atividade *in vitro* de fosfatases ácidas (APases) de quatro clones de batata cultivados em três sistemas de cultivo. Os clones Macaca, SMIC148-A, Dakota Rose e *Solanum microdontum* foram cultivados *in vitro*, em sistema hidropônico e em vasos em casa de vegetação. A enzima foi analisada na presença de 0, 50, 100, 150 e 200 mg Al L^{-1}. Em todos os clones, as APases de raízes de plantas derivadas do cultivo *in vitro* foram inibidas por Al. Na parte aérea, o Al inibiu a atividade de APases somente nos clones *S. microdontum* e Dakota Rose, enquanto no clone Macaca, um aumento foi observado em todos os níveis de Al. Em raízes de plantas cultivadas em sistema hidropônico, a atividade de APases aumentou em 50 mg Al L^{-1} no clone Macaca, enquanto em *S. microdontum* a atividade foi reduzida em todos os níveis de Al. Em raízes de plantas crescendo em casa de vegetação, a atividade de APases foi reduzida nos clones *S. microdontum* e SMIC148-A em 200 mg Al L^{-1}, enquanto no clone Dakota Rose, a atividade foi reduzida em 100, 150 e 200 mg L^{-1}. A atividade de APases de parte aérea foi reduzida nos clones Macaca e SMIC148-A, enquanto aumentou em 50 e 100 mg Al L-1 no clone Dakota Rose. Esses resultados mostram que a toxicidade do Al na atividade *in vitro* de APases depende não somente da concentração de Al, mas também do clone e do sistema de crescimento utilizado.

Termos para indexação: fosfatases ácidas, alumínio, fósforo, *Solanum tuberosum, S. microdontum.*

Introduction

Phosphorus (P) plays important roles as a structural and regulatory element in plant growth and development. P deficiency limits plant growth more frequently than any other nutrient except nitrogen (Raghothama & Karthikeyan, 2005). In natural ecosystems, P availability is seldom optimal for plant growth because of limited P content in minerals, chemical and biological reactions. The inorganic phosphorus (Pi) level in soil solutions is regulated mainly by its interaction with organic or inorganic surfaces in the soil. Aluminum and iron ions in acid soils and calcium ions in alkaline soils interact strongly with Pi and render it unavailable to plants (Sousa et al., 2007).

Organic phosphorus compounds in the soil, representing approximately 50% of total soil phosphate, are mainly present as phosphoric esters such as inositol phosphates (Halstead & McKercher, 1975), which must be hydrolyzed to Pi by hydrolases of phosphoric esters (phosphatases, E.C.3.1.3) before P can be taken up by plants. The hydrolysis of phosphoric organic esters is catalyzed by both acidic and alkaline phosphatases (Marzadori et al., 1998). Synthesis of phosphatase enzymes is dependent on environmental conditions and the physiological state of the plant, among other factors (Grierson & Comerford, 2000).

Acid phosphatases (orthophosphoric-monoester phosphohydrolases, E.C.3.1.3.2) are a group of enzymes that catalyze the hydrolysis of a variety of phosphate esters releasing Pi from phosphorylated substrates (Yoneyama et al., 2007) in acidic environments and are widely distributed in plants. They appear to be important in the production, transport and recycling of Pi (Tejera García et al., 2004). Phosphorus deficiency in higher plants has been shown to increase the activity of acid phosphatases (Duff et al., 1994). Moreover, metals such as Hg and Zn can affect acid phosphatase activity (Tabaldi et al., 2007a).

Toxic concentrations of aluminum (Al), generally found in acid soils (pH < 5.0), inhibit root growth (Dong et al., 2002), restricting water and nutrient uptake and leading to poor growth and yield. Aluminum can bind to the pectic residues or proteins in the cell wall, displacing other ions from critical sites on the cell wall or membranes (Emmanuel & Peter, 1995). Aluminum can also be transported across the root plasma membrane and interact with components in the cell sap such as nuclear DNA, enzymes, calmodulin, tubulin and ATP (Emmanuel & Peter, 1995). Aluminum causes oxidative stress (Tabaldi et al., 2007b) and mineral deficiency in higher plants (Guo et al., 2007), probably because it affects enzyme-mediated reactions, especially those involving carbon, nitrogen and phosphorus metabolisms (Dong et al., 2002). In addition, Al can interfere with PO_4^{3-} binding (Rai et al., 1998). Therefore, it is important to study key enzymes involved in these processes, such as acid phosphatases (APases).

Plant protein and enzyme responses to a variety of environmental factors may be useful in predicting the survival capacity of a plant species or variety to stress conditions. There are many reports in the literature showing the enzymatic activity level of several processes involving plant metabolism (Dong et al., 2002; Tejera Garcia et al., 2004). Hydrolysis of phosphate esters is a crucial process in the energy metabolism and metabolic regulation of plant cells and the expression of their respective enzymes (phosphatases). This process is regulated by a variety of developmental and environmental factors (Murata & Los, 1997).

The aim of this work was to evaluate the effect of Al on the *in vitro* activity of acid phosphatases of four potato clones cultivated in three growth systems.

Material and Methods

Three adapted (2n=4x=48) (Macaca, SMIC148-A and Dakota Rose) and one diploid (2n=2x=24) clone (PI595511-5 of *Solanum microdontum*) potato clones were evaluated. The *S. microdontum* clone was identified as highly resistant to *Phytophora infestans* (Bisognin et al., 2005) and has been used in our breeding program. This clone will be referred to as *S. microdontum*. These clones were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, RS, Brazil. The effect of the aluminum on APases was determined by assays in the presence of 0, 50, 100, 150 and 200 mg L^{-1} of Al as $AlCl_3.6H_2O$. These concentrations were chosen after preliminary tests in our laboratory (data not shown).

To *in vitro* culture system, nodal segments (1.0 cm long) were micropropagated in MS medium (Murashige & Skoog, 1962), supplemented with 30 g L^{-1} of sucrose, 0.1 g L^{-1} of myo-inositol and 6 g L^{-1} of agar. Plantlets were grown in a growth chamber at 25±2ºC on a 16/8-h light/dark cycle with 35 μmol m^{-2} s^{-1} of irradiance during 30 days. At harvest, plantlets were colleted, divided into roots and shoot and utilized for enzymatic assay.

To hydroponic system, twenty-day-old plantlets from *in vitro* culture were transferred into plastic boxes (10 L) filled with aerated full nutrient solution of low ionic strength. The nutrient solution had the following composition (in μM): 6090.5 of N; 974.3 of Mg; 4986.76 of Cl; 2679.2 of K; 2436.2 of Ca; 359.9 of S; 243.592 of P; 0.47 of Cu; 2.00 of Mn; 1.99 of Zn; 0.17 of Ni; 24.97 of B; 0.52 of Mo; 47.99 of Fe ($FeSO_4$/Na-EDTA). These *ex vitro* cultured plantlets were grown in a growth chamber at 25±2ºC on a 16/8-h light/dark cycle with 35 μmol m^{-2} s^{-1} of irradiance. After 10 days of culture in hydroponic system, plantlets were colleted, divided into roots and shoot and utilized for enzymatic assay.

To greenhouse culture system, ten tubers of each clone, with approximately 1.0 cm^3, were sowed separately in plastic pots of 300 mL, employing sand as substrate. The irrigation was made with the same nutritive solution utilized in the hydroponic culture. After 30 days of

cultivation, all plantlets were colleted and divided into shoot and roots for enzymatic analysis. Roots were rinsed with aliquots of distilled water to remove the substrate.

Enzyme extraction and assay were carried out at 4ºC. For extraction, fresh potato samples of roots and shoot were centrifuged at 43200 x *g* for 30 min and the resulting supernatant was used for enzyme assay. Acid phosphatases activity was determined according to Tabaldi et al. (2007a) in a reaction medium consisting of 3.5 mM sodium azide, 2.5 mM calcium chloride, 100 mM citrate buffer, pH 5.5, and 20 μL of metals, except in controls, at a final volume of 200 μL. A 20 μL aliquot of the enzyme preparation (10-20 μg protein) was added to the reaction mixture, except in controls, and preincubated for 10 min at 35°C. The reaction was started by the addition of substrate and stopped by the addition of 200 μL of 10% trichloroacetic acid (TCA) to a final concentration of 5%. Inorganic phosphate (Pi) was measured at 630 nm using malachite green as the colorimetric reagent and KH_2PO_4 as standard for the calibration curve. Controls were carried out to correct for nonenzymatic hydrolysis by adding enzyme preparation after TCA addition. Enzyme specific activities are reported as nmol Pi released min^{-1} mg^{-1} protein. All assays were performed in triplicate using PPi as substrate at a final concentration of 3.0 mM. In all the enzyme preparations, protein was determined by the method of Bradford (1976) using bovine serum albumin.

Data were submitted to variance analyses and treatment means compared by Tukey's range test at 5% of error probability. Treatments were presented as mean ± S.D. of at least three independent replicates.

Results

The growth systems were shown to have significant effects on acid phosphatase (APases) activity. Under Al stress, APase activity of plantlets grown *in vitro* was affected in both roots and shoot (Fig. 1). APase activity was higher in the shoot (Fig. 1B) than in roots of all clones studied (Fig. 1A). Root APases were inhibited by Al in all clones, but the degree of inhibition differed (Fig. 1A).

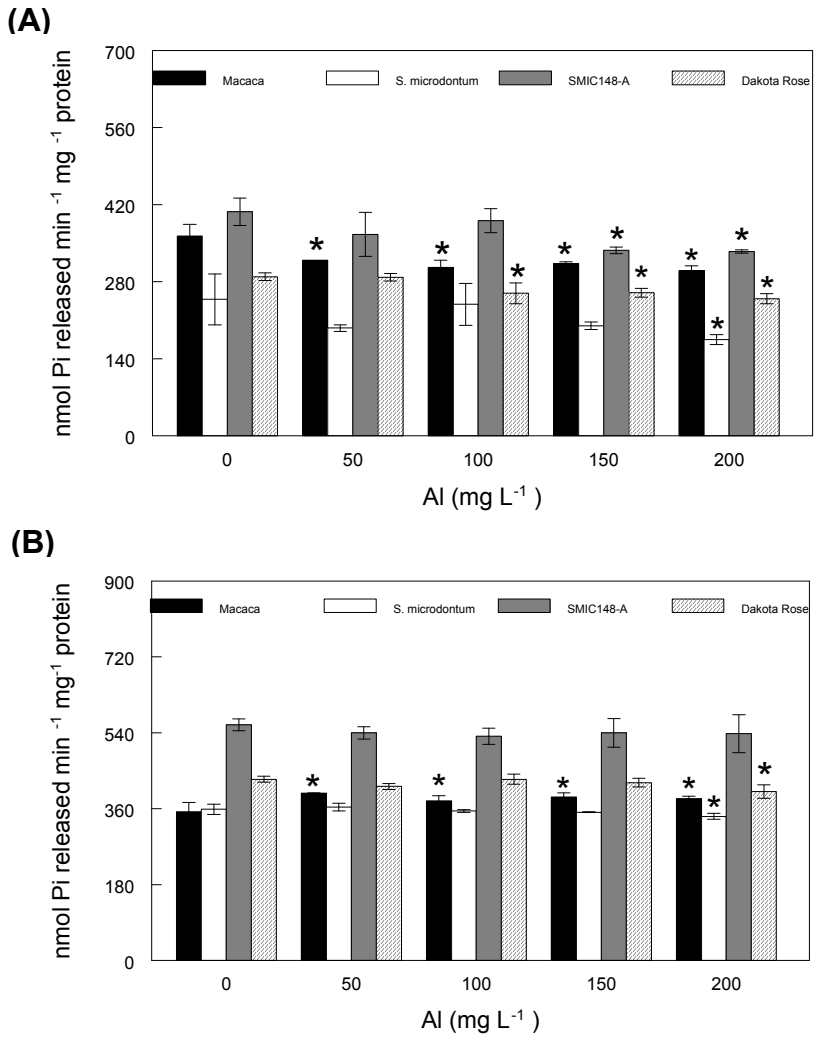

Figure 1. Effect of increasing Al concentration on the *in vitro* acid phosphatase activity of roots (A) and shoot (B) of potato plantlets (Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones) grown *in vitro*. Data represent the mean ± S.D. of three different replicates. *Different from control at $p<0.05$.

The Macaca clone was the most Al-sensitive, where APase activity was reduced at all Al levels. At 200 mg Al L^{-1}, there was a reduction of about 17%. In the *S. microdontum* clone, root and shoot APase activity was only reduced (30%) at 200 mg Al L^{-1}. In the SMIC148-A clone, root APases were reduced by 16% at 150 and 200 mg Al L^{-1}, and in the Dakota Rose clone there was a reduction of 16% at 100, 150 and 200 mg Al L^{-1}. In *S. microdontum* and Dakota Rose clones, shoot APase activity was only reduced (7%) at the 200 mg Al L^{-1} (Fig. 1B). This inhibition was lower than that observed in roots. On the other hand, APase activity in the shoot of Macaca increased (10%) at all Al levels. In the SMIC148-A clone, APase activity was not altered with increasing Al levels.

APase activity of plantlets grown in hydroponics was higher in shoot than in roots (Fig. 2A and 2B). In Macaca, root APases increased by about 35% at 50 mg Al L^{-1} (Fig. 2A), whereas in *S. microdontum*, there was a reduction at all Al levels, and inhibition was about 43% at 200 mg Al L^{-1}. APases of SMIC148-A and Dakota Rose were not affected by Al treatment. In the shoot (Fig. 2B), Al did not affect the APase activity in any of the clones evaluated.

APase activity was higher in roots than in shoot of greenhouse grown plantlets (Fig. 3A and 3B). In roots (Fig. 3A), Al reduced APase activity in *S. microdontum* (24%) and SMIC148-A (13%) only at 200 mg Al L^{-1}. In Dakota Rose, APase activity was reduced at 100, 150 and 200 mg Al L^{-1}, and inhibition was of about 15% at 200 mg L^{-1}. In roots of Macaca, Al did not affect APase activity. On the other hand, shoot APase activity (Fig. 3B) was reduced in Macaca (200 mg Al L^{-1}) and SMIC148-A (50, 100 and 200 mg Al L^{-1}). At 200 mg Al L^{-1}, the inhibition was about 22% and 11% for Macaca and SMIC148-A clones, respectively. Conversely, in Dakota Rose, APase activity increased at 50 and 100 mg Al L^{-1}, while in *S. microdontum*, no alteration was observed.

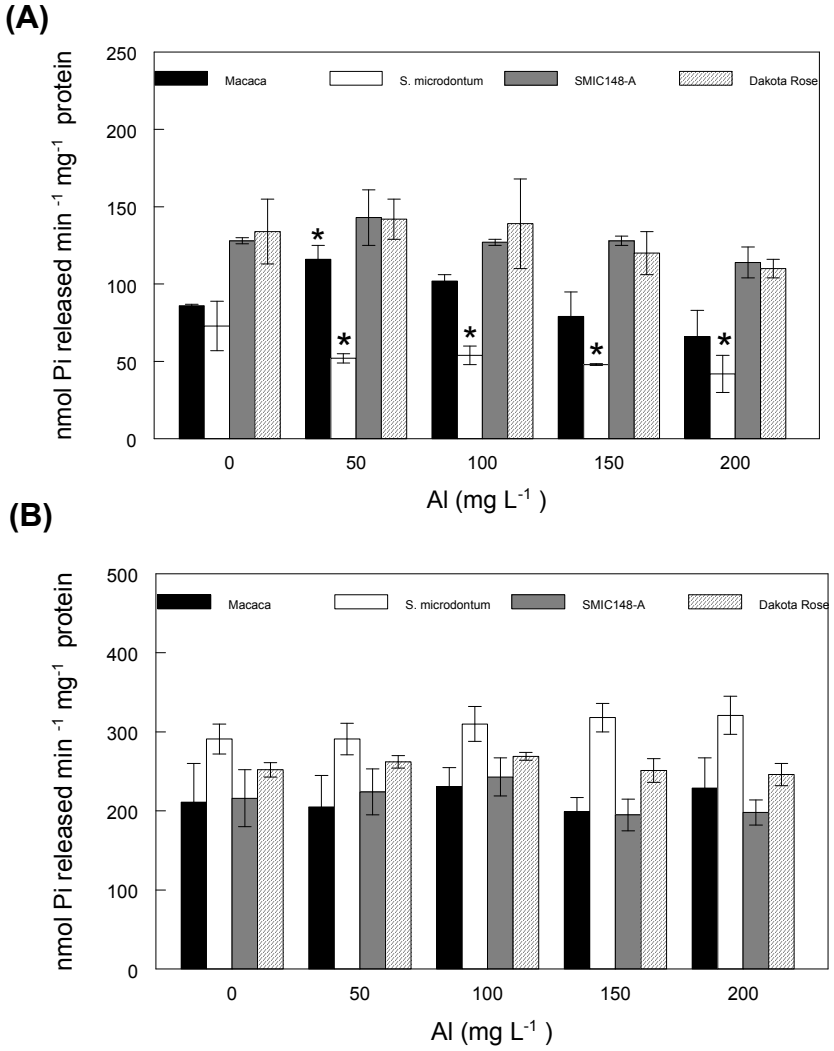

Figure 2. Effect of increasing Al concentration on the *in vitro* acid phosphatase activity of roots (A) and shoot (B) of potato plantlets (Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones) grown in hydroponics. Data represent the mean ± S.D. of three different replicates. *Different from control at $p<0.05$.

Discussion

Phosphorus (P) is qualitatively and quantitatively one of the most important nutrients of many organisms, making up about 0.2% of plant dry weight. It forms part of key biomolecules and, in the form of Pi, PPi, ATP, ADP or AMP, plays a crucial role in energy transfer and metabolic regulation (Raghothama & Karthikeyan, 2005). In this context, higher plants possess

the innate ability to mineralize organic P compounds through acid phosphatases, which encompass a broad group of hydrolytic enzymes that catalyze the

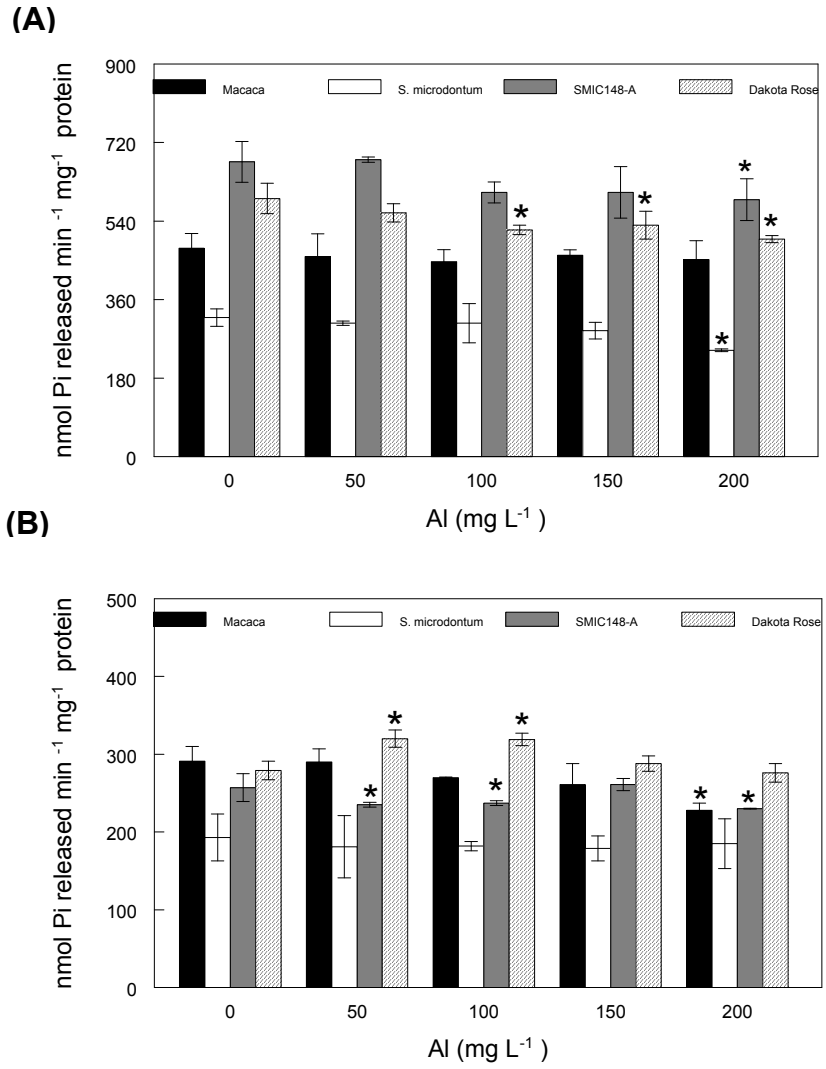

Figure 3. Effect of increasing Al concentration on the *in vitro* acid phosphatase activity of roots (A) and shoot (B) of potato plantlets (Macaca, *S. microdontum*, SMIC148-A and Dakota Rose clones) grown in greenhouse. Data represent the mean ± S.D. of three different replicates. *Different from control to $p<0.05$.

breakdown of P-monoesters with acid pH optima (Duff et al., 1994). Therefore, phosphatase activity plays a significant role in P bioavailability to plants from native organic P compounds.

Acid phosphatases (APases) are expressed under a variety of conditions and in response to many stimuli (Zhang et al., 2001). It is believed that APases play an important role during cell starvation for P scavenging and remobilization, and in some other conditions that promote phosphate mobilization and/or oxidative stress (del Pozo et al., 1999).

The mode of action of metals varies with enzymes and little is known about the exact mechanisms by which metals interact with the multitude of enzymes that exist. In this study, Al stress provoked significant effects on APase activity in the four potato clones, but these effects depended on the growth system utilized. In the Macaca clone, Al-mediated APase inhibition occurred either in roots of *in vitro* grown plantlets or in the shoot of plantlets grown in the greenhouse. On the other hand, in the *S. microdontum* clone, Al inhibited APase activity in roots of plantlets grown in all three systems used, as well as in the shoot of *in vitro* grown plantlets. In the SMIC148-A clone, APase activity was inhibited only in roots of *in vitro* grown plantlets and in roots and shoot of plantlets grown in the greenhouse. In the Dakota Rose clone, Al inhibited APase activity in roots and shoot of *in vitro* grown plantlets and in shoot of plantlets grown in the greenhouse.

Enzyme reactions can be inhibited by metals, which can form a complex with the substrate, combine with protein-active groups of the enzymes, or react with the enzyme-substrate complex. Purified APase from leaves and nodules of common bean was strongly inhibited by Al (Tejera Garcia et al., 2004). Phosphatases are generally metalloenzymes dependent on Ca^{2+} or Mg^{2+}. One possible mechanism explaining Al-toxicity may be the replacement of Mg^{2+} by Al in the active site of the enzyme. Another possibility, suggested by Rai et al. (1998), is that Al may interfere with the PO_4^{3-} binding sites. Other metals, such as Hg and Zn, also inhibited *in vitro* APase activity in cucumber seedlings, possibly replacing Mg^{2+} in the active site of enzyme, or interfering with the PO_4^{3-} binding sites (Tabaldi et al., 2007a). In

addition, once within the cell, Al may affect a range of mechanisms, such as the complexation of ligands required by Ca^{2+}-dependent enzymes (Rengel, 1992). Therefore, it is possible to suggest that Al-mediated inhibition of APase activity in potato may impair phosphate mobilization, since this enzyme is involved in the metabolism of P, an essential element for plant growth and development (Duff et al., 1994).

In the Macaca clone, Al induced an increase in APase activity in shoot of *in vitro* plantlets, as well as in roots of hydroponic plantlets. Conversely, in shoot of the Dakota Rose clone, APase activity increased only in greenhouse plantlets. Intra- and/or extracellular APases of plants are induced under various environmental and developmental conditions (Duff et al., 1994), including exposure to cations, salt stress and in response to phosphate starvation (Gabbrielli et al., 1989; Yoneyama et al., 2007). In the present study, it was shown that *in vitro* APase activity was affected by Al supply, but it depended on other factors such as: the growth system, genetic background, and plant organ analyzed. Therefore, all of these factors must be considered in the development of protocols for the characterization of Al tolerant potato clones. In *Arabidopsis*, it was suggested that purple acid phosphatase had a bifunctional role, acting in phosphate mobilization and in the metabolism of reactive oxygen species (del Pozo et al., 1999). SAP_1 and SAP_2, two secreted purple acid phosphatase isozymes from *Lycopersicum esculentum* may also be multifunctional proteins that operate as: (a) scavengers of Pi from extracellular phosphate-esters during Pi deprivation, or (b) alkaline peroxidases that participate in the production of extracellular reactive oxygen species during the oxidative burst associated with the defense response of the plant to pathogen infection (Bozzo et al., 2002). Moreover, Dong et al. (2002) reported an increase in APase activity in plants exposed to Al. These authors suggested that the increased activity of acid phosphatase was an indication of membrane damage, which leads to liberation of enzymes from lysosomes, mitochondria and other phosphate-containing cell structures.

In all clones studied, the APase activity of *in vitro* and hydroponic plantlets was higher in shoot than in roots. On the contrary, in greenhouse plants, APase activity was higher in roots than in shoot. This result might be related to different physiological characteristics of plants grown in these three environments. Zimmermann et al. (2004) reported a different expression of three purple acid phosphatases (PAP) from potato plants grown aeroponically either with or without Pi, where $StPAP_1$ was expressed more abundantly in root and stem than in young leaves, stolons and flowers. This gene was not responsive to P deprivation. $StPAP_2$, in contrast, responded strongly but locally to P deficiency stress and also showed a higher expression in roots. Similar to $StPAP_2$, $StPAP_3$ was induced by P starvation, but showed a higher expression in the stem than in roots and leaves.

Major differences exist between the environment of plants grown in tissue culture and those grown in a greenhouse. These include differences in lighting, both quantity and quality; relative humidity; nutrients and other growth promoters; the gaseous composition; and the medium substrate (Hazarika, 2003). Differences between these two environments and their effect on plants have been recognized in numerous studies (Pospísilová et al., 1999; Hazarika, 2003). In the present study, greenhouse plants, grown in uncontrolled temperatures, had a higher transpiration rate than *in vitro* and hydroponic plants, where the temperature was maintained at 25ºC. During acclimatization to *ex vitro* conditions, the transpiration rate usually decreases gradually because stomatal regulation of water loss becomes more effective and cuticle and epicuticular waxes develop (Pospísilová et al., 1999). In addition, chlorophyll a and b contents increase after transplantation (Pospísilová et al., 1998). The net photosynthetic rate in *Solanum tuberosum* decreased in the first week after transplantation and increased thereafter (Baroja et al., 1995). An increase in the transpiration rate may enhance the uptake and translocation of mineral elements in the xylem (Marschner, 1995). As APases are enzymes involved in the production, transport and recycling of Pi (Bozzo et al., 2002), it might be suggested that Pi taken

up by roots was not efficiently transported to the shoot in plants grown *in vitro* and in hydroponics due to the lower transpiration rate. Therefore, it can be suggested that APase activity in the shoot was higher than in the roots because Pi was more available in the latter. In addition, these data also suggest that the pool of acid phosphatases in roots and shoot is either different or that the mechanism of regulation of these enzymes is tissue specific. Navarro-De la Sancha et al. (2007) found the presence of at least three Mg-dependent inorganic pyrophosphatase activity groups in roots and leaves of 3-week-old *Arabidopsis* plants. These authors also presented interesting data about the expression of the AtPPa genes (https://www.genevestigator.ethz.ch/), as follow: $AtPPa_1$ showed a high expression throughout plant development, with a slight reduction during senescence, whereas $AtPPa_4$ and $AtPPa_6$ presented a lower level of expression, though present at all ages, and their expression was lower in young plants and reached a maximum during flowering.

Conclusions

1. Aluminum interferes with APase activity in potato clones.
2. Such effects are related to the three factors tested: the growth system utilized, the genetic background, and the plant organ analyzed.

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References

BISOGNIN, D.A.; DOUCHES, D.S.; BUSZKA, L.; BRYAN, G.; WANG, D. Mapping late blight resistance in *Solanum microdontum* Bitter. **Crop Science,** v.45, p.340-345, 2005.

BOROJA, M.E.; AGUIRREOLEA, J.; SÁNCHEZ-DÍAZ, M. CO_2 exchange of *in vitro* and acclimatized potato plantlets. In: CARRE, F.; CHAGVARDIEFF, P. (Ed.). **Ecophysiology and Photosynthetic *in vitro* cultures.** CEA: Centre d'Études de Cadarache, Saint-Paul-lez-Durance, 1995. p.187-188.

BOZZO, G.G.; RAGHOTHAMA, K.G.; PLAXTON, W.C. Purification and characterization of two secreted purple acid phosphatase isozymes from phosphate-starved tomato (*Lycopersicon esculentum*) cell cultures. **European Journal of Biochemistry,** v.269, p.6278-6286, 2002.

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantity of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v.72, p.248-254, 1976.

DEL POZO, J.C.; ALLONA, I.; RUBIO, V.; LEYVA, A.; de la PEÑA, A.; ARAGONCILLO, C.; PAZ-ARES, J. A type 5 acid phosphatase gene from *Arabidopsis thaliana* is induced by phosphate starvation and by some other types of phosphate mobilizing/oxidative stress conditions. **The Plant Journal**, v.19, p.579-589, 1999.

DONG, B.; SNG, W.L.; JIANG, X.; ZHOU, J.M.; KONG, F.X.; HU, W.; WANG, L.S. Effects of aluminum on physiological metabolism and antioxidant system of wheat (*Triticum aestivum* L.). **Chemosphere**, v.47, p.87-92, 2002.

DUFF, S.M.G.; SARATH, G.; PLAXTON, W.C. The role of acid phosphatase in plant phosphorus metabolism. **Physiologia Plantarum**, v.90, p.791-800, 1994.

EMMANUEL, D.; PETER, R.R. Aluminum toxicity and tolerance in plants. **Plant Physiology**, v.107, p.315-321, 1995.

GABBRIELLI, R.; GROSSI, L.; VERGNANO, O. The effects of nickel, calcium and magnesium on the acid phosphatase activity of two *Alyssum* species. **New Phytologist**, v.111, p.631-636, 1989.

GRIERSON, P.F.; COMERFORD, N.B. Non-destructive measurement of acid phosphatase activity in the rhizosphere using nitrocellulose membranes and image analysis. **Plant and Soil,** v.218, p.49-57, 2000.

GUO, T-R.; ZHANG, G-P.; ZHOU, M-X.; WU, F-B.; CHEN, J-X. Influence of aluminum and cadmium stresses on mineral nutrition and root exudates in two barley cultivars. **Pedosphere,** v.17, p.505-512, 2007.

HALSTEAD, R.L.; MCKERCHER, R.B. Biochemistry and cycling of phosphorus. In: PAUL, E.A.; McLAREN, A.D. (Ed.). **Soil Biochemistry.** Dekker, New York, 1975. p.31-63.

HAZARICA, B.N. Acclimatization of tissue-cultured plants. **Current Science,** v.85, p.1704-1712, 2003.

MARSCHNER, H. **Mineral Nutrition of Higher Plants**. 2nd ed. Academic Press, London, 1995. 889p.

MARZADORI, C.; GESSA, C.; CIURLI, S. Kinetic properties and stability of potato acid phosphatase immobilized on Ca-polygalacturonate. **Biology and Fertility of Soils,** v.27, p.97-103, 1998.

MURASHIGE, T.; SKOOG, F. A revised medium for rapid growth and bioassays with tobacco tissue culture. **Plant Physiology,** v.15, p.473-497, 1962.

MURATA, N.; LOS, D.A. Membrane fluidity and temperature perception. **Plant Physiology**, v.115, p.875–879, 1997.

NAVARRO-De la SANCHA, E.; COELHO-COUTIÑO, M.P.; VALENCIA-TURCOTTE, L.G.; HERNANDEZ-DOMÍNGUEZ, E.E.; TREJO-YEPES, G.; RODRÍGUEZ-SOTRES, R. Characterization of two soluble inorganic pyrophosphatases from *Arabidopsis thaliana*. **Plant Science,** v.172, p.796-807, 2007.

POSPÍLOVÁ, J.; WILHEMOVÁ, N.; SYNCOVÁ, H.; CATSKÝ, J.; KREBS, D.; TICHÁ, I.; HANÁCKOVÁ, B.; SNOPEK, J. Acclimation of tobacco plantlets to *ex vitro* conditions as affected by application of abscisic acid. **Journal of Experimental Botany**, v.49, p.863-869, 1998.

POSPÍSILOVÁ, J.; TICHÁ, I.; KADLECÉK, P.; HAIZEL, D.; PLZÁKOVÁ, S. Acclimatization of micropropagated plants to *ex vitro* conditions. **Biologia Plantarum**, v.42, p.481-497, 1999.

RAGHOTHAMA, K.G., KARTHOKEYAN, A.S. Phosphate acquisition. **Plant Soil,** v.274 p.37–49, 2005.

RAI, L.C.; HUSAINI, Y.; MALLICK, N. pH-altered interaction of aluminum and fluoride on nutrient uptake, photosynthesis and other variables of *Chlorella vulgaris*. **Aquatic Toxicology,** v.42, p.67-84, 1998.

RENGEL, Z. Role of calcium in aluminium toxicity. **New Phytologist,** v.121, p.499-513, 1992.

SOUSA, M.F.; FAÇANHA, A.R.; TAVARES, R.M.; NETO, T.L.; GERÓS, H. Phosphate transport by proteoid roots of *Hakea sericea*. **Plant Science,** v.173, p.550-558, 2007.

TABALDI, L.A.; RUPPENTHAL, R.; CARGNELUTTI, D.; MORSCH, V.M.; PEREIRA, L.B.; SCHETINGER, M.R.C. Effects of metal elements on acid phosphatase activity in cucumber (*Cucumis sativus* L.) seedlings. **Environmental and Experimental Botany**, v.59, p.43-48, 2007.

TABALDI, L.A.; NICOLOSO, F.T.; Y CASTRO, G.; CARGNELUTTI, D.; GONÇALVES, J.F.; RAUBER, R.; SKREBSKY, E.C.; SCHETINGER, M.R.C.; MORSCH, V.M.; BISOGNIN, D.A. Physiological and oxidative stress responses of four potato clones to aluminum in nutrient solution. **Brazilian Journal of Plant Physiology**, v.19, p.211-222, 2007.

TEJERA GARCIA, N.A.; OLIVEIRA, M.; IRIBARNE, C.; LLUCH, C. Partial purification and characterization of a non-specific acid phosphatase in leaves and root nodules of *Phaseolus vulgaris*. **Plant Physiology and Biochemistry**, v.42, p.585-591, 2004.

YONEYAMA, T.; TAIRA, M.; SUZUKI, T.; NAKAMURA, M.; NIWA, K.; WATANABE, T.; OHYAMA, T. Expression and characterization of a recombinant unique acid phosphatase from kidney bean hypocotyl exhibiting chloroperoxidase activity in the yeast *Pichia pastoris*. **Protein Expression and Purification**, v.53, p.31-39, 2007.

ZHANG, R.G.; SKARINA, T.; KATZ, J.E.; BEASLEY, S.; KHACHATRYAN, A.; VYAS, S.; ARROWSMITH, C.H.; CLARKE, S.; EDWARDS, A.; JOACHIMIAK, A.; SAVCHENKO, A. Structure of *Thermotoga maritime* stationary phase survival protein SurE: a novel acid phosphatase. **Structure**, v.9, p.1095-1106, 2001.

ZIMMERMANN, P.; REGIERER, B.; KOSSMANN, J.; FROSSARD, E.; AMRHEIN, N.; BUCHER, M. Differential expression of three purple acid phosphatases from potato. **Plant Biology,** v.6, p.519-528, 2004.

Capítulo III

Influência do estresse de alumínio no teor de micronutrientes em plântulas de batata

Manuscrito III

Micronutrient concentration in potato clones with distinct physiological sensitivity to Al stress

Micronutrient concentration in potato clones with distinct physiological sensitivity to Al stress

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This study aimed to evaluate the effects of aluminum (Al) on zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu) concentrations in potato clones, Macaca, SMIC148-A, Dakota Rose and *Solanum microdontum,* grown in a nutrient solution (pH 4.00) with 0, 50, 100, 150 and 200 mg Al L^{-1}. Root Zn and Fe concentrations decreased linearly with increasing Al levels in Macaca, SMIC148-A and Dakota Rose and increased linearly in *S. microdontum*. Shoot Zn concentration showed a quadratic relationship with Al in *S. microdontum* and SMIC148-A, but a curvilinear response in Dakota Rose. Shoot Fe concentration showed a quadratic relationship with Al in *S. microdontum*, SMIC148-A and Dakota Rose. Root Mn concentration decreased linearly in Macaca and SMIC148-A, and increased linearly in *S. microdontum*. Mn concentration showed a quadratic relationship with Al in roots of Dakota Rose and in shoot of SMIC148-A, and

increased curvilinearly with Al levels in shoot of Dakota Rose. In shoot, there was no alteration in Zn, Fe and Mn in Macaca or in Mn concentration in *S. microdontum.* Root and shoot Cu concentration increased linearly in Dakota Rose, and showed a quadratic relationship in Macaca. Root Cu concentration showed a quadratic relationship with Al levels in *S. microdontum* and SMIC148-A. Shoot Cu concentration increased linearly in *S. microdontum* and decreased linearly in SMIC148-A. Therefore, Al accumulation in potato tissues affects the rate of uptake and distribution of certain micronutrients in roots and shoot of potato clones, which may affect the growth and plant yield.

Key words: Aluminum; copper; iron; manganese; potato; zinc.

Concentração de micronutrientes em clones de batata com distinta sensibilidade fisiológica ao estresse de alumínio: O objetivo deste estudo foi caracterizar o efeito do alumínio (Al) na concentração de zinco (Zn), manganês (Mn), ferro (Fe) e cobre (Cu) em quatro clones de batata (Macaca, SMIC148-A, Dakota Rose e *Solanum microdontum*) crescendo em solução nutritiva (pH 4,00) com 0, 50, 100, 150 e 200 mg Al L^{-1}. A concentração de Zn e Fe em raízes diminuiu linearmente com o aumento nos níveis de Al nos clones Macaca, SMIC148-A e Dakota Rose e aumentou linearmente em *S. microdontum*. Na parte aérea, a concentração de Zn mostrou resposta quadrática ao Al em *S. microdontum* e SMIC148-A, enquanto no clone Dakota Rose, houve uma resposta cúbica. Nos clones *S. microdontum*, SMIC148-A e Dakota Rose, a concentração de Fe mostrou resposta quadrática ao Al. A concentração de Mn em raízes diminuiu linearmente em relação ao Al nos clones Macaca e SMIC148-A, e aumentou linearmente em *S. microdontum*. Para Dakota Rose e SMIC148-A, a concentração de Mn mostrou uma resposta quadrática em relação ao suprimento de Al em raízes e parte aérea. A concentração de Mn na parte aérea aumentou de forma cúbica com os níveis de Al no clone Dakota Rose. Na parte aérea, não houve alteração na concentração de Zn e Fe na Macaca e de

Mn nos clones Macaca e *S. microdontum*. Em raízes e parte aérea, a concentração de Cu aumentou linearmente no clone Dakota Rose, e mostrou resposta quadrática no clone Macaca. A concentração de Cu mostrou resposta quadrática com os níveis de Al em raízes dos clones *S. microdontum* e SMIC148-A. Na parte aérea, a concentração de Cu aumentou linearmente no clone *S. microdontum* e diminuiu linearmente no clone SMIC148-A com o aumento nos níveis de Al. Portanto, o Al afeta a taxa de absorção e distribuição de alguns micronutrientes em raízes e parte aérea de clones de batata, podendo consequentemente afetar o crescimento e a produtividade das plantas.

Palavras-chave: Alumínio; batata; cobre; ferro; manganês; zinco.

INTRODUCTION

The mineral nutrient status of plants is directly related to their growth and productivity. Micronutrients are basic requirements for plant growth and development and their status is controlled by their genetically fixed nutrient uptake potential, availability of the nutrient in the soil, and other environmental factors (Mengel and Kirkby, 2001). Environmental variation of nutrient availability is expected to result in changes for plant physiology and morphology, and consequently in changes in the yield.

On the other hand, availability of most micronutrients depends, among other factors, on the pH of the soil solution, as well as the nature of binding sites on organic and inorganic particle surfaces (Tuna et al., 2008). Trace elements are adsorbed by inorganic constituents such as iron (Fe) and aluminum (Al) oxides and form complexes with organic matter (Omil et al., 2007).

Acid soils are found throughout the world. It is estimated that about 40% of the world's arable soils and 12% of the land in crop production have a pH below 5.5 (von Uexküll and Mutert, 1995). Moreover, soil acidification is increasing globally. These soils are often characterized by reduced availability of several nutrients (Kamprath, 1984; Foy, 1992). In fact,

soil acidification may bring about many other changes in the physical and chemical properties of the soil, which in turn affect plant growth and development.

In acid soil, some nutrients, such as P, Ca, and Mg, may be deficient, whereas others, such as Mn and B, could be toxic to plants. In addition, the continuing acidification of soils with low buffering capacity leads to an increase of Al mobilization in the environment and may be potentially hazardous to all terrestrial and aquatic systems (Matús et al., 2006). These effects are further complicated by interactions of Al with other ions in different plant genotypes and under stress conditions (Foy, 1992). Although Al is the most abundant metal in the Earth's crust, it is nonessential for plants. Although Al occurs in various chemical species, the Al cation Al^{3+} is regarded as the most toxic soluble form of Al (Parker et al., 1988). Al^{3+} is toxic to many plants at micromolar concentrations, primarily affecting the normal functioning of roots. The rapid inhibition of Al-mediated root growth (Ryan et al., 1993) results in poor nutrient and water acquisition and transport, consequently leading to nutrient deficiencies and decreasing crop yields (Kochian, 1995). Aluminum toxicity may be manifested as a deficiency of essential nutrients such as Ca, Mg, Fe, Zn or Mo; decreased availability of P or as toxicity of Mn and H^+ (Guo et al., 2007; Schöll et al., 2005). Aluminum at high levels competes with cationic (mono or bivalents) ions for absorption sites in channels or transporters (Kochian, 1995).

Al's interference in uptake, transport and utilization efficiency of most of the macronutrients has been well documented (McColl et al., 1991; Guo et al., 2007). On the other hand, there is a lack of studies connecting the effects of Al on the uptake and transport of micronutrients in plants. Thus, the objective of this study was to analyze the influence of exposure to Al in a nutrient solution on micronutrient concentrations in roots and shoot of four potato clones.

MATERIAL AND METHODS

Plant materials and growth conditions: Three adapted (2n=4x=48) clones (Macaca, SMIC148-A and Dakota Rose) and one wild species (2n=2x=24) clone (PI595511-5 of *Solanum microdontum*) were evaluated. *S. microdontum* was identified as highly resistant to *Phytophora infestans* (Bisognin et al., 2005) and has been used in our breeding program. This clone will be referred to as *S. microdontum*. Tissue culture plantlets were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, Brazil. Nodal segments (1.0 cm long) were micropropagated in MS medium (Murashige and Skoog, 1962), supplemented with 30 g L^{-1} of sucrose, 0.1 g L^{-1} of myo-inositol and 6 g L^{-1} of agar.

Twenty-day-old plantlets from *in vitro* culture were transferred into plastic boxes (10 L) filled with aerated full nutrient solution of low ionic strength. The nutrient solution had the following composition (in μM): 6090.5 of N; 974.3 of Mg; 5229.5 of Cl; 2679.2 of K; 2436.2 of Ca; 359.9 of S; 0.47 of Cu; 2.00 of Mn; 1.99 of Zn; 0.17 of Ni; 24.97 of B; 0.52 of Mo; 47.99 of Fe ($FeSO_4$/Na-EDTA). Treatments consisted of the addition of 0, 50, 100, 150 or 200 mg Al L^{-1} as $AlCl_3.6H_2O$. The solution pH was adjusted daily to 4.0±0.1 by titration with HCl or NaOH solutions of 0.1 M. Both *in vitro* and *ex vitro* cultured plants were grown in a growth chamber at 25±2ºC on a 16/8-h light/dark cycle with 35 μmol m^{-2} s^{-1} of irradiance. Aluminum-treated plantlets remained in each treatment for 7 days. At harvest, the plantlets were divided into shoot and roots. Roots were rinsed twice with distilled water. Subsequently, micronutrient concentrations were determined, according to Tedesco et al., 1995.

Determination of micronutrient concentration: After Al treatment, samples (roots and shoot) were dried at 60ºC until reaching a constant weight. The dried tissues were weighed and ground into a fine powder before nitric-percloric digestion. Micronutrient concentrations were determined by atomic absorption spectrometry.

Statistical analysis: All data were analyzed by ANOVA procedures. The effects of Al on micronutrient concentrations in roots and shoot of potato plantlets were quantified using regression analysis with the SOC statistic package (Software Científico: NTIA/EMBRAPA). Coefficients were included in a regression equation when their values were significant ($P < 0.05$).

RESULTS AND DISCUSSION

In our previous study (Tabaldi et al., 2007), based on relative root growth, *S. microdontum* and SMIC148-A were shown to be Al-tolerant clones, whereas Macaca and Dakota Rose were shown to be Al-sensitive clones. In addition, regression analysis showed that the concentration of Al in both roots and shoot of these clones increased linearly with increasing Al levels, and the increase in tissue Al was much steeper for Macaca and SMIC148-A. However, the maximum concentrations of Al, 49,300 mg kg^{-1} in roots and 17,900 mg kg^{-1}in shoot, were found in Dakota Rose at 200 mg Al L^{-1}.

Plants require an adequate supply of micronutrients for their normal physiological and biochemical functions. Deficiencies of essential micronutrients induce abnormal pigmentation, size, and shape of plant tissues, reduce leaf photosynthetic rates, and lead to various detrimental conditions (Masoni et al., 1996). In the present study, the concentration of some micronutrients in the tissue of roots and shoot of four potato clones was examined after 7 days of Al exposure. A micronutrient- and organ-dependent response to Al toxicity was observed in all potato clones. Micronutrient concentrations were higher in roots than in shoot of all potato clones tested, suggesting that more micronutrients were retained in the roots and smaller amounts were transported to the shoot.

Regression analysis showed that the concentration of zinc (Zn) decreased linearly with increasing Al levels in roots of Macaca, SMIC148-A and Dakota Rose clones (Fig. 1A). This result is similar to that reported by Kolawole et al. (2000) and Jemo et al. (2007), who observed a reduction in nutrient acquisition in cowpea genotypes exposed to Al. High concentrations of Al in the substrate decreased the uptake of Ca, K, P, Fe, and Zn in birch seedlings (*Betula pendula* Roth.), limiting the growth of roots and shoot (Bojaczuk et al., 2002).

As was observed for Zn concentration, root iron (Fe) concentration decreased linearly with increasing Al levels in Macaca, SMIC148-A and Dakota Rose clones (Fig. 1C). At 200 mg Al L^{-1}, root Fe concentration decreased by about 20%, 47% and 30%, in Macaca, SMIC148-A and Dakota Rose clones, respectively, when compared to the control. Metal-metal interactions may occur when cations compete for negatively charged binding sites at the cell surface (Kinraide and Parker, 1987; Kinraide et al., 1992). Since the cell wall is the major site of metal accumulation (Kochian, 1995; Taylor, 1988) and provides the bulk of charged surfaces in the apoplasm, the metal-metal interactions should affect total metal accumulation. These data might indicate a direct competition between Al and essential nutrients for the same uptake site. At high levels, Al competes with cationic (mono or bivalent) ions for absorption sites in channels or transporters (Kochian, 1995). This competition may reduce ion absorption and utilization. In addition, Al ions may bind to the phospholipid heads of the plasma membrane, alter the lipid-protein interaction, and modify transporter activity (Suhayda and Haug, 1986). Another possibility is that Al binds directly to the transport proteins, thereby impairing their function (Schroeder, 1988).

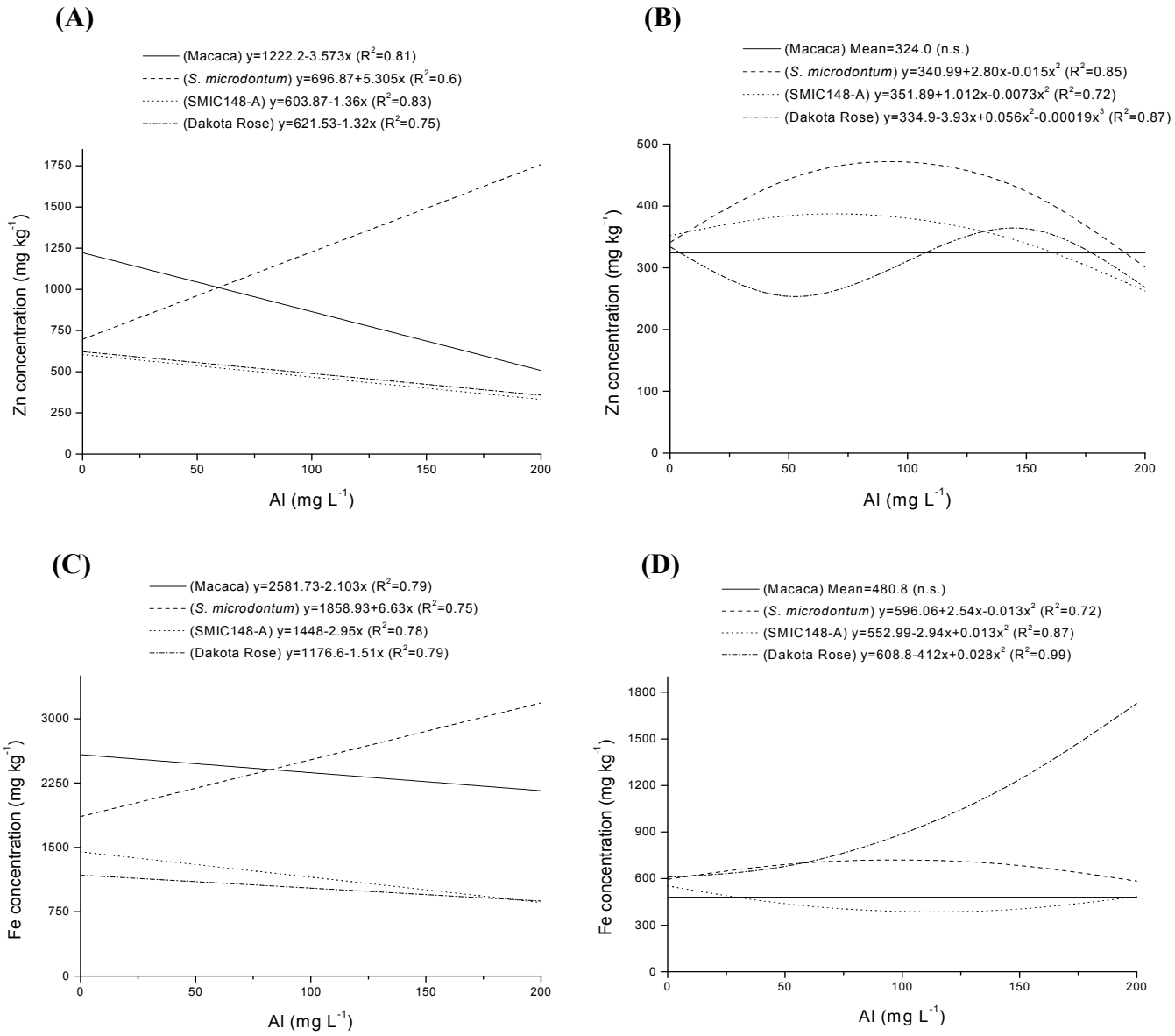

Figure 1- Effect of increasing Al concentration on the root (A) and shoot (B) zinc (Zn) concentration and root (C) and shoot (D) iron (Fe) concentration of potato clones, Macaca, *S. microdontum*, SMIC148-A and Dakota Rose, submitted to increasing Al concentrations for 7 days. n.s.: not significant.

Root Zn and Fe concentrations increased linearly with increasing Al levels in *S. microdontum* (Fig. 1A and Fig. 1C, respectively). In this study, *S. microdontum* presented greater concentrations of most micronutrients analyzed. Therefore, as this potato clone is Al-

tolerant (Tabaldi et al., 2007), it seems that the Al levels tested were not high enough to cause severe alteration in the metabolism. Thus, higher levels of mineral nutrients may be connected with Al tolerance, as suggested by Giannakoula et al. (2008). Shoot Zn concentration showed a quadratic relationship in the *S. microdontum* and SMIC148-A clones, increasing at intermediary Al levels (Fig. 1B). In Dakota Rose, shoot Zn concentration showed a curvilinear response, decreasing at approximately 50 mg Al L^{-1} and increasing at approximately 150 mg Al L^{-1} (Fig. 1B).

In SMIC148-A clone, shoot Fe concentration decreased between Al levels of approximately 50 and 150 mg Al L^{-1} and showed a quadratic increase in Dakota Rose. On the other hand, shoot Fe concentration slightly increased at Al levels between approximately 50 and 100 mg Al L^{-1} in *S. microdontum*.

Root manganese (Mn) concentration decreased linearly in both Macaca and SMIC148-A clones, and increased linearly with increasing Al levels in *S. microdontum* (Fig. 2A). However, in Dakota Rose, Mn concentration showed a quadratic relationship to Al supply, decreasing at levels between approximately 50 and 100 mg Al L^{-1} and increasing at 200 mg Al L^{-1}. Shoot Mn concentration showed a quadratic relationship with Al levels in SMIC148-A (Fig. 2B), decreasing at levels between approximately 50 and 100 mg Al L^{-1} and increasing at 200 mg Al L^{-1}. For Dakota Rose, shoot Mn concentration showed a curvilinear response to Al supply, increasing at levels between approximately 100 and 150 mg Al L^{-1}, while in *S. microdontum* there was no alteration in Mn concentration.

In our previous study (Tabaldi et al., 2007), shoot growth in Macaca decreased linearly with increasing Al levels. However, there was no alteration in shoot Zn (Fig. 1B), Fe (Fig. 1D) or Mn (Fig. 2B) concentrations in this clone. Therefore, the interference of Al in root growth and absorption and transport of water and other nutrients may have brought about lower shoot growth.

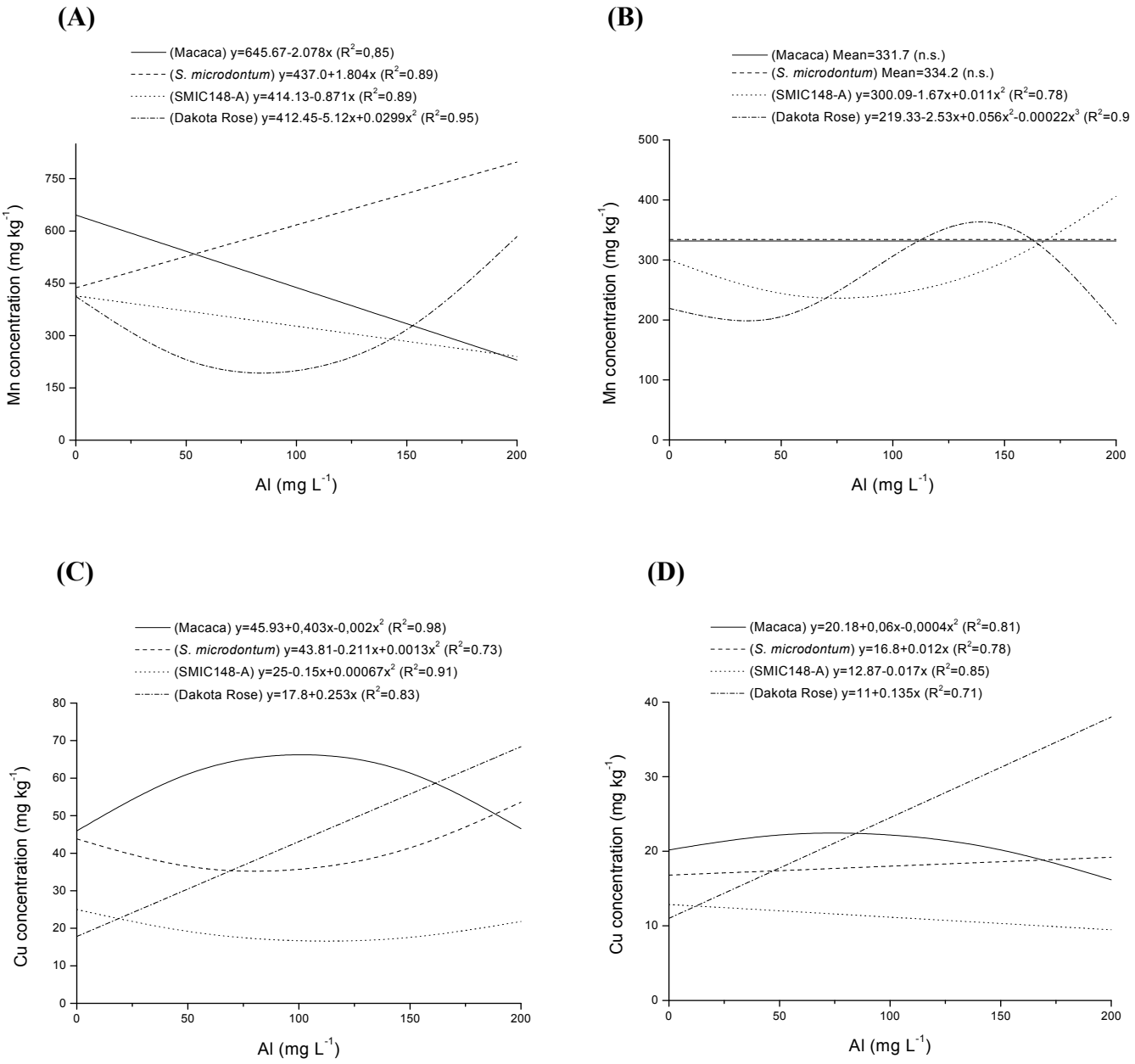

Figure 2- Effect of increasing Al concentration on the root (A) and shoot (B) manganese (Mn) concentration and root (C) and shoot (D) copper (Cu) concentration of potato clones, Macaca, *S. microdontum*, SMIC148-A and Dakota Rose, submitted to increasing Al concentrations for 7 days. n.s.: not significant.

Copper (Cu) is an essential plant micronutrient that plays an important role in both photosynthetic and respiratory electron transport, being a cofactor for many enzymes (Owen,

1982). However, when present at elevated concentrations it affects different parameters of plant metabolism, such as dry mass accumulation (Ali et al., 2002; Zheng et al., 2004), chlorophyll (Lou et al., 2004), and water content (Burzynski and Klobus, 2004) and the balance of macro- and micronutrient levels (Ali et al., 2002; Bernal et al., 2007). Being a redox active metal, Cu generates reactive oxygen species (ROS) by Fenton reaction, which may result in oxidative stress leading to peroxidation of membrane lipids (Stohs and Bagchi, 1995). The response of root Cu concentration to Al in Dakota Rose was linear and positive (Fig. 2C), whereas in Macaca it increased only at levels between approximately 50 and 150 mg Al L^{-1}. By contrast, in both Al-tolerant clones, root Cu concentration generally showed an inverse relationship with increasing Al levels, with exception of *S. microdontum*, which showed an increased Cu concentration at 200 mg Al L^{-1} when compared to the control (Fig. 2C). Shoot Cu concentration in Dakota Rose increased linearly with increasing Al levels (Fig. 2D), while in Macaca it slightly increased at Al levels between approximately 50 and 100 mg Al L^{-1}. The response of shoot Cu concentration to Al in *S. microdontum* was linear and positive (Fig. 2D), while in SMIC148-A it was linear and negative (Fig. 2D). However, shoot Cu concentration was less altered in both of these Al-tolerant clones than was in the Al-sensitive clones. Therefore, the increase in tissue Cu concentration observed in the potato clones exposed to Al might have caused a disturbance in the metabolism, and hence in plant growth and development, mainly in the Al-sensitive clones.

Plants vary in their sensitivity to toxic compounds in the soil. Many studies have shown that sensitivity depends on many factors, such as physicochemical properties of the soil, concentration of organic matter and nutrients, but primarily on soil pH (Göransson and Eldhuset, 2001; Rengel, 1996). Aluminum is widespread in the Earth's crust and its availability to plants increases with decreasing pH of the soil (Boudot et al., 1994). The toxic Al ions present in the substrate can damage root cells, which become inefficient in absorption and

translocation of both nutrients and water (Mossor-Pietraszewska et al., 1997), blocking their participation in important metabolic processes, such as photosynthesis and respiration (Rengel, 1996).

Therefore, in the present work, the excessive Al accumulation observed could have affected the rate of uptake and distribution of certain micronutrients in roots and shoot of potato clones, and consequently would be responsible for mineral deficiencies/imbalance and depression of the plant growth. Selection of plants tolerant to toxic ions contained in the soil may enable more effective management of degraded habitats.

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REFERENCES

Ali NA, Bernal MP, Ater M (2002) Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. Plant and Soil 239:103-111.

Bernal M, Cases R, Picorel R, Yruela I (2007) Foliar and root Cu supply affect differently Fe- and Zn-uptake and photosynthetic activity in soybean plants. Environmental and Experimental Botany 60:145-150.

Bisognin DA, Douches DS, Buszka L, Bryan G, Wang D (2005) Mapping late blight resistance in *Solanum microdontum* Bitter. Crop Science 45:340-345.

Bojarczuk K, Karalewski P, Oleksyn J, Kieliszewska-Rokicka B, Żyrkowlak R, Tjoelker MG (2002) Effect of polluted and fertilization on growth and physiology of silver birch (*Betula pendula* Roth.) seedlings. Polish Journal of Environmental Studies 11:483-492.

Boudot JP, Becquer T, Merlet D, Rouiller J (1994) Aluminium toxicity in declining forests: a general overview with a seasonal assessment in a silver fir forest in the Vosges mountains (France). Annals of Forest Science 51:27-51.

Burzynski M, Klobus G (2004) Changes of photosynthetic parameters in cucumber leaves under Cu, Cd and Pb stress. Photosynthetica 42:505-510.

Foy CD (1992) Soil chemical factors limiting plant root growth. Advances in Soil Science 19:97-149.

Giannakoula A, Moustakas M, Mylona P, Papadakis I, Yupsanis T (2008) Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and praline, and decreased levels of lipid peroxidation and Al accumulation. Journal of Plant Physiology 165:385-396.

Göransson A, Eldhuset TD (2001) Is the $Ca^{+}K^{+}Mg/Al$ ratio in the soil solution a predictive tool for estimating damage? Water, Air and Soil Pollution 1:57-74.

Guo TR, Zhang GP, Zhang YH (2007) Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. Colloids and Surfaces B: Biointerfaces 57:182-188.

Jemo M, Abaidoo RC, Nolte C, Horst WJ (2007) Aluminum resistance of cowpea as affected by phosphorus-deficiency stress. Journal of Plant Physiology 164:442-451.

Kamprath EJ (1984) Crop response to lime on soils in the tropic. In: Adams A (ed), Soil acidity and liming, pp.349-368. American Society of Agronomy, Madison, WI.

Kinraide TB, Parker DR (1987) Cation amelioration of aluminum toxicity in wheat. Plant Physiology 83:546-551.

Kinraide TB, Ryan PR, Kochian LV (1992) Interactive effects of $A1^{3+}$, H^{+}, and other cations on root elongation considered in terms of cell-surface electrical potential. Plant Physiology 99:1461-1468.

Kochian LV (1995) Cellular mechanisms of aluminum toxicity and resistance in plants. Annual Review of Plant Physiology and Plant Molecular Biology 46:237-260.

Kolawole GO, Tian G, Singh BB (2000) Differential response of cowpea lines to aluminum and phosphorus application. Journal of Plant Nutrition 23:731-740.

Lou L, Shen Z, Li X (2004) The copper tolerance mechanisms of *Elsholtzia haichowensis*, a plant from copper-enriched soils. Environmental and Experimental Botany 51:111-120.

Masoni A, Ercoli L, Mariotti M. (1996) Special properties of leaves deficient in iron, sulfur, magnesium, and manganese. Agronomy Journal 88:937-943.

Matús P, Kubová J, Bujdos M, Medved J (2006) Free aluminium extraction from various reference materials and acid soils with relation to plant availability. Talanta 70:996–1005.

McColl JG, Waldren RP, Wafula NJ, Sigunga DO (1991) Aluminium effect on six wheat cultivars in Kenyan soils. Communications in Soil Science and Plant Analysis 22:1701-1719.

Mengel K, Kirkby EA (2001) Principles of Plant Nutrition. 5th ed. Kluwer Academic Publishers, Dordrecht.

Mossor-Pietraszewska T, Kwit M, Legiewicz M (1997) The influence of aluminium ions on activity changes of some dehydrogenases and aminotransferases in yellow lupine. Biological Bulletin of Poznan 34:47-48.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiology 15:473-497.

Omil B, Piñeiro V, Merino A (2007) Trace elements in soils and plants in temperate forest plantations subjected to single and multiple applications of mixed wood ash. Science of the Total Environment 381:157-168.

Owen C (1982) Biochemical Aspects of Copper. 1st ed. Noyes Publications, Park Ridge, NJ.

Parker DR, Kinraide TB, Zelazny LW (1988) Aluminum speciation and phytotoxicity in dilute hydroxy-aluminum solutions. Soil Science Society of America Journal 52:438-444.

Rengel Z (1996) Uptake of aluminium by plant cells. New Phytologist 134:389-406.

Ryan PR, DiTomaso JM, Kochian LV **(**1993) Aluminum toxicity in roots: an investigation of spatial sensitivity and the role of the root cap. Journal of Experimental Botany 44:437-446.

Schoeder JI (1988) K^+ transport properties of K^+ channels in the plasma membrane of *Vicia faba* guard cells. The Journal of General Physiology 92:667-683.

Schöll L, Keltjens WG, Hoffland E, Breemen NV (2005) Effects of ectomycorrhizal colonization on the uptake of Ca, Mg and Al by *Pinnus sylvestris* under aluminum toxicity. Forest Ecology and Management 215:352-360.

Stohs SJ, Bagchi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radical in Biology and Medicine 18:321-336.

Suhayda CG, Haug A (1986) Organic acids reduce aluminum toxicity in maize root membranes. Physiologia Plantarum 68:189-195.

Tabaldi LA, Nicoloso FT, Y Castro G, Cargnelutti D, Gonçalves JF, Rauber R, Skrebsky EC, Schetinger MRC, Morsch VM, Bisognin DA (2007) Physiological and oxidative stress responses of four potato clones to aluminum in nutrient solution. Brazilian Journal of Plant Physiology 19:211-222.

Taylor, GJ (1998) The physiology of aluminum tolerance. In: Sigel, H, Sigel, A (eds.) Metal ions in biological systems. Aluminum and its role in Biology, pp. 165-198. Marcel Dekker, New York.

Tedesco, MS, Gianello, C, Bissani, CA, Bohnen, H, Volkweiss, SJ (1995) Análise de solo, plantas e outros materiais. 2nd ed. Porto Alegre, Universidade Federal do Rio Grande do Sul, 174p.

Tuna AL, Kaya C, Dikilitas M, Higgs D (2008) The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environmental and Experimental Botany 62:1-9.

Von Uexküll HR, Mutert E (1995) Global extent, development and economic impact of acid soils. Plant and Soil 171:11-15.

Zheng Y, Wang L, Dixon MA (2004) Response to copper toxicity for three ornamental crops in solution culture. HortScience 39:1116-1120.

Capítulo IV

Respostas localizadas e sistêmicas de estresse oxidativo induzidas por alumínio em clones de batata (*Solanum tuberosum* L.) cultivados em sistema de raízes divididas

Manuscrito IV

Local and systemic oxidative stress responses induced by aluminum in two potato clones (S*olanum tuberosum* L.) that differ in Al-avoidance

Local and systemic oxidative stress responses induced by aluminum in two potato clones (S*olanum tuberosum* L.) that differ in Al-avoidance

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Abstract

The objective of this study was to check whether Al oxidative stress differs in the potato clones Macaca (Al-sensitive) and SMIC148-A (Al-tolerant), which present distinct degrees of Al-avoidance. Plants were cultivated in a split-root system for 10 days with five treatments of varying concentrations and locations of Al (in mg L^{-1}): T1 - pot 1: 0.0, pot 2: 0.0; T2- pot 1: 50, pot 2: 50; T3- pot 1: 0.0, pot 2: 100; T4- pot 1: 100, pot 2: 100; T5- pot 1: 0.0, pot 2: 200. At 200 mg Al L^{-1}, a significant decrease in chlorophyll concentration and increase in protein oxidation was observed only for Macaca. At 200 mg L^{-1} supplied to half of the root system, shoot H_2O_2 concentration was lower than that with both root halves treated by 100 mg L^{-1} for both clones, but this effect was much less pronounced in Macaca. Shoot lipid peroxidation in Macaca

increased with increasing Al supply. In SMIC148-A, plants treated with 100 and 200 mg Al L^{-1} in only one root half showed lower shoot lipid peroxidation. The 200 side of 0/200 plants demonstrated significantly greater lipid peroxidation than that untreated with Al, mainly in Macaca. The increase observed in the concentration of NPSH in shoot of SMIC148-A seemed to present a higher correlation with lipid peroxidation than that for Macaca. At 100 mg Al L^{-1} at both root halves, Macaca showed an inefficient tolerance response in terms of CAT activity, protein oxidation, lipid peroxidation, H_2O_2 concentration and APase activity. These results show that SMIC148-A, despite lower Al-avoidance when compared to Macaca, presented a stronger local and systemic antioxidant response to Al supply.

Keywords: aluminum; oxidative stress; *Solanum tuberosum*; split-root.

Introduction

Potatoes (*Solanum tuberosum* L.) rate fourth in world production among various agricultural products, following wheat, rice and corn (FAO, 2004), with an overall annual production of nearly 327 million tons and about 19 million ha planted. The most widely cultivated species of potato are very sensitive to abiotic stress, whereas several wild or primitive cultivated species from different ploidy levels adapt well to grow under unfavorable conditions (Li and Fennel, 1985). Potato is the main horticultural crop in Brazil in terms of area and food preference, with about 98% of the producers located in the southern states of Minas Gerais, São Paulo, Paraná and Rio Grande do Sul. Potato crops tolerate moderate acidity in the soil, growing well at a pH of 5.0 to 6.5. On the other hand, in very acid soils (pH below 5.0) a decrease in yield occurs (Castro, 1983).

Acid soils, which comprise 30–40% of the world's arable lands (Vitorello et al., 2005) are a limiting factor to crop growth and are usually associated to low inherent

levels of plant-available phosphorus (P) (Jemo et al., 2007) and high levels of aluminum (Al) (von Uexküll and Mutert, 1995), which is solubilized in acidic pH into the toxic cation Al^{3+}. Aluminum is known to inhibit plant growth (Ciamporova, 2002), mainly that of the root (Balestrasse et al., 2006; Tabaldi et al., 2007b). Symptoms of Al toxicity are also manifested in the shoot and are regarded as a consequence of injuries to the root system (Vitorello et al., 2005). In addition, Al also alters water relations (Barceló and Poschenrieder, 2002), reduces stomatal opening, decreases photosynthetic activity and causes chlorosis and necrosis of leaves, decreasing carbon sequestration and biomass formation (Vitorello et al., 2005). Potential alternatives to the direct amelioration of subsoil acidity include the use of Al-tolerant germplasm (Foy, 1988).

Although the physiological mechanism of Al toxicity is still unclear, several reports suggest a role of Al in the induction of oxidative stress (Yamamoto et al., 2002; Tabaldi et al., 2007b) and, consequently, formation of reactive oxygen species (ROS) in plants, including superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) and hydroxygen peroxide (H_2O_2). These ROS can cause oxidative damage to the biomolecules such as lipids, proteins (Tabaldi et al., 2007b), photosynthetic pigments and nucleic acids, which leads to cell membrane peroxidation, loss of ions, protein hydrolysis, and even DNA strand breakage (Guo et al., 2007). To mitigate the oxidative damage initiated by ROS, plants have developed a complex defense antioxidant system, including low-molecular mass antioxidants as well as antioxidant enzymes. Many plant species vary in their ability to withstand Al toxicity. The antioxidant defense system might possibly protect cells from Al toxicity.

Plant roots are characterized by very high adaptability. Their growth and development involve complex interactions with both the soil environment and the shoot (Marschner, 1996). Under natural soil conditions, roots are able to respond to the

heterogeneous soil environment by improving root growth in more favorable pockets (Kerley et al., 2000), which is described as a plastic response of the root system (Feldman, 1984). Hairiah et al. (1993) showed that velvet bean (*Mucuna pruriens*) was Al-resistant when the whole root system was exposed to homogeneous Al supply. However, when Al was supplied to only one part of the root system, roots avoided Al by preferential development of roots not in contact with Al, accompanied by marked inhibition of roots exposed to Al. This relative Al avoidance, rather than absolute Al tolerance or toxicity, explains root response to acid subsoil conditions in the field. Al-avoidance reactions in this sense may help to explain why selection of Al-tolerant genotypes based on experiments with homogeneous media may fail to be successful for field trials.

In our previous study (Tabaldi et al., 2007b), utilizing a homogeneous supply of Al to the roots of potato clones grown in a hydroponic growth system, it was demonstrated that the SMIC148-A clone was Al-tolerant, whereas the Macaca clone was Al-sensitive. Moreover, it was observed that Al supply induced oxidative stress, mainly in the Al-sensitive clone. Therefore, we formulated the hypothesis that potato clones with distinct physiological sensitivity to Al stress and growing in a heterogeneous root environment (split-root experiment) would show contrasting Al-avoidance responses. A consequence of this hypothesis is that both Al avoidance and Al oxidative stress should be less pronounced for the Al-tolerant clone, since the response to local supply of Al is reduced under this condition. The aim of the present paper is to test this hypothesis.

Material and Methods

Plant materials and growth conditions: Microtubers of potato clones (*Solanum tuberosum* L.) Macaca (Al-sensitive) and SMIC148-A (Al-tolerant) were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, Santa Maria, RS, and were sowed in plastic pots of 300 mL, employing sand as substrate. The plants were irrigated with a complete nutrient solution. The nutrient solution had the following composition (in μM): 6090.5 of N; 974.3 of Mg; 4986.76 of Cl; 2679.2 of K; 2436.2 of Ca; 359.9 of S; 243.592 of P; 0.47 of Cu; 2.00 of Mn; 1.99 of Zn; 0.17 of Ni; 24.97 of B; 0.52 of Mo; 47.99 of Fe ($FeSO_4$/Na-EDTA). After about 3 weeks, uniform plants were chosen and transferred to a split-root system, in which the two halves of the root system, each in a pot of 1 L, were exposed to an aerated complete nutrient solution for 1 week. After that acclimatization period, these plants with split-roots were cultivated for 10 days in a new nutrient solution (without P and pH 4.0±0.1) with five treatments (six replicates for each treatment) of varying concentrations and locations of Al, as follows: Treatment 1 (control) - pot 1: 0.0 mg Al L^{-1}, pot 2: 0.0 mg Al L^{-1}; Treatment 2- pot 1: 50 mg Al L^{-1}, pot 2: 50 mg Al L^{-1}; Treatment 3- pot 1: 0.0 mg Al L^{-1}, pot 2: 100 mg Al L^{-1}; Treatment 4- pot 1: 100 mg Al L^{-1}, pot 2: 100 mg Al L^{-1}; Treatment 5- pot 1: 0.0 mg Al L^{-1}, pot 2: 200 mg Al L^{-1}. With exception of Al, the concentrations of the other mineral elements in the nutrient solution were the same for all treatments. Nutrient solutions were replaced every 48 hours and pH was evaluated daily. Aluminum-treated plantlets remained in each treatment for 10 d. At harvest, the plants of both clones were divided into shoot, left root and right root to evaluate biochemical parameters.

Chlorophyll and carotenoid determination: Chlorophyll (a+b) and carotenoids were extracted following the method of Hiscox and Israeslstam (1979) and estimated with the help of Lichtenthaler's formulae (Lichtenthaler, 1987). Fresh leaves (0.1 g) were incubated at 65ºC in dimethylsulfoxide (DMSO) until tissues were completely bleached. Absorbance of the solution was then measured at 663 and 645 nm for chlorophyll and 470 nm for carotenoids on a spectrophotometer (Celm E-205D). Chlorophyll and carotenoid concentrations were expressed as mg g^{-1} fresh weight.

Catalase assay: Catalase (CAT) activity was assayed following the modified Aebi (1984) method. Fresh roots and shoot samples (1 g) were homogenized in 5 mL of 50 mM KH_2PO_4/ K_2HPO_4 (pH 7.0), 10 g L^{-1} of polyvinylpyrrolidone (PVP), 0.2 mM EDTA and 10 mL L^{-1} Triton X-100. The homogenate was centrifuged at 12000 x g for 20 min at 4ºC and the supernatant was used for enzyme assay. Activity of CAT was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture with a final volume of 2 mL containing 15 mM H_2O_2 in KPO_4 buffer (pH 7.0) and 30 µL extract. Activity was expressed as ΔE min^{-1} mg^{-1} protein.

Protein oxidation: Samples of roots and shoot (1 g) were homogenized with 25 mM K_2HPO_4 (pH 7.0) containing 10 mL L^{-1} Triton X-100, at a proportion of 1:2 (w/v) (Levine et al., 1990). After the homogenate was centrifuged at 15000 x g for 10 min at 4ºC, the supernatant was used for immediate determination of protein oxidation, which was expressed as nmol carbonyl mg^{-1} protein.

Determination of hydrogen peroxide: The H_2O_2 concentration was determined according to Loreto and Velikova (2001). Approximately 0.1 g of both roots and shoot was homogenized at 4ºC in 2 mL of 0.1% trichloroaceti acid (TCA) (w/v). The homogenate was centrifuged at 12000 x g for 15 min at 4ºC. Then, 0.5 mL of the supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1M KI. The H_2O_2 concentration of the supernatant was evaluated by comparing its absorbance at 390 nm with a standard calibration curve. Hydrogen peroxide concentration was expressed as μmol g^{-1} fresh weight.

Estimation of lipid peroxides: The degree of lipid peroxidation was estimated following the method El-Moshaty et al. (1993). Fresh roots and shoot samples of 0.1 g were homogenized in 20 mL of 0.2 M citrate-phosphate buffer (pH 6.5) containing 0.5% Triton X-100, using mortar and pestle. The homogenate was filtered with two paper layers and centrifuged for 15 min at 20000 x g. One milliliter of the supernatant fraction was added to an equal volume of 20% (w/v) TCA containing 0.5% (w/v) of thiobarbituric acid (TBA). The mixture was heated at 95ºC for 40 min and then quickly cooled in an ice bath for 15 min, and centrifuged at 10000 x g for 15 min. The absorbance of the supernatant at 532 nm was read and corrected for unspecific turbidity by subtracting the value of the absorbance at 600 nm. The lipid peroxides were expressed as nmol MDA mg^{-1} protein, by using an extinction coefficient of 155 L $mmol^{-1}$ cm^{-1}.

Non-protein thiol groups (NPSH) contents: Roots and shoot of potato plants were homogenized in a solution containing 50 mmol L^{-1} Tris-HCl and 10 mL L^{-1} Triton X-100 (pH 7.5), centrifuged at 6800 x g for 10 min. To the resulting supernatant 10% TCA

was added at proportion 1:1 (v/v) followed by centrifugation (6800 x g for 10 min) to remove protein. Non-protein thiols content was measured spectrophotometrically with Ellman's reagent (Ellman, 1959). An aliquot of the sample (400 µL) was added in a medium containing 550 µL 1 mol L^{-1} Tris-HCl (pH 7.4). The developed color was read at 412 nm after the addition of 10 mmol/L 5-5-dithio-bis 2-nitrobenzoic acid (DTNB) (0.05 mL). A standard curve using cysteine was used to calculate the content of thiol groups in samples.

Acid phosphatases assay: Fresh root and shoot samples were centrifuged at 43200 x g for 30 min at 4ºC and the supernatant was used for enzyme assay. Acid phosphatases activity was determined according to Tabaldi et al. (2007a) in a reaction medium consisting of 3.5 mM sodium azide, 2.5 mM calcium chloride, 100 mM citrate buffer, pH 5.5, at a final volume of 200 µL. A 20 µL aliquot of the enzyme preparation (10-20 µg protein) was added to the reaction mixture, and preincubated for 10 min at 35°C. The reaction was started by the addition of PPi as substrate and stopped by the addition of 200 µL of 10% trichloroacetic acid (TCA) to a final concentration of 5%. Inorganic phosphate (Pi) was measured at 630 nm using malachite green as the colorimetric reagent and KH_2PO_4 as standard for the calibration curve. Controls were carried out to correct for nonenzymatic hydrolysis by adding enzyme preparation after TCA addition. Enzyme specific activities were reported as nmol Pi released min^{-1} mg^{-1} of protein. All assays were performed in triplicate using PPi as substrate at a final concentration of 3.0 mM.

Protein determination: In all the enzyme preparations, protein was determined following the method of Bradford (1976) using bovine serum albumin.

Statistical analysis: Data were submitted to variance analyses and treatment means compared by Tukey's range test at 5% of error probability. Treatments were presented as mean ± S.D. of three replicates.

Results

Chlorophyll and carotenoid concentrations: After 10 d in a split-root system, a significant decrease in the chlorophyll concentration was observed in the Al-sensitive clone (Macaca) both when plants were treated at 100 mg Al L^{-1} in both halves of the root system (100/100; decrease of 40%) and with Al supplied to only half of the root system at 200 mg L^{-1} (0/200; decrease of 34%) (Fig. 1A). On the other hand, no significant difference was observed in chlorophyll concentration in the Al-tolerant clone (SMIC148-A) exposed to varying Al concentrations (Fig. 1B).

In the Al-sensitive clone, there was no alteration in carotenoid concentration at any Al concentration (Fig. 1C). In the Al-tolerant clone, it increased significantly, by about 60%, both when only one root half of the plant was supplied with Al at 100 mg L^{-1} (0/100) and when both sides of the root system were supplied (100/100 mg Al L^{-1}) (Fig. 1D). Moreover, plants in which only half of the root system was treated with 200 mg Al L^{-1} (0/200) showed a slight, but not significant, increase in carotenoid concentration.

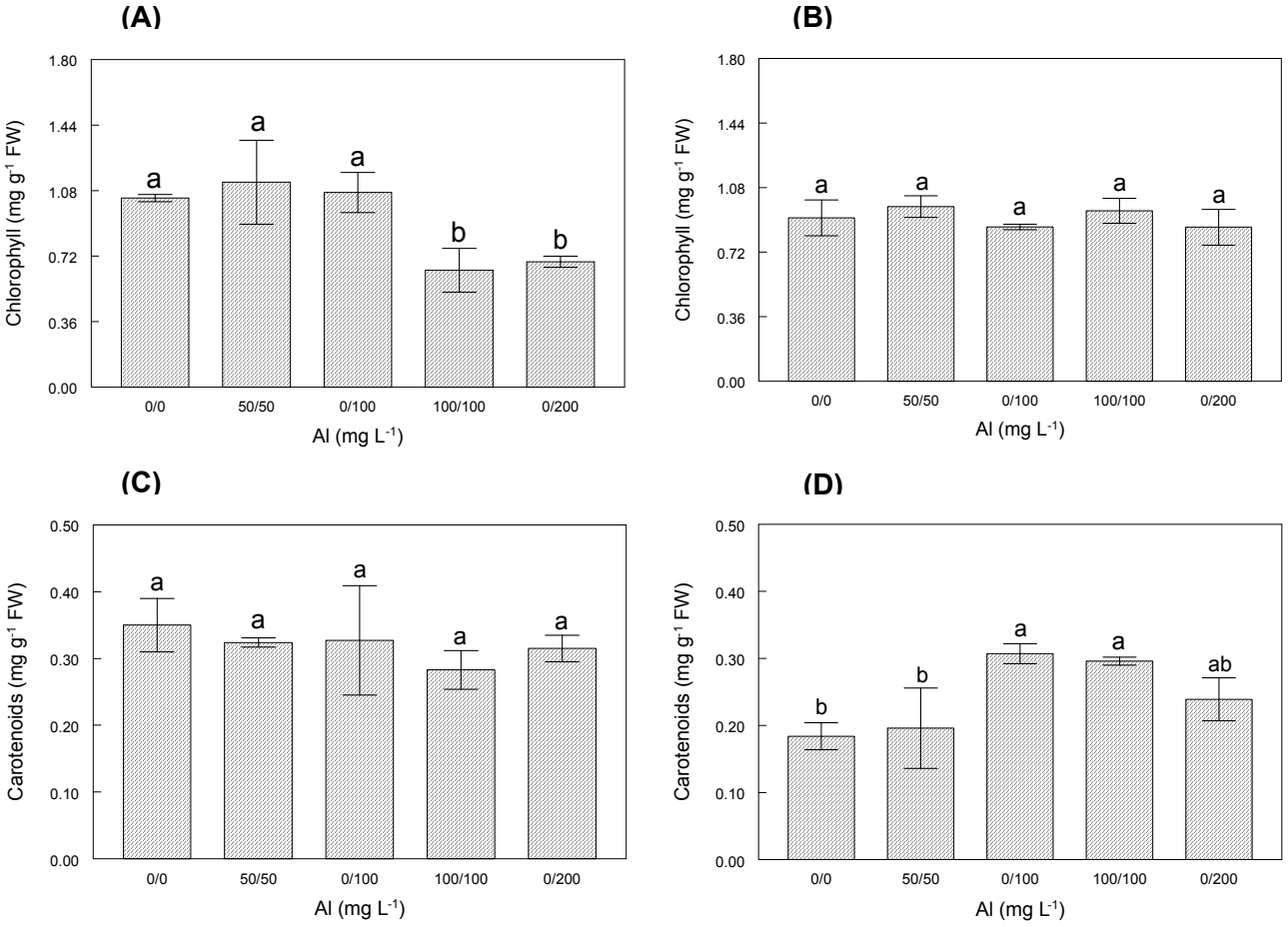

Figure 1. Effect of varying Al concentrations on chlorophyll concentration of the (A) Al-sensitive (Macaca) and (B) Al-tolerant (SMIC148-A) clones, and carotenoid concentration of the (C) Al-sensitive (Macaca) and (D) Al-tolerant (SMIC148-A) clones in a split-root system. Data are means ± SD at $p<0.05$, Tukey test.

Catalase activity and protein oxidation: In general, shoot catalase (CAT) activity was greater for the Al-sensitive clone than for the Al-tolerant clone. In the Al-sensitive clone, shoot CAT activity was significantly decreased in the treatments with both sides of the root system exposed to Al (50/50 and 100/100 mg L^{-1}) (Fig. 2A), whereas for plants where Al was supplied to only half of the root system (0/100 and 0/200 mg Al L^{-1}), it was slightly, but not significantly, reduced when compared to the control plants

(0/0 mg Al L^{-1}). In the Al-tolerant clone, CAT activity decreased when plants were supplied either with 100 mg Al L^{-1} or 200 mg Al L^{-1} to only half of the root system (0/100 and 0/200), as well as when both halves of the root system were treated with 100 mg Al L^{-1} (100/100) (Fig. 2B). In addition, plants in which both halves of the root system were treated with 50 mg Al L^{-1} (50/50) showed a slight, but not significant, reduction in CAT activity when compared to the control plants.

A significant increase in shoot carbonyl concentration (i.e. protein oxidation) was only observed in the Al-sensitive clone at all Al concentrations (Fig. 2C). The highest increase in protein oxidation (about 114%) was seen in the treatments with both halves of the root system exposed to 100 mg Al L^{-1} (100/100) and when only half of the root system (0/200) was supplied with 200 mg Al L^{-1}. In the Al-tolerant clone, no significant difference was observed in shoot protein oxidation when the roots were either completely or partly exposed to Al, as compared to the control (0/0) (Fig. 2D). Interestingly, the basal level of protein oxidation was significantly lower for this clone than for the Al-sensitive clone.

Hydrogen peroxide concentration: In general, shoot H_2O_2 concentration was higher in the Al-sensitive clone (Fig. 3A) than in the Al-tolerant clone (Fig. 3B). In both clones, an increase in shoot H_2O_2 concentration was found both upon supply of Al to only one half of the root system and to the entire root system. When both sides of the root system were treated with 100 mg Al L^{-1} (100/100), an increase of about 150% and 101% in shoot H_2O_2 concentration was observed in the Al-sensitive and Al-tolerant clones, respectively, while in the treatment where plants were supplied with 200 mg Al L^{-1} to only half of the root system (0/200), an increase of about 122% and 28% was found in the Al-sensitive and Al-tolerant clones, respectively.

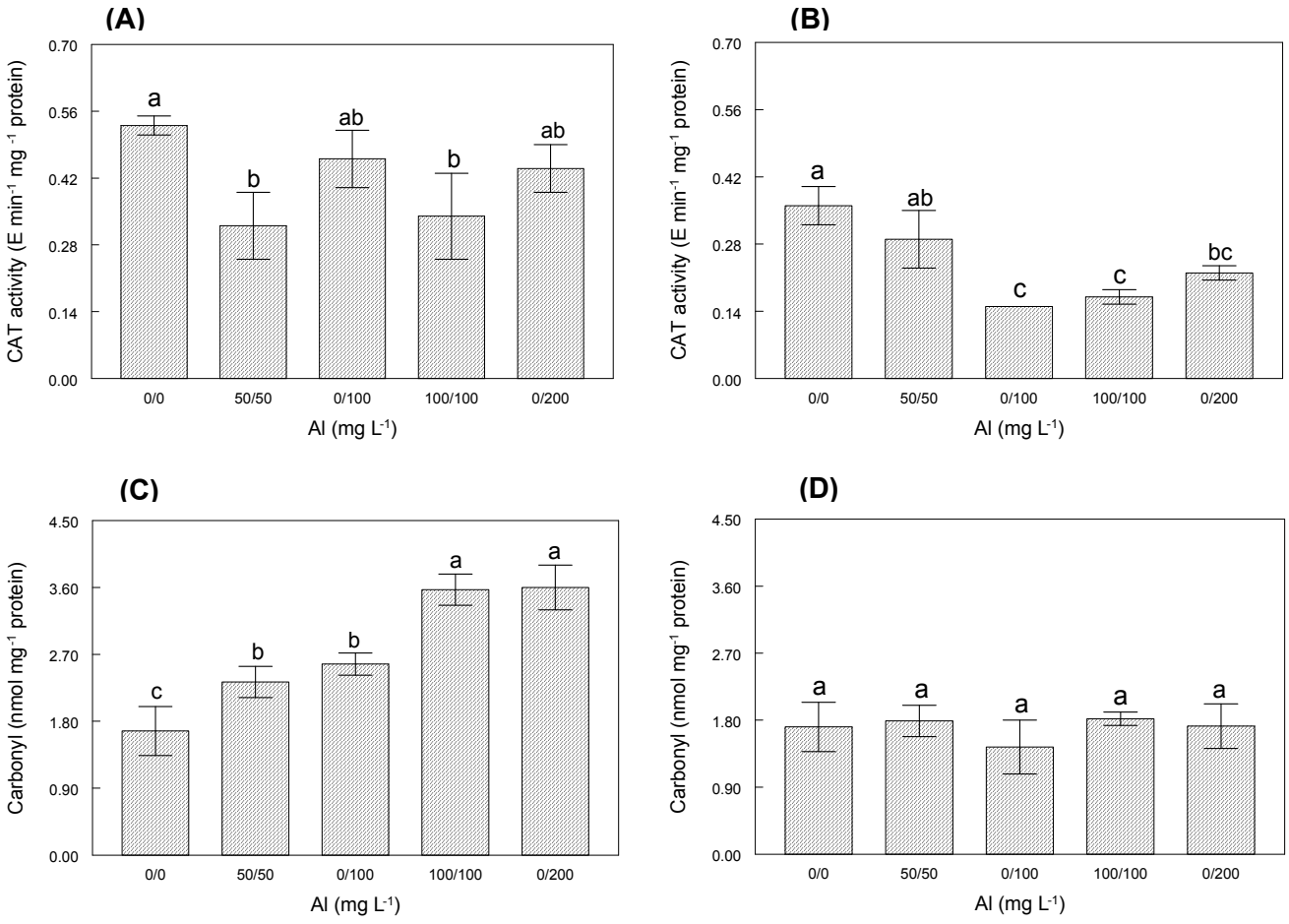

Figure 2. Effect of varying Al concentrations on shoot catalase (CAT) activity of the (A) Al-sensitive (Macaca) and (B) Al-tolerant (SMIC148-A) clones, and protein oxidation of the (C) Al-sensitive (Macaca) and (D) Al-tolerant (SMIC148-A) clones in a split-root system. Data are means ± SD at $p<0.05$, Tukey test.

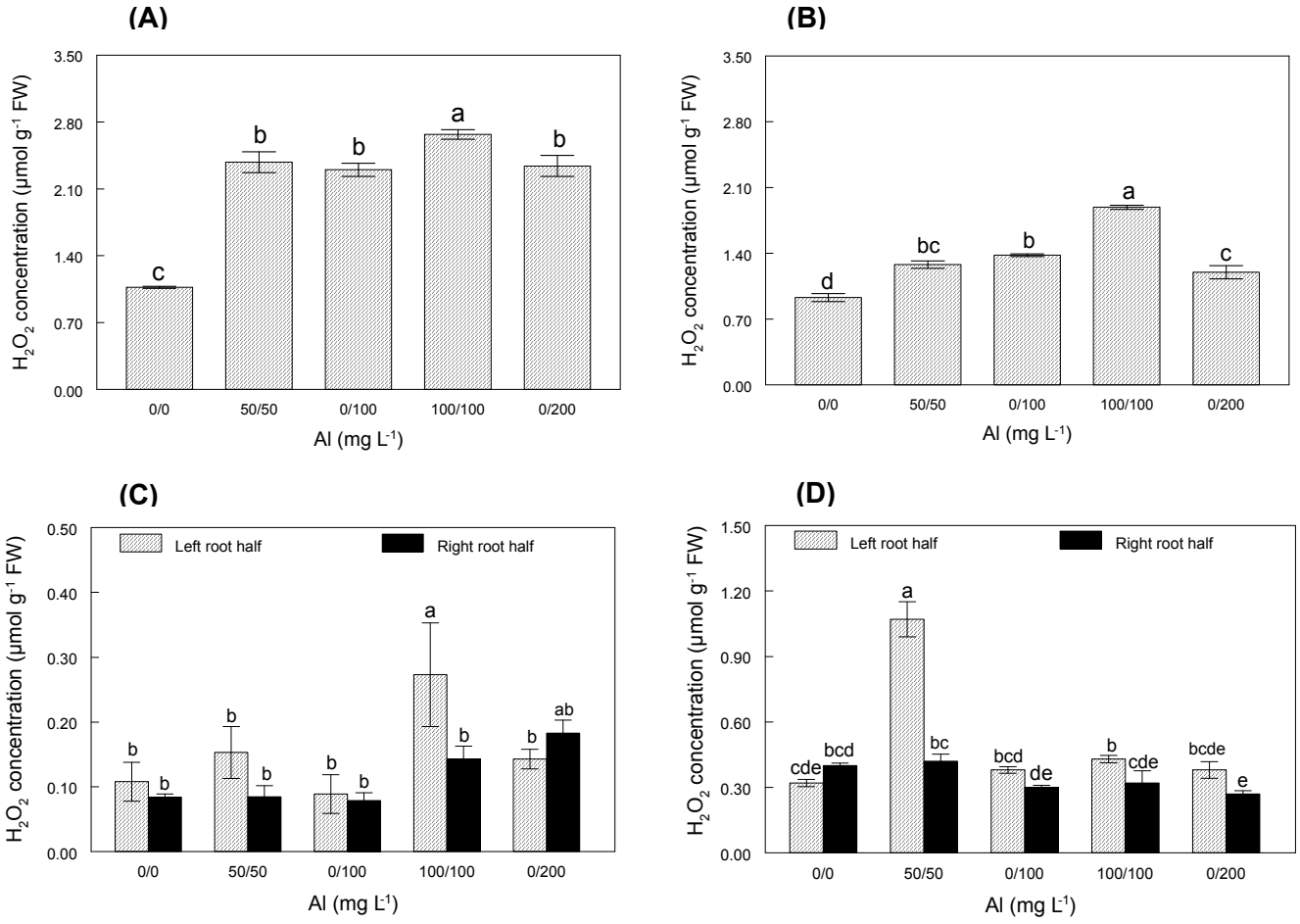

Figure 3. Hydrogen peroxide concentration in shoot of the (A) Al-sensitive (Macaca) and (B) Al-tolerant (SMIC148-A) clones, and roots of the (C) Al-sensitive (Macaca) and (D) Al-tolerant (SMIC148-A) clones under varying Al concentrations in a split-root system. Data are means ± SD at $p<0.05$, Tukey test.

In contrast to that observed in shoot, root H_2O_2 concentration was greater in the Al-tolerant clone than in the Al-sensitive clone. Root H_2O_2 concentration in the Al-sensitive clone increased in one root half of plants supplied with Al at 100 mg L^{-1} to both root halves (100/100) (Fig. 3C). In addition, in plants supplied with Al at 200 mg L^{-1} to only one half of the root system (0/200), root H_2O_2 concentration was slightly, but not significantly, increased in that root half treated by Al, when compared to the control plants (0/0). In roots of the Al-tolerant clone, a significant increase in H_2O_2

concentration was only observed in one root half of plants supplied with Al at 50 mg L^{-1} to both root halves (50/50) (Fig. 3D).

Lipid peroxidation: The basal level of shoot MDA concentration (i.e. lipid peroxidation) in the Al-tolerant clone was higher than in the Al-sensitive clone. On the other hand, a greater increase in shoot lipid peroxidation in response to Al treatment was observed in the Al-sensitive clone. A significant increase in shoot lipid peroxidation in the Al-sensitive clone was observed either by applying Al to only one half of the root system (0/100 and 0/200) or when the entire root system (50/50 and 100/100) was exposed to Al (Fig. 4A). The highest increase in lipid peroxidation (of about 162%) was seen in the Al-sensitive clone when Al was supplied to both sides of the root system at 100 mg L^{-1} (100/100). On the other hand, in the Al-tolerant clone, a significant increase in shoot lipid peroxidation (about 40%) was observed only in treatments with both sides of the root system exposed to Al (50/50 and 100/100), when compared to the control plants (Fig. 4B).

In roots of the Al-sensitive clone, lipid peroxidation increased significantly in both halves of the root system supplied with 100 mg Al L^{-1} (100/100) and in one half of the root treated with 200 mg Al L^{-1} (0/200) (Fig. 4C). In the Al-tolerant clone, a significant increase in root lipid peroxidation was only observed in one half of the root in all Al treatments (50/50, 0/100, 100/100 and 0/200) (Fig. 4D).

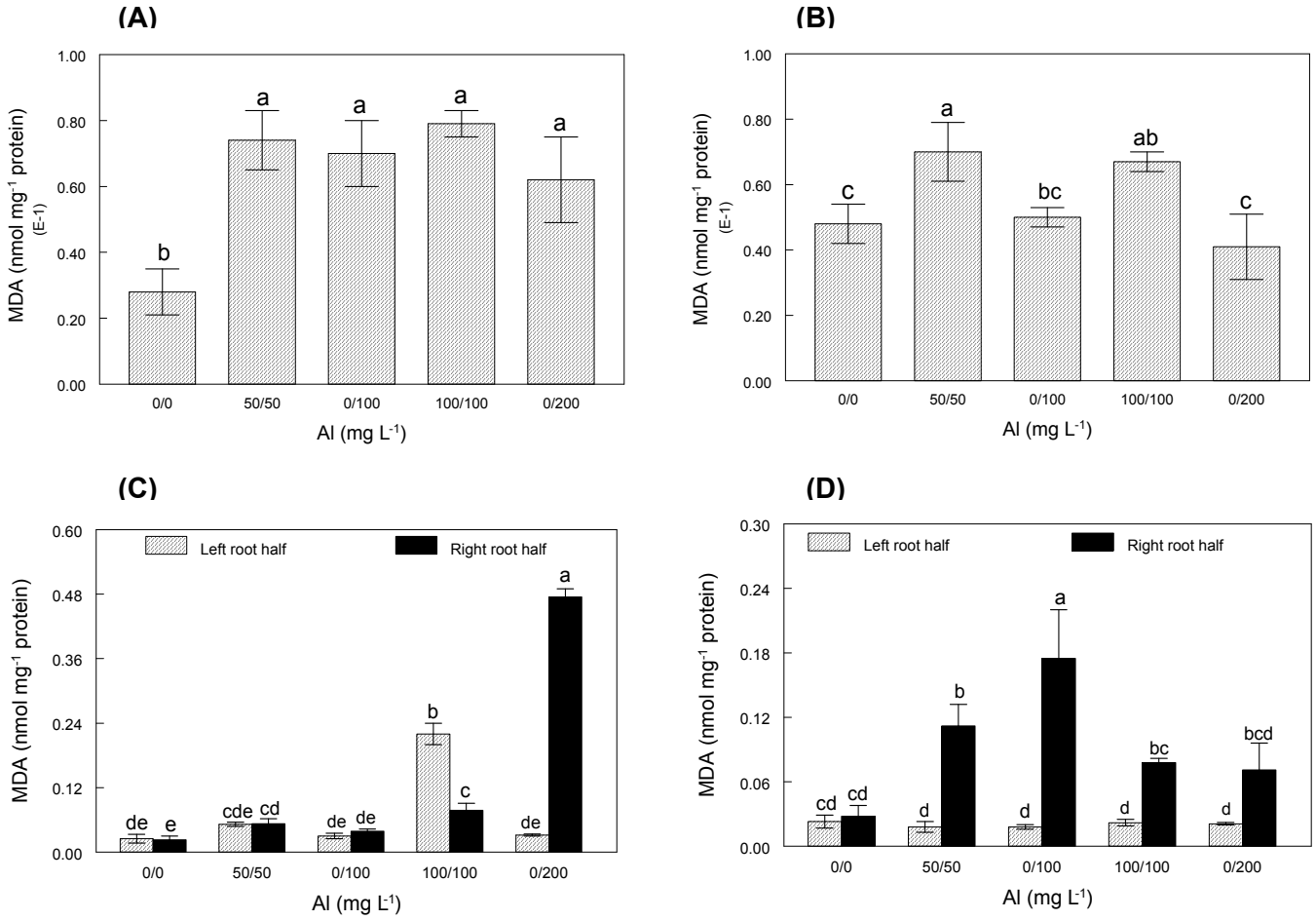

Figure 4. Lipid peroxidation in shoot of the (A) Al-sensitive (Macaca) and (B) Al-tolerant (SMIC148-A) clones, and roots of the (C) Al-sensitive (Macaca) and (D) Al-tolerant (SMIC148-A) clones under varying Al concentrations in a split-root system. Data are means ± SD at $p<0.05$, Tukey test.

Non-protein thiol groups (NPSH) concentration: In the Al-sensitive clone, a significant increase in shoot NPSH concentration was observed upon supply of Al both to one half of the root system and to the entire root system (Fig. 5A). The greatest increases in NPSH concentration, of about 207% and 153%, were seen when Al was supplied to both sides of the root system at 100 mg L^{-1} (100/100) and to only one half of the root system at 200 mg L^{-1} (0/200), respectively. In the Al-tolerant clone (Fig. 5B),

shoot NPSH concentration increased significantly (about 100%) only in the treatments with both root halves exposed to Al (50/50 and 100/100).

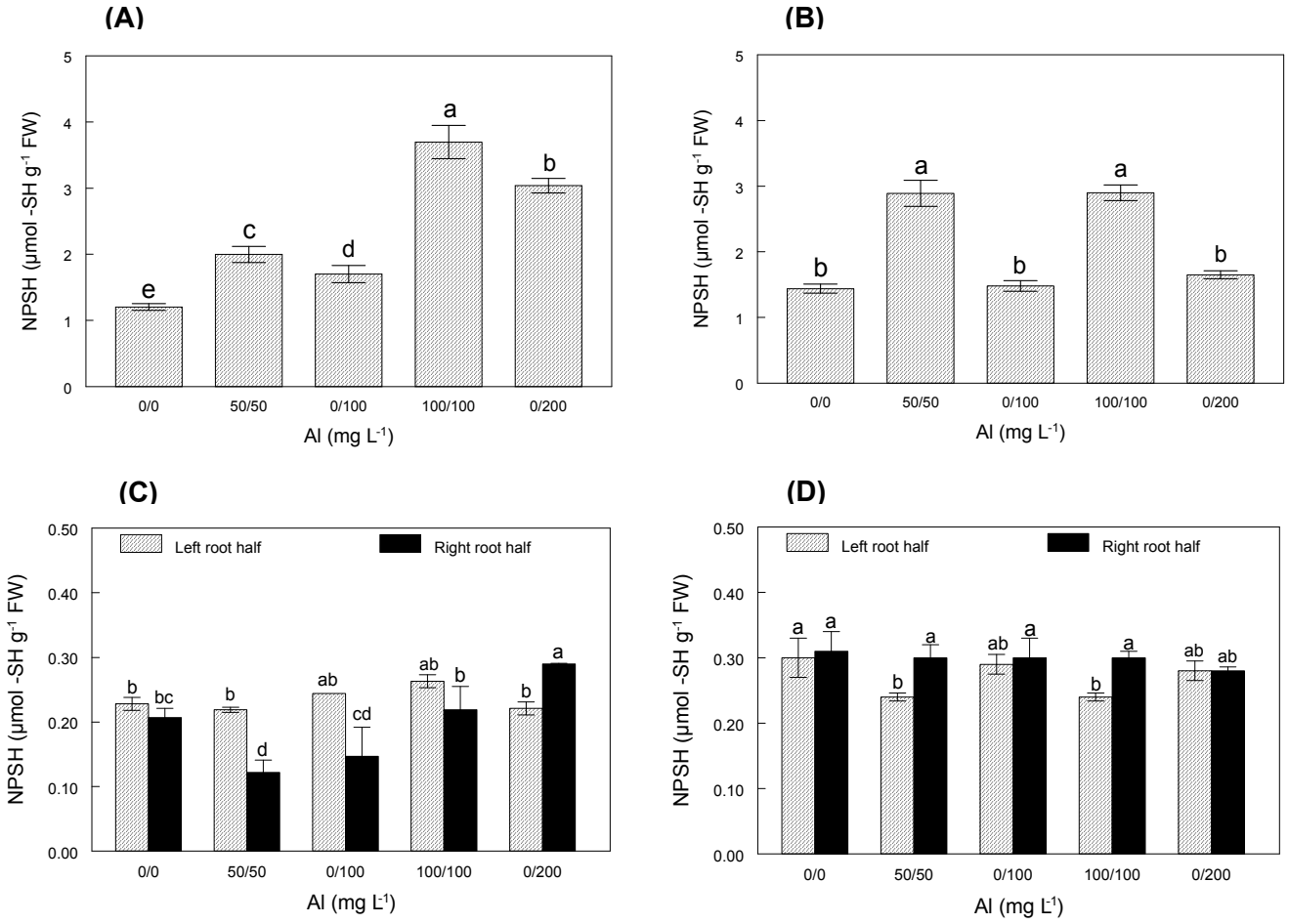

Figure 5. Non-protein thiol group (NPSH) concentration in shoot of the (A) Al-sensitive (Macaca) and (B) Al-tolerant (SMIC148-A) clones, and roots of the (C) Al-sensitive (Macaca) and (D) Al-tolerant (SMIC148-A) clones under varying Al concentrations in a split-root system. Data are means ± SD at $p<0.05$, Tukey test.

In roots of the Al-sensitive clone, NPSH concentration decreased significantly in the right root half of the 50/50 and 0/100 treatments. On the other hand, in the 0/200 treatment, a significant increase in NPSH concentration was observed in the root half supplied at 200 mg Al L^{-1} (Fig. 5C). In the Al-tolerant clone, NPSH concentration

decreased significantly only in one half of the root supplied with 50/50 and 100/100 (Fig. 5D).

Acid phosphatase (APases) activity: In roots and shoot of potato plants, the basal activity of APases in the Al-tolerant clone was higher than in the Al-sensitive clone. A significant decrease (about 21%) in shoot APase activity was observed in the Al-sensitive clone only when plants were supplied with 200 mg Al L^{-1} to half of the root system (0/200) (Fig. 6A). On the other hand, in the Al-tolerant clone, shoot APase activity decreased in all Al treatments, either by applying Al only to one half of the root system or when the entire root system was exposed to Al (Fig. 6B).

In roots of both clones, in general, an inhibition of APase activity was observed in all Al treatments, but this effect was less pronounced in the Al-tolerant clone (Fig. 6C and 6D). Plants of the Al-sensitive clone which had only half of the root system exposed to Al (treatments 0/100 and 0/200 mg Al L^{-1}) showed lower APase activity in that root half treated by Al. Moreover, plants with both root halves exposed to Al at 100 mg L^{-1} presented reduced APase activity in both root halves, whereas plants treated with Al at 50 mg L^{-1} (50/50) showed a decrease in APase acitivity only in one root half, when compared to the control.

In the Al-tolerant clone, root APase activity was either reduced at least in one root half exposed to Al (treatments 50/50 and 100/100) or in both root halves (treatments 0/100 and 0/200), when compared to the control. Moreover, the 200 half of 0/200 plants presented significantly higher root APase activity than that half untreated with Al, whereas the 0 half of 0/100 plants presented higher root APase activity than did the 100 side.

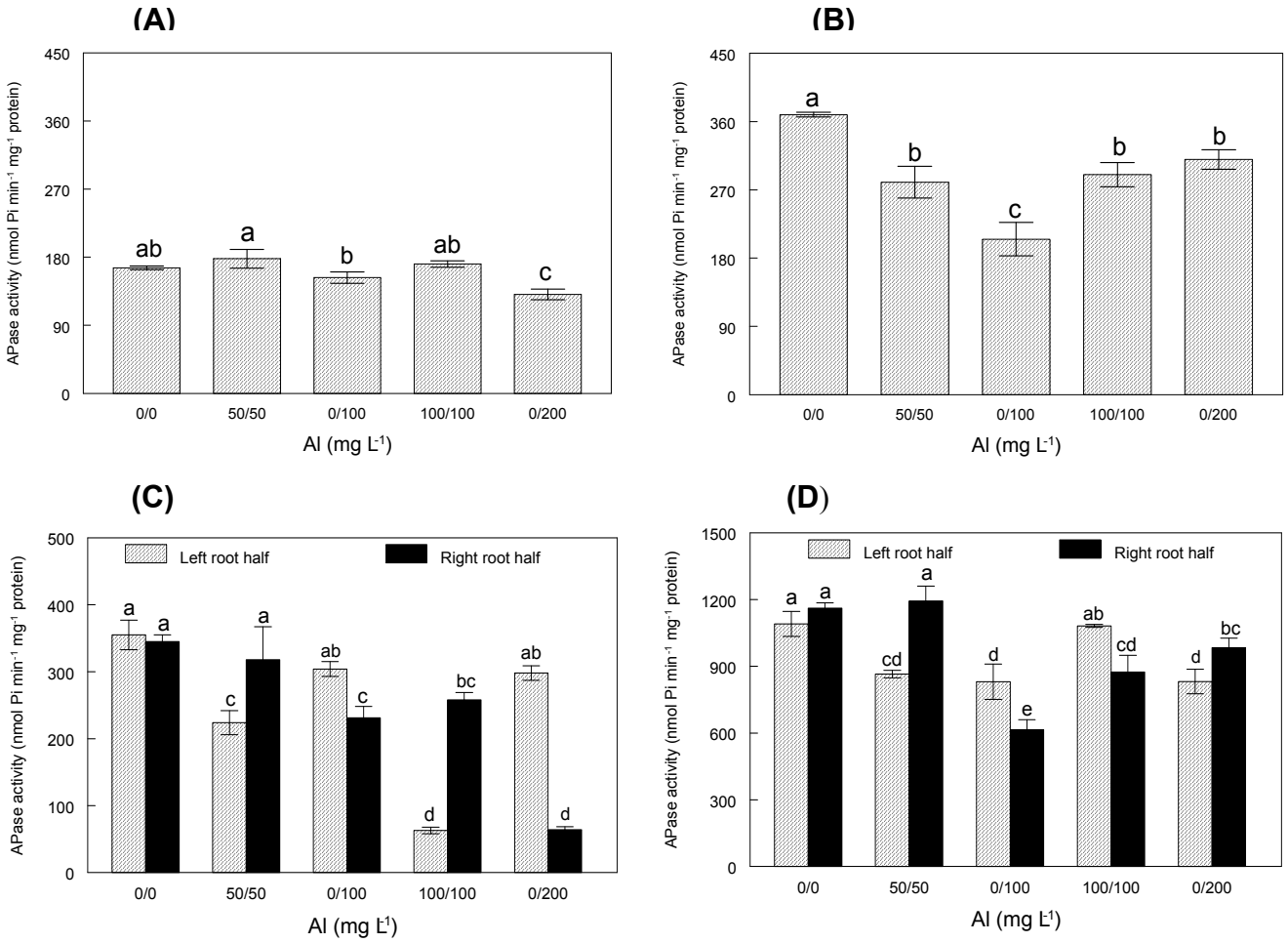

Figure 6. Effect of varying Al concentrations on acid phosphatase (APases) activity in shoot of the (A) Al-sensitive (Macaca) and (B) Al-tolerant (SMIC148-A) clones, and roots of the (C) Al-sensitive (Macaca) and (D) Al-tolerant (SMIC148-A) clones in a split-root system. Data are means ± SD at $p<0.05$, Tukey test.

Discussion

Growth inhibition, which is a physiological response to acidic soils, especially due to aluminum (Al) toxicity, is a serious problem for the production of most important crops in the world. Some screening techniques can be used to readily quantify the response of different species or different clones of the same species towards environmental stresses. In a previous study, utilizing a homogeneous supply of Al to the roots of potatoes grown in a hydroponic growth system and based on relative root growth, it was demonstrated that there was a significant difference in Al tolerance among the four potato clones. In addition, it was shown that Al treatment induced oxidative stress, mainly in the Al-sensitive clones (Tabaldi et al., 2007b). Hairiah et al. (1993) observed that the response of root growth for *Mucuna pruriens* to the presence of Al in its environment was positive when no other choice was given, and negative in the absence of Al around other parts of the same root system. This relative Al avoidance was related to the response of local P sources of plants with an overall insufficient P supply. This hypothesis is actually based on internal P shortage in Al-exposed roots, due to precipitation of Al phosphates. In the present study, to avoid the interaction between P and Al in the nutrient solution, the experimental setup determined that plants be grown for about 4 weeks in the presence of 250 μM of P, and, subsequently, during the Al exposure (for 10 days), P was omitted from the nutrient solution. In a previous experiment, we observed that potato plants that were very well nourished with P could withstand 10 days in the absence of P in the nutrient solution without showing visible symptoms of P deficiency. In this study, calculations with 'Visual MINTEQ' showed that 83-90% of the nominal Al concentration (based on initial ion concentration) are in the monomeric form (Supplement A).

Based on the results, a stronger visible (based on biomass) Al avoidance was seen in the 0/100 and 0/200 plants of the Al-sensitive clone (Macaca) than in the Al-tolerant clone (SMIC148-A) (Fig. 7 and 8). The 0 side of 0/100 and 0/200 plants showed a significantly higher fresh biomass than that half of the root system supplied with Al. Moreover, shoot growth of the Al-sensitive clone was significantly affected by the Al treatments, while it was slightly decreased in the Al-tolerant clone. The nutrient solutions were renewed every 48 h and the pH of the nutrient solutions was evaluated at 24 h intervals. From the onset of the Al treatments, the pH of the nutrient solutions decreased similarly for both potato clones upon presence of Al. However, in general, within 10 d of Al treatment, the difference in solution pH between root halves in 0/100 and 0/200 was less pronounced for the Al-tolerant clone (Supplement B). Interestingly, in these treatments, at 10 d of Al exposure, that root half of the Al-tolerant clone supplied with either 100 or 200 mg Al L^{-1} showed higher capacity to buffer the pH of the nutrient solution (Supplement B).

Changes in the degree of lipid, protein and pigment oxidation, in the concentration of non-enzymatic antioxidants and in the activity of antioxidant enzymes are symptomatic of the plant oxidative stress response in relation to several biotic and abiotic factors (Smirnoff, 1993). In the present study, potato plants grown under varying Al concentrations revealed signs of oxidative stress. After 10 days of Al exposure in a split-root system, Al supply at 100 mg L^{-1} in both halves of the root system (100/100) and at 200 mg L^{-1} in only one root half (0/200) resulted in a significant decrease in the shoot chlorophyll concentration

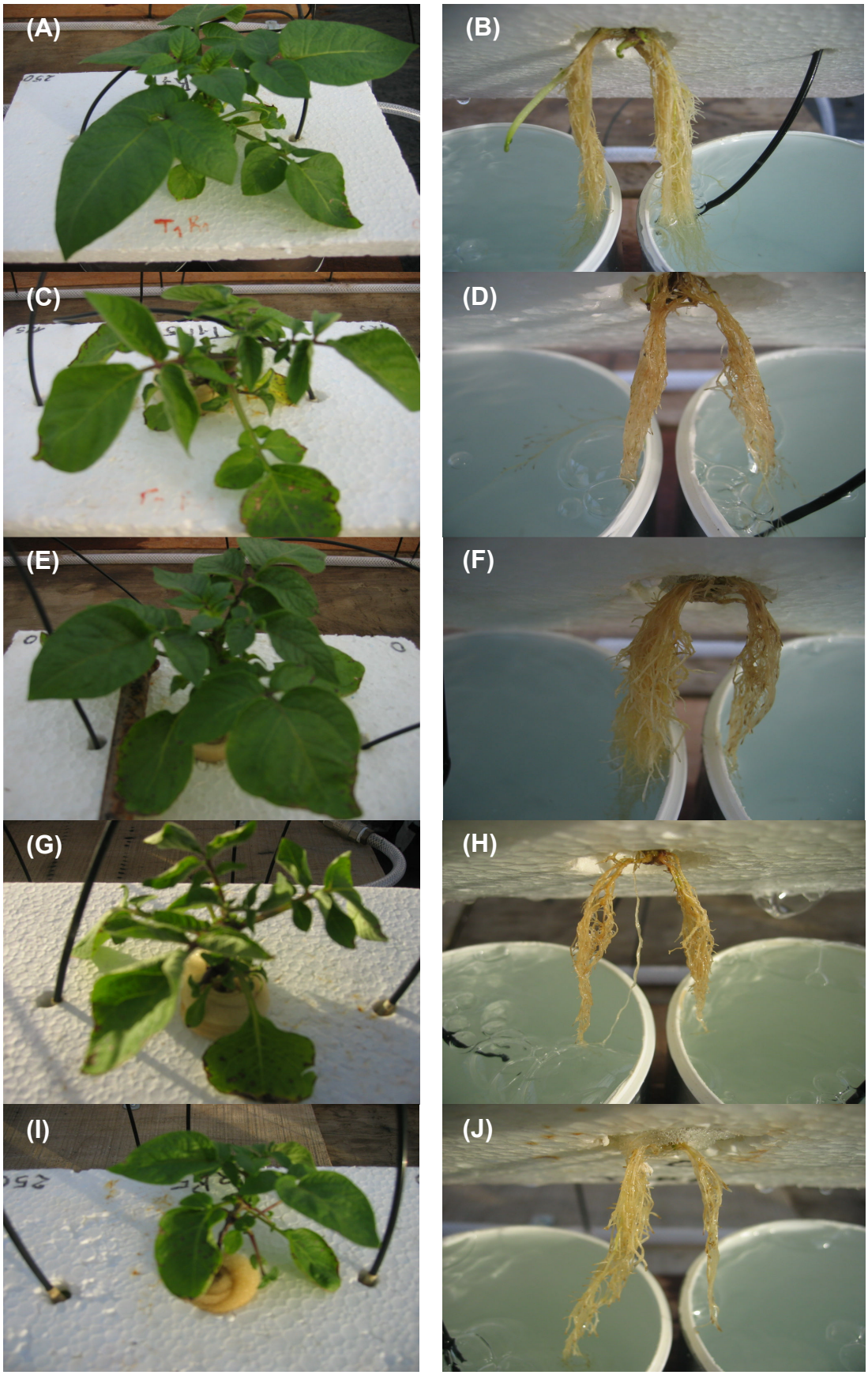

Figure 7 – Shoot and roots of potato plants, Macaca clone (Al-sensitive), exposed to 0/0 mg Al L^{-1} (A,B), 50/50 mg Al L^{-1} (C,D), 0/100 mg Al L^{-1} (E,F), 100/100 mg Al L^{-1} (G,H) and 0/200 mg Al L^{-1} (I,J), in split-root system.

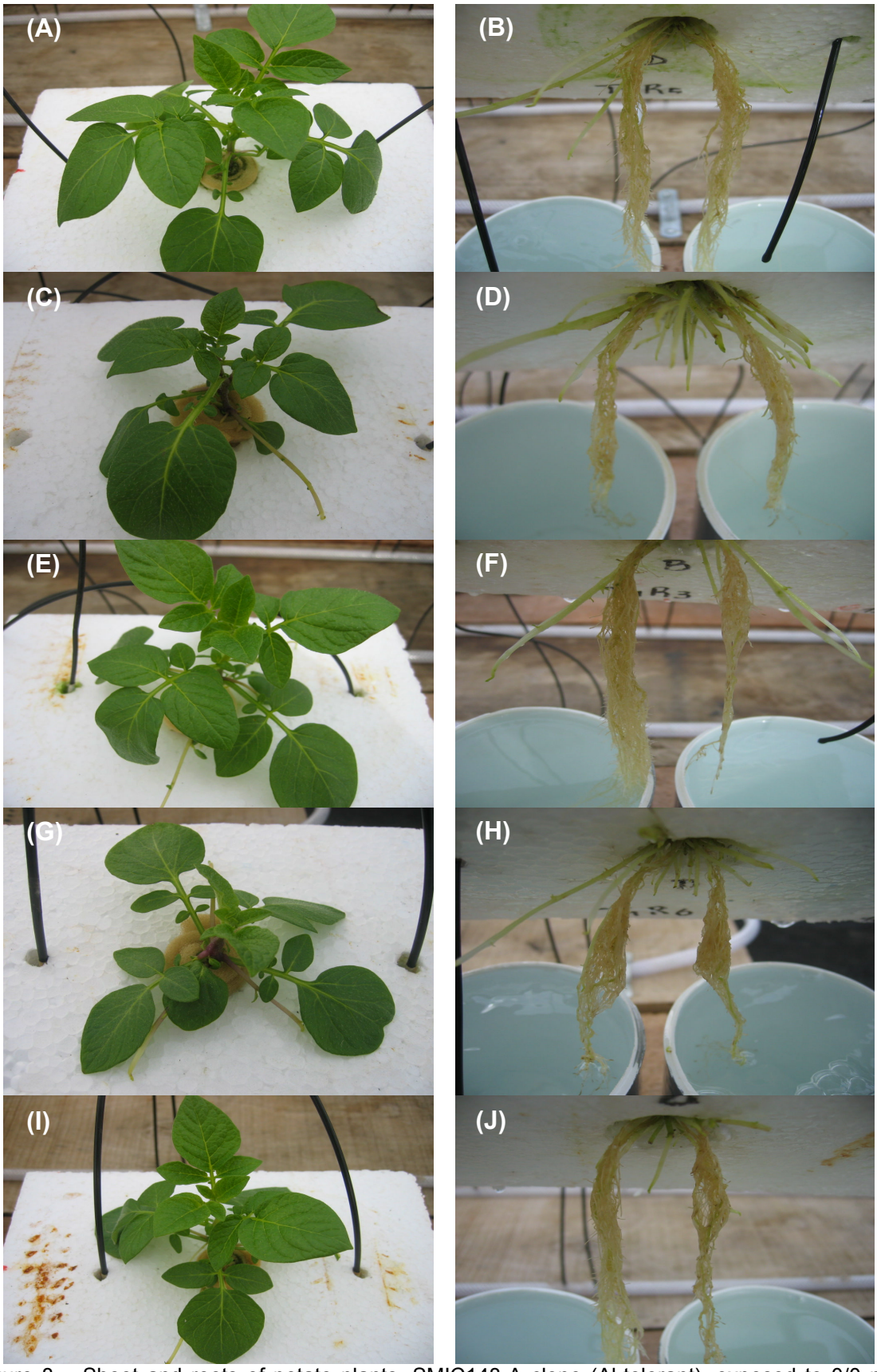

Figure 8 – Shoot and roots of potato plants, SMIC148-A clone (Al-tolerant), exposed to 0/0 mg Al L^{-1} (A,B), 50/50 mg Al L^{-1} (C,D), 0/100 mg Al L^{-1} (E,F), 100/100 mg Al L^{-1} (G,H) and 0/200 mg Al L^{-1} (I,J), in split-root system.

only for the Al-sensitive clone (Fig. 1A). In contrast, in the Al-tolerant clone, no significant difference in chlorophyll concentration was observed (Fig. 1B). This decrease in the chlorophyll concentration in the Al-sensitive clone demonstrates the poisoning effect of high concentrations of Al. According to Smirnoff (1993) and Brito et al. (2003), the decrease in chlorophyll concentration is a typical symptom of oxidative stress and may either be the result of chlorophyll degradation or lower chlorophyll synthesis associated with changes in the thylakoid membrane structure. Carotenoids, besides acting as accessory light harvesting pigments, show antioxidant properties (de Pascale et al., 2001). In the present study, the increase in carotenoid concentration with increasing Al supply observed only for the Al-tolerant clone (Fig. 1D) suggests that this clone has greater potential for detoxification of toxic oxidation radicals formed in response to Al treatments.

H_2O_2 is a toxic reactive oxygen species (ROS), which has deleterious effects on plant tissue (Salin, 1988). For both potato clones tested, shoot H_2O_2 concentration increased in a similar pattern upon Al treatment. This effect was dependent on the Al concentration and, to a lesser degree, on the distribution of roots exposed to Al. At the highest Al concentration supplied (200 mg L^{-1}) to only half of the root system (0/200), shoot H_2O_2 concentration was lower than that with both root halves treated by 100 mg L^{-1} (100/100). However, this effect was less pronounced in the Al-sensitive clone. Therefore at levels of Al that are toxic to potato, based on shoot chlorophyll concentration (Fig. 1A), the amount of root exposed to Al was well correlated with several biochemical alterations linked to oxidative stress.

Shoot CAT activity in the Al-sensitive clone was significantly reduced in the treatments with both sides of the root system exposed to Al (50/50 and 100/100) when compared to treatments where Al was supplied to only one root half (0/100 and 0/200

mg L^{-1}). This result was correlated with the shoot H_2O_2 concentration. The decreased activity of the H_2O_2 scavenging enzyme CAT upon Al treatment might have contributed to the observed increases in shoot H_2O_2 concentration. Thus, a decrease in shoot CAT activity would result in accumulation of H_2O_2, which can react with O_2^- to produce hydroxyl-free radicals via the Herbert–Weiss reaction (Bowler et al., 1992). The hydroxyl-free radicals can directly damage the membrane by attacking unsaturated fatty acids of lipids to induce lipid peroxidation (Okuda et al., 1991). During oxidative stress, H_2O_2 is a strong toxic oxidant causing cell damage or even cell death and can also contribute to the carbonylation of proteins (Bienert et al., 2006). The decrease, at least, in CAT activity could be due to the blocking of essential functional groups in the enzyme such as –SH or the displacement of essential metal ions from enzymes, as suggested for other metals (Schützendübel and Polle, 2002).

It is worthy to note that plants respond to Al stress by various antioxidant mechanisms, including the enzymatic ROS-scavenging system and by non-enzymatic antioxidants, which function to interrupt the cascades of uncontrolled oxidation in each organelle. In a previous study (Tabaldi et al., unpublished), it was observed that in roots of the SMIC148-A potato clone (Al-tolerant), the inadequate response of CAT activity to Al was compensated by the increased activity of ascorbate peroxidase. Moreover, the enzymatic and non-enzymatic (acid ascorbic and non-protein thiol group concentrations) antioxidants seem to compensate for each other.

Interestingly, in the 100/100 and 0/200 mg Al L^{-1} treatments, the Al-sensitive potato clone showed a significant increase in H_2O_2 concentration in only one part of the root system (right root half in the 100/100 treatment) when compared to the control plants. Such an effect could be related to an uneven split of roots used in the split-root experiment, even though care was taken in the division of the root system. Moreover,

root H_2O_2 concentration was slightly, but not significantly, increased in the root half treated by 200 mg Al L^{-1} (treatment 0/200). These data suggest that, despite the fact that the potato roots accumulate more Al than does the shoot (Tabaldi et al., 2007b), some antioxidant systems in the roots were more efficient than those of shoot in scavenging Al stress side effects. In addition, in those Al treatments (100/100 and 0/200) where shoot chlorophyll concentration was decreased in the Al-sensitive clone, the H_2O_2 concentration in the roots seemed to respond locally to Al supply.

Protein carbonylation is one of the markers of oxidative stress that results from excessive production of ROS in the cell which is not balanced by an increased efficiency of the antioxidant system (Juszczuk et al., in press). Although the basal level of root H_2O_2 concentration was higher for the Al-tolerant clone than for the Al-sensitive clone (Fig. 3), the former showed no alteration in shoot protein oxidation at any Al concentration (Fig. 2). On the other hand, in the Al-sensitive clone, shoot protein oxidation was strongly correlated to Al concentrations in the nutrient solution rather than to the distribution of roots exposed to Al.

Independently of the Al concentration and distribution of roots exposed to Al treatments, shoot MDA concentration (i.e. lipid peroxidation) significantly increased in the Al-sensitive clone, even in those treatments (50/50 and 0/100) which did not alter the shoot chlorophyll concentration. However, root lipid peroxidation presented a different pattern, where it increased upon Al supply either with both root halves exposed at 100 mg Al L^{-1} (100/100) or only with one root half exposed at 200 mg Al L^{-1} (0/200). This indicates that Al toxicity of one half of the roots resulted in a local response. In the Al-tolerant clone, the effect of Al on shoot lipid peroxidation was dependent on the concentration and location of Al. Plants treated with 100 and 200 mg Al L^{-1} in only one root half (0/100 and 0/200) showed lower shoot lipid peroxidation than

those which had both root halves treated by Al at concentrations of 50 (50/50) and 100 (100/100) mg L^{-1}. Such data suggest, to a certain degree, that potato roots responded locally to Al stress, whereas the shoot showed a systemic response.

The major pool of non-protein thiol groups (NPSH) in most plant species is represented by reduced glutathione. NPSH are known to be affected by the presence of several metals (Xiang and Oliver, 1998). In the present study, the effect of Al on shoot NPSH concentration in both potato clones (Fig. 5A, 5B) showed a pattern similar to that shown for H_2O_2 concentration (Fig. 3A, 3B). However, the increase observed in shoot NPSH concentration in the Al-tolerant clone (Fig. 5B) seemed to have a higher correlation with lipid peroxidation (Fig. 4B) than that presented for the Al-sensitive clone. The increase in NPSH concentration is associated with tolerance against several stresses (Agrawal and Rathore, 2007), and they may function as reducers of oxidative damage (Ali et al., 2005). Therefore, the antioxidant system of the Al-sensitive clone was less efficient to remove the excess of ROS than that of Al-tolerant clone.

In the Al-tolerant clone, the Al treatment which caused the highest negative effect on both shoot and root APase activity was that with only one root half exposed at 100 mg Al L^{-1} (0/100). Although the root half not exposed to Al showed higher APase activity than that treated with 100 mg Al L^{-1}, the levels of APase activities were lower than those of the control plants. Therefore, this result suggests that roots of potato responded both locally and systemically to Al stress. This assumption is corroborated by the effect of other Al treatments, suggesting that the Al transported to the shoot might be retranslocated to roots not treated by Al. This hypothesis should be further tested.

The expectation that the Al-tolerant clone (SMIC148-A) would be more tolerant to Al than the Al-sensitive clone (Macaca) was confirmed by the results. At 100 mg Al

L^{-1} supplied to both root halves, the Al-sensitive clone showed an inefficient tolerance response, based on tissue CAT activity, protein oxidation, lipid peroxidation, H_2O_2 concentration and APase activity. In contrast, the Al-tolerant clone showed a comparatively small negative effect on both shoot and root biochemical parameters at this concentration. However, an apparently significant Al-avoidance reaction was presented, though less pronounced than for the Al-sensitive clone at the higher Al concentrations. The higher Al tolerance of SMIC148-A might be related to a more efficient oxidative scavenging capacity and the higher basal APase activity level than that found in Macaca. Acid phosphatases are a group of enzymes involved in the production, transport and recycling of inorganic phosphate (Yoneyama et al., 2007). In natural ecosystems, P availability is seldom optimal for plant growth because of limited P content in the soil solution. Aluminum and iron ions in acid soils interact strongly with P and render it unavailable to plants (Sousa et al., 2007). Indeed, Hairiah et al. (1993) observed that Al avoidance for *Mucuna pruriens* and *Centrosema pubescens* was related to a response to local P sources in plants with an overall insufficient P supply. Moreover, the present study shows that the Al-tolerant potato clone presented a stronger local and systemic antioxidant response to Al supply. Therefore, the SMIC148-A clone, which is more Al-tolerant than the Macaca clone, might show a deeply rooted system in surroundings with toxic levels of Al.

References

Aebi H. 1984. Catalase *in vitro*. Methods Enzymology **105**, 121-126.

Agrawal SB, Rathore D. 2007. Changes in oxidative stress defense in wheat (*Triticum aestivum* L.) and mung bean (*Vigna radiata* L.) cultivars grown with and without mineral nutrients and irradiated by supplemental ultraviolet-B. Environmental and Experimental Botany **59**, 21-33.

Ali MB, Hahn E-J, Paek K-Y. 2005. Effects of temperature on oxidative stress defense systems, lipid peroxidation and lipoxygenase activity in *Phalaenopsis*. Plant Physiology and Biochemistry **4**3, 213-223.

Balestrasse KB, Gallego SM, Tomaro ML. 2006. Aluminium stress affects nitrogen fixation and assimilation in soybean (*Glycine max* L.). Plant Growth Regulation **48,** 271-281.

Barceló J, Poschenrieder C. 2002. Fast root growth responses, root exudates and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. Environmental and Experimental Botany **48**, 75-92.

Bienert GP, Schjoerring JK, Jahn TP. 2006. Membrane transport of hydrogen peroxide. Biochimica et Biophysica Acta **1758**, 994-1003.

Bowler CM, Montagu V, Inze D. 1992. Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology **43,** 83-116.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantity of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72**, 248-254.

Brito G, Costa A, Fonseca HMAC, Santos CVV. 2003. Response of *Olea europaea* ssp. maderensis *in vitro* shoots exposed to osmotic stress. Scientia Horticulturae **97**, 411-417.

Castro JD. 1983. Acidez e calagem para a batatinha (*Solanum tuberosum* L.). In: XV Reunião Brasileira de Fertilidade do Solo, Campinas/SP. Anais..., Campinas: Sociedade Brasileira de Ciência do Solo, 79-85.

Ciamporova M. 2002. Morphological and structural responses of plant roots to aluminium at organs, tissue and cellular levels. Biologia Plantarum **45**, 161-171.

de Pascale S, Maggio A, Fogliano V, Ambrosino P, Ritieni A. 2001. Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. The Journal of Horticultural Science & Biotechnology **76,** 447-453.

Ellman GL. 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics **82**, 70-77.

El-Moshaty FIB, Pike SM, Novacky AJ, Sehgal OP. 1993. Lipid peroxidation and superoxide production in cowpea (*Vigna unguiculata*) leaves infected with tobacco ringspot virus or southern bean mosaic virus. Physiology Molecular and Plant Pathology **43,** 109-119.

FAO. FAOSTAT. Agriculture. Rome. (http://faostat.fao.org/), 2004.

Feldman LJ. 1984. Regulation of root development. Annual Review of Plant Physiology **35,** 223-242.

Foy CD. 1988. Plant adaptation to acid, aluminum-toxic soils. Communications in Soil Science and Plant Analysis **19,** 959-987.

Guo TR, Zhang GP, Zhang YH. 2007. Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. Colloids and Surfaces B: Biointerfaces **57,** 182-188.

Hairiah K, Van Noordwijk M, Stulen I, Meijboom FW, Kuiper PJC. 1993. Phosphate nutrition effects on aluminium avoidance of *Mucuna pruriens var. utilis*. Environmental and Experimental Botany **33**, 75-83.

Hiscox JD, Israelstam GF. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany **57,** 1132-1334.

Jemo M, Abaidoo RC, Nolte C, Horst WJ. 2007. Aluminum resistance of cowpea as affected by phosphorus-deficiency stress Journal of Plant Physiology **164,** 442-451.

Juszczuk IM, Tybura A, Rychter AM. 2007. Protein oxidation in the leaves and roots of cucumber plants (*Cucumis sativus* L.), mutant MSC16 and wild type. Journal of Plant Physiology, doi:10.1016/j.jplph.2007.06.021.

Kerley SJ, Leach JE, Swain JL, Huyghe C. 2000. Investigations into the exploitation of heterogeneous soils by *Lupinus albus* L. and *L. pilosus* Murr. and the effects upon plant growth. Plant Soil **222**, 241-253.

Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shaltiel S, Stadtman ER. 1990. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymology **186,** 464-478.

Li PH, Fennel A. 1985. Potato Frost Hardiness. In: Li PH, ed. *Potato Physiology*, Academic Press, Orlando, 457-479.

Lichtenthaler HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomemranes. Methods in Enzymology **148,** 350-382.

Loreto F, Velikova V. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiology **127,** 1781-1787.

Marschner H. 1996. “Mineral nutrition of higher plants”, 2nd Ed., Academic Press, London.

Salin ML. 1988. Toxic oxygen species and protective systems of the chloroplast. Physiologia Plantarum **72,** 681-689.

Schützendübel A, Polle A. 2002. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization. Journal of Experimental Botany **53,** 1351-1365.

Smirnoff N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist **125,** 27-58.

Sousa MF, Façanha AR, Tavares RM, Neto TL, Gerós H. 2007. Phosphate transport by proteoid roots of *Hakea sericea*. Plant Science **173,** 550-558.

Tabaldi LA, Ruppenthal R, Cargnelutti D, Morsch VM, Pereira LB, Schetinger MRC. 2007. Effects of metal elements on acid phosphatase activity in cucumber (*Cucumis sativus* L.) seedlings. Environmental and Experimental Botany **59,** 43-48.

Tabaldi LA, Nicoloso FT, Y Castro G, Cargnelutti D, Gonçalves JF, Rauber R, Skrebsky EC, Schetinger MRC, Morsch VM, Bisognin DA. 2007. Physiological and oxidative stress responses of four potato clones to aluminum in nutrient solution. Brazilian Journal of Plant Physiology **19,** 211-222.

Vitorello VA, Capald FR, Stefanuto VA. 2005. Recent advances in aluminium toxicity and resistance in higher plants. Brazilian Journal of Plant Physiology **17,** 129-143.

von Uexküll HR, Mutert E. 1995. Global extent, development and economic impact of acid soils. Plant and Soil **171,** 11–15.

Xiang C, Oliver DJ. 1998. Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in *Arabidopsis*. Plant Cell **10,** 1539-1550.

Yamamoto Y, Kobayashi Y, Devi SR, Rikiishi S, Matsumoto H. 2002. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. Plant Physiology **128,** 63–72.

Yoneyama T, Taira M, Suzuki T, Nakamura M, Niwa K, Watanabe T, Ohyama T. 2007. Expression and characterization of a recombinant unique acid phosphatase from kidney bean hypocotyl exhibiting chloroperoxidase activity in the yeast *Pichia pastoris*. Protein Expression and Purification **53,** 31–39.

CONSIDERAÇÕES FINAIS

O uso do alumínio em solução nutritiva mostrou-se uma técnica adequada para estudos bioquímicos e fisiológicos de toxicidade desse metal. Esse estudo contribuiu para uma melhor compreensão dos mecanismos causais da toxicidade do alumínio (Al) em clones de batata. Além disso, a determinação de parâmetros bioquímicos mostrou ser uma ferramenta importante para correlacionar inibição do crescimento com alterações bioquímicas provocadas pelo Al em plantas de batata.

O Al foi absorvido pelas raízes e transportado para a parte aérea de todos os clones, mas os efeitos tóxicos desse metal foram diferenciados entre os clones. Os maiores sintomas de toxidez se manifestaram na raiz, justamente onde foram detectadas as maiores concentrações desse metal.

A inibição do crescimento da raiz é o primeiro sintoma visível de toxicidade do Al. Utilizando-se o crescimento relativo da raiz como critério, foi possível separar os clones de batata em sensíveis (Macaca e Dakota Rose) e tolerantes (*Solanum microdontum* e SMIC148-A) ao Al. Os dados sugerem que o(s) mecanismo(s) de tolerância ao Al existentes nos clones tolerantes (*S. microdontum* e SMIC148-A) é(são) interno(s), uma vez que o alumínio foi absorvido pelas raízes e também transportado para a parte aérea das plantas. O crescimento da parte aérea foi bem menos afetado. Entretanto, vários parâmetros bioquímicos mostraram-se alterados pela presença de Al, tanto em clones tolerantes como sensíveis ao Al.

As diferenças entre os clones foram observadas especialmente em nível de dano oxidativo a biomoléculas e na expressão da quantidade e tipo de antioxiante. Esse dano pode ser uma conseqüência do aumento na concentração de peróxido de hidrogênio, ou de outras espécies reativas formadas a partir do peróxido de hidrogênio, observado nesses clones.

Nos clones tolerantes ao Al, as biomoléculas como lipídios de membrana, as proteínas e os pigmentos sofreram menor dano oxidativo, comparado com os clones sensíveis ao Al. Nesses clones, pode-se observar que há sempre um componente do sistema antioxidante protegendo as plantas do estresse de Al, o mesmo não acontecendo com os clones sensíveis ao Al. Além disso, o clone *S. microdontum*

manteve uma concentração maior de micronutrientes em raízes e parte aérea, sugerindo um mecanismo adicional de tolerância.

Da mesma forma, quando os clones Macaca (sensível ao Al) e SMIC148-A (tolerante ao Al) foram cultivados em sistema de raízes divididas com variação na concentração e distribuição de Al ao sistema radicular, as plantas apresentaram sinais de estresse oxidativo, os quais foram observados principalmente no clone sensível ao Al. Baseado em parâmetros bioquímicos de raízes e parte aérea, o clone tolerante ao Al sofreu danos oxidativos menores, em comparação com o clone sensível, apresentando respostas antioxidativas sistêmicas e locais mais evidentes ao suprimento de Al, mesmo tendo uma reação de escape ao Al menor que o clone sensível ao Al. Portanto, a maior tolerância ao Al do clone SMIC148-A pode ser relacionada à sua eficiente capacidade antioxidante. Além disso, por apresentar uma reação de escape ao Al menor, o clone tolerante pode apresentar um sistema de enraizamento mais profundo em solos com níveis tóxicos de Al.

Baseado nessas observações pode-se afirmar que o estresse oxidativo pode ser um importante mecanismo de toxicidade do Al, principalmente em clones sensíveis ao metal. Essa toxicidade depende da disponibilidade de Al, do clone e do sistema de crescimento analisado. Além disso, pode-se observar que os efeitos adversos do Al não desapareceram quando parte do sistema radicular não está em contato com o Al. Portanto, todos esses fatores devem ser considerados no desenvolvimento de protocolos para a caracterização de clones de batata tolerantes ao Al.

Experimentos futuros devem ser realizados para investigar se esses clones possuem outros mecanismos de tolerância, como por exemplo, a complexação interna do Al com ácidos orgânicos. Além disso, é interessante observar o comportamento desses clones em um solo caracteristicamente ácido e com alta saturação em Al.

REFERÊNCIAS

ABREU Jr, C. H.; MURAOKA, T.; LAVORANTE, A. Relationship between acidity and chemical properties of brazilian soils. **Scientia Agricola,** Piracicaba, v. 60, n. 2, p. 337-343, apr./june, 2003.

BARCELÓ, J. et al. Aluminum phytotoxicity. A challenge for plant scientists. **Fertilizer Research**, The Hague, v. 43, n. 1-3, p. 217-223, jan., 1996.

BARCELÓ, J.; POSCHENRIEDER, C. H. Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. **Environmental and Experimental Botany**, New York, v. 48, n. 1, p. 75-92, jan./mar., 2002.

BISOGNIN, D. A. (Ed.) **Recomendações técnicas para o cultivo da batata no Rio Grande do Sul e Santa Catarina.** Santa Maria: Universidade Federal de Santa Maria, 1996. 64 p.

_____ **Simpósio de melhoramento genético e previsão de epifitias em batata**. Santa Maria: Universidade Federal de Santa Maria, 2006. 101 p.

BOLAN, N. S.; HEDLEY, M. J. Role of carbon, nitrogen and sulfur cycles in soil acidification. In: RENGEL, Z. (Ed.). **Handbook of soil acidity.** New York: Marcel Dekker, 2003. p. 29-56.

BOLAN, N. S.; HEDLEY, M. J.; WHITE, R. E. Process of soil acidification during nitrogen cycling with emphasis on legume based pasture. **Plant and Soil**, The Hague, v. 134, n. 1, p. 53-63, july, 1991.

BONA, L. et al. Screening wheat and other small grains for acid soil tolerance. **Landscape and Urban Planning**, Amsterdam, v. 27, n. 2/4, p. 175-178, dec., 1993.

BOSCOLO, P. R. S.; MENOSSI, M.; JORGE, R. A. Aluminum-induced oxidative stress in maize. **Phytochemistry: chemistry biochemistry, molecular biology**, New York, v. 62, n. 2, p. 181-189, jan., 2003.

BOZZO, G. G.; RAGHOTHAMA, K. G.; PLAXTON, W. C. Purification and characterization of two secreted purple acid phosphatase isozymes from phosphate-starved tomato (*Lycopersicon esculentum*) cell cultures. **European Journal of Biochemistry,** Berlin, v. 269, n. 24, p. 6278-6286, dec., 2002.

CAKMAK, I.; HORST, W. J. Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). **Physiologia Plantarum**, Copenhagen, v. 83, n. 3, p. 463-468, nov., 1991.

CASTRO, J. D. Acidez e calagem para a batatinha (*Solanum tuberosum* L.). In: REUNIÃO BRASILEIRA DE FERTILIDADE DO SOLO, 15., 1983, Campinas/SP. **Anais...,** Campinas: Sociedade Brasileira de Ciência do Solo, 1983. p. 79-85.

CHAOUI, A.; FERJAN, E. E. Effects of cadmium and copper on antioxidant capacities lignification and auxin degradation in leaves of pea (*Pisum sativum* L.) seedlings. **Comptes Rendus Biologies,** Amsterdam, v. 328, n. 1, p. 23-31, jan., 2005.

COBBETT, C. S.; GOLDSBOROUGH, P. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. **Annual Review of Plant Biology,** Palo Alto, v. 53, n. 1, p. 159–182, jun., 2002.

COELHO, A. H. R.; VILELA E. R.; CHAGAS, S. J. de Qualidade de batata (*Solanum tuberosum* L.) para fritura, em função dos níveis de açúcares redutores e amido, durante o armazenamento refrigerado e à temperatura ambiente com atmosfera modificada. **Ciência e Agrotecnologia**, Lavras, v. 23, n. 4, p. 899-910, nov., 1999.

DAVIES Jr., F. T. et al. Influence of a flavonoid (formononetin) on mycorrhizal activity and potato crop productivity in the highlands of Peru. **Scientia Horticulturae**, Amsterdam, v. 106, n. 3, p. 318-329, oct., 2005.

DELHAIZE, E.; GRUBER, B. D.; RYAN, P. R. The roles of organic anion permeases in aluminium resistance and mineral nutrition. **FEBS Letters**, Amsterdam, v. 581, n. 12, p. 2255-2262, may 2005.

DEL POZO, J.C. et al. A type 5 acid phosphatase gene from *Arabidopsis thaliana* is induced by phosphate starvation and by some other types of phosphate mobilizing/oxidative stress conditions. **Plant Journal: for cell and molecular biology**, Oxford, v. 19, n. 5, p. 579-589, sept., 1999.

DUFF, S. M. G.; SARATH, G.; PLAXTON, W. C. The role of acid phosphatases in plant phosphorus metabolism. **Physiologia Plantarum**, Copenhagen, v. 90, n. 4, p. 791–800, dec., 1994.

FAGERIA, N. K.; ZIMMERMANN, F. J. P. Seleção de cultivares de arroz para tolerância à toxidez de alumínio em solução nutritiva. **Pesquisa Agropecuária Brasileira**, Rio de Janeiro, v. 14, n. 2, p. 141-147, fev., 1979.

FOY, C. D. Plant adaptation to acid, aluminum-toxic soils. **Communications in Soil Science and Plant Analysis**, Philadelphia, v. 19, n. 7, p. 959-987, dec., 1988.

FOY, C. D.; CHANEY, R. L.; WHITE, M. C. The physiology of metal toxicity in plants. **Annual Review of Plant Physiology**, Palo Alto, v. 29, n. 1, p. 511-566, jun., 1978.

FOYER, C. H.; DESCOUVIERES, P.; KUNERT, K. J. Protection against oxygen free radicals: an important defense mechanism studied in transgenic plants. **Plant, Cell and Environment**, Oxford, v. 17, n. 5, p. 507–523, may, 1994.

FURLANI, P. R.; FURLANI, A. M. C. Tolerância a alumínio e eficiência a fósforo em milho e arroz: características independentes. **Bragantia**, Campinas, v. 50, n. 2, p. 331-340, set., 1991.

GELLATLY, K. S. et al. Purification and characterization of a potato tuber acid phosphatase having significant phosphotyrosine phosphatase activity. **Plant Physiology,** Minneapolis, v. 106, n. 1, p. 223–232, sept., 1994.

GIANNAKOULA, A. et al. Aluminum tolerance in maize is correlated with increased levels of mineral nutrients, carbohydrates and proline, and decreased levels of lipid peroxidation and Al accumulation. **Journal of Plant Physiology,** Stuttgart, v. 165, n. 4, p. 385-396, mar., 2008.

GUO, T. R.; ZHANG, G. P.; ZHANG, Y. H. Physiological changes in barley plants under combined toxicity of aluminum, copper and cadmium. **Colloids and Surfaces B: Biointerfaces,** Amsterdam, v. 57, n. 2, p. 182-188, june, 2007.

HAIRIAH, K. et al. Phosphate nutrition effects on aluminium avoidance of *Mucuna pruriens var. utilis*. **Environmental and Experimental Botany**, Elmsford, v. 33, n. 1, p. 75-83, jul. 1993.

HALLIWELL, B.; GUTTERIDGE, J. M. C. **Free radicals in biology and medicine,** 3rd ed. New York: Oxford Science Publications, 1999.

HAYNES, R. J.; MOKOLOBATE, M. S. Amelioration of Al toxicity and P deficiency in acid soils by additions of organic residues: a critical review of the phenomenon and the mechanisms involved. **Nutrient Cycling in Agroecosystems,** Dordrecht, v. 59, n. 1, p. 47-63, jan., 2001.

HEGEDÜS, A.; ERDEI, S.; HORVÁTH, G. Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. **Plant Science,** Limerick, v. 160, n. 6, p. 1085-1093, may, 2001.

HORST, W. J. The role of the apoplast in aluminum toxicity and resistance of higher plants: a review. **Zeitchrift für Pflanzenernährung und Bodenkunde,** Weinheim, v. 158, n. 5, p. 419-428, nov., 1995.

IBGE. **Levantamento sistemático da produção agrícola, confronto das safras de 2003 e das estimativas para 2004**. Online. Disponível na Internet http://www.ibge.gov.br. Acesso em: jan/2008.

JANSEN, S. et al. Aluminum hyperaccumulation in Angiosperms: A Review of its phylogenetic significance. **The Botanical Review**, Bronx, v. 68, n. 2, p. 235-269, sept., 2002.

KINRAIDE, T. B. Identity of the rhizotoxic aluminium species. **Plant and Soil,** The Hague, v. 134, n. 1, p. 167-178, july, 1991.

KINRAIDE, T. B.; PARKER, D. R. Assessing the phytotoxicity of mononuclear hydroxyaluminum. **Plant, Cell and Environment**, Oxford, v. 12, n. 5, p. 479-487, july, 1989.

KOCHIAN, L. V.; HOEKENGA, O. A.; PIÑEROS, M. A. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorus efficiency. **Annual Review of Plant Biology**, Palo Alto, v. 55, n. 1, p. 459–493, jun., 2004.

LI, P. H.; FENNELL, A. Potato Frost Hardiness. In: Li, P.H. (Ed.). **Potato Physiology**. Orlando: Academic Press, 1985. p. 457–479.

MA, J.F.; FURUKAWA, J. Recent progress in the research of external Al detoxification in higher plants: a minireview. **Journal of Inorganic Biochemistry**, New York, v. 97, n. 6, p. 46-51, june, 2003.

MA, J .F.; RYAN, P. R.; DELHAIZE, E. Aluminum tolerance in plants and the complexing role of organic acids. **TRENDS in Plant Science,** Oxford, v. 6, n. 6, p. 273-278, june, 2001.

MA, Q. F.; RENGEL, Z.; KUO, J. Aluminum toxicity in rye (*Secale cereale*): Root growth and dynamics of cytoplasmic Ca^{2+} in intact root tips. **Annals of Botany**, London, v. 89, n. 2, p. 241-244, feb., 2002.

MARRONI, N. P. (Ed.) **Estresse oxidativo e antioxidantes.** Canoas: Ed. ULBRA, 2002. 189 p.

MELLO, P. E. Aptidão de cultivares de batata para consumo in natura e para processamento. In: SEMINÁRIO DE ATUALIZAÇÃO NA CULTURA DA BATATA, 1997, Santa Maria. **Anais...,** Santa Maria: Sociedade de Agronomia de Santa Maria, 1997, p. 27-38.

MENDOZA, H. A. ESTRADA, R.N. Breeding potatoes for tolerance to stress: heat and frost. In: MUSSELL, H.; STAPLES, R. C. (Eds.). **Stress Physiology in Crop Plants.** New York: Wiley, 1979. p. 227–264.

MITTLER, R. Oxidative stress, antioxidants and stress tolerance. **TRENDS in Plant Science**, Oxford, v. 7, n. 9, p. 405-410, sept., 2002.

NOCTOR, G.; FOYER, C. Ascorbate and glutathione: keeping active oxygen under control. **Annual Review of Plant Physiology and Plant Molecular Biology,** Palo Alto, v. 49, n. 1, p. 249-279, june, 1998.

NOCTOR, G. et al. Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signaling. **Journal of Experimental Botany**, Oxford, v. 53, n. 372, p. 1283-1304, may, 2002.

PARKER, D. R. Root growth analysis: an underutilized approach to understanding aluminum rhizotoxicity. **Plant and Soil**, The Hague, v. 171, n. 1, p. 151-157, apr., 1995.

PASTORI, G. M.; FOYER, C. H. Commom components, networks, and pathwaysof cross-tolerance to stress. The central role of “redox” and abscisic acid-mediated controls. **Plant Physiology**, Minneapolis, v. 129, n. 2, p. 460-468, jun., 2002.

PENHEITER, A. R.; DUFF, S. M. G.; SARATH, G. Soybean root nodule acid phosphatase. **Plant Physiology,** Minneapolis, v. 114, n. 2, p. 597–604, jun., 1997.

POLAK, T. B. et al. The uptake and speciation of various Al species in the *Brassica rapa pekinensis*. **Phytochemistry: chemistry biochemistry, molecular biology**, New York, v. 57, n. 2, p. 189-198, may, 2001.

PREZOTTI, L. C.; CARMO, C. A. S. do; ANDRADE NETO, A. P. M. de. **Nutrição mineral da batata**. Vitória, EMCAPA, 1986. 44 p.

RAUSER, W. E. Structure and function of metal chelators produced by plants. **Cell Biochemistry and Biophysics,** Durham, v. 31, n. 1, p. 19–33, feb., 1999.

REDDY, A. R.; CHAITANYA, K. V.; VIVEKANANDAN, M. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. **Journal of Plant Physiology**, Stuttgart, v. 161, n. 11, p. 1189–1202, nov., 2004.

RENGEL, Z. Uptake of aluminum by plant cells. **New Phytologist**, Cambridge, v. 134, n. 3, p. 389-406, nov., 1996.

RENGEL, Z.; ZHANG, W-H. Role of dynamics of intracellular calcium in aluminum-toxicity syndrome. **New Phytologist,** Cambridge, v. 159, n. 2, p. 295-314, aug., 2003.

ROSSIELLO, R. O. P.; NETTO, J. J. Toxidez de alumínio em plantas: novos enfoques para um velho problema. In: FERNANDES, M. S. (Ed.). **Nutrição Mineral de Plantas.** Viçosa: SBCS, 2006. 432 p.

SAIRAM, R. K.; DESHMUKH, P. S.; SAXENA, D. C. Role of antioxidant systems in wheat genotypes tolerance to water stress. **Biologia Plantarum,** Praha, v. 41, n. 3, p. 387–394, apr., 1998.

SAIRAM, R. K. et al. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. **Biologia Plantarum,** Praha,v. 49, n. 1, p. 85–89, mar., 2005.

SALLES, L. A. B. Panorama mundial da produção, consumo e comércio a batata. In: SEMINÁRIO DE ATUALIZAÇÃO NA CULTURA DA BATATA, 1997, Santa Maria. **Anais...** Santa Maria: Sociedade de Agronomia de Santa Maria: UFSM, Centro de Ciências Rurais, 1997. p. 1-12.

SALVADOR, J.O. et al. Influência do alumínio no crescimento e na acumulação de nutrientes em mudas de goiabeira. **Revista Brasileira de Ciência do Solo**, Campinas, v. 24, n. 4, p. 787-796, nov., 2000.

SALVADOR, M.; HENRIQUES, J. A. P. (Eds.) **Radicais livres e a resposta celular ao estresse oxidativo.** Canoas: Ed. ULBRA, 2004. 204 p.

SANCHEZ, P. A.; SALINAS, J. G. Low input technology for Oxisols and Ultisols in Tropical America. **Advances in Agronomy,** San Diego, v. 34, n. 1, p. 279-305, mar., 1981.

SCOTT, N. et al. Reactions of arsenic (III) and arsenic (V) species with glutathione. **Chemical Research in Toxicology,** Washington, v. 6, n. 1, p. 102-106, jan., 1993.

SHAH, K. et al. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. **Plant Science**, Limerick, v. 161, n. 6, p. 1135-1144, nov., 2001.

SIECZA, J. B.; THORNTON, R. E. (Eds.). **Commercial Potato Production in North America: Potato Association of America Handbook**. Maine: Potato Assoc. of America, Orono, 1993.

TAMÁS, L. et al. Aluminium stimulated hydrogen peroxide production of germinating barley seeds. **Environmental and Experimental Botany**, Elmsford, v. 51, n. 3, p. 281-288, jun., 2004.

TAMÁS, L. et al. Aluminium-induced drought and oxidative stress in barley roots. **Journal of Plant Physiology,** Stuttgart, v. 163, n. 7, p. 781-784, may, 2006.

TANG, C.; RENGEL, Z. Role of plant cation/anion uptake ratio in soil acidification. In: RENGEL, Z. (Ed.). **Handbook of soil acidity.** New York: Marcel Dekker, 2003. p. 57-81.

TAYLOR, G. J. The physiology of aluminum tolerance. In: SIGEL, H.; SIGEL, A. (Eds.). **Metal Ions in Biological systems. Aluminum and its role in Biology.** New York: Marcel Dekker, 1988. p. 165-198, v. 24.

TAYLOR, G. J. Current views of the aluminum stress response: the physiological basis of tolerance. In: RANDALL, D. D.; BLEVINS, D. G.; MILES, C. D. (Eds.). **Current Topics in Plant Biochemistry and Physiology.** Columbia: University of Missouri, 1991. p. 57-93, v. 10.

TURNER, W. L.; PLAXTON, W. C. Purification and characterization of banana fruit acid phosphatase. **Planta,** Berlin, v. 214, n. 2, p. 243–249, dec., 2001.

VINCENT, J. B.; CROWDER, M. W.; AVERILL, B. A. Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. **Trends in Biochemical Sciences,** Amsterdam, v. 17, n. 3, p. 105–110, mar., 1992.

VITORELLO, V. A.; CAPALDI, F. R.; STEFANUTO, V. A. Recent advances in aluminum toxicity and resistance in higher plants. Brazilian **Journal of Plant Physiology**, Piracicaba, v. 17, n. 1, p. 129-143, jan., 2005.

VOLKWEISS, S. J. Química da acidez dos solos. In: SEMINÁRIO SOBRE CORRETIVOS DA ACIDEZ DO SOLO, 2., 1989, Santa Maria. **Anais...** Santa Maria: Edições UFSM, 1989. p. 7-38.

VON UEXKÜLL, H. R.; MUTERT, E. Global extent, development and economic impact of acid soils, **Plant and Soil**, The Hague, v. 171, n. 1, p. 11–15, apr., 1995.

WENZL, P. et al. The high level of aluminum resistance in *Signalgrass* is not associated with known mechanisms of external aluminum detoxification in root apices. **Plant Physiology**, Minneapolis, v. 125, n. 3, p. 1473-1484, mar., 2001.

WILLIAMS, R. J. P. What is wrong with aluminum? The J. D. Birchall memorial lecture. Journal of Inorganic Biochemistry, New York, v. 76, n. 2, p. 81-88, aug., 1999.

YAMAMOTO, Y. et al. Aluminum toxicity is associated with mitochondrial dysfunction and the production of reactive oxygen species in plant cells. **Plant Physiology**, Minneapolis, v. 128, n. 1, p. 63-72, jan., 2002.

YAMAMOTO, Y. et al. Oxidative stress triggered by aluminum in plant roots. **Plant and Soil**, The Hague, v. 255, n. 1, p. 239-243, aug., 2003.

YONEYAMA, T. et al. Expression and characterization of a recombinant unique acid phosphatase from kidney bean hypocotyl exhibiting chloroperoxidase activity in the yeast *Pichia pastoris*. **Protein Expression and Purification**, Wisconsin, v. 53, n.1, p. 31–39, may, 2007.

ZENK, M. H. Heavy metal detoxication in higher plants, a review. **Gene**, Amsterdam, v. 179, n. 1, p. 21-30, jan., 1996.

APÊNDICES

APÊNDICE A - Porcentagem de distribuição de espécies de alumínio (Al) dissolvidas e adsorvidas, segundo programa Visual Minteq.

Concentrações de Al	Espécies de alumínio							
	Al^{3+}	$AlOH^{2+}$	$Al(OH)^{2+}$	$Al(OH)_4{}^{5+}$	$Al_2(OH)_2{}^{4+}$	$AlCl^{2+}$	$AlSO_4{}^{+}$	$Al(SO_4)^{2-}$
	……%……							
50 mg L^{-1}	83,413	4,124	0,136	0,058	0,392	0,075	11,76	0,041
100 mg L^{-1}	87,716	3,941	0,123	0,261	0,835	0,070	7,038	0,016
150 mg L^{-1}	89,255	3,705	0,110	0,599	1,257	0,065	5,0	0
200 mg L^{-1}	89,772	3,485	0,099	1,054	1,651	0,061	3,872	0

APÊNDICE B – Valores de pH da solução nutritiva durante o período de exposição ao Al (10 dias) de clones de batata (Macaca e SMIC148-A) em sistema de raízes divididas. As soluções foram trocadas a cada 48 horas.

Tratamentos	1º dia (24 h)				2º dia (48 h)			
	Macaca		SMIC148-A		Macaca		SMIC148-A	
(mg Al L^{-1})	RE	RD	RE	RD	RE	RD	RE	RD
RE 0, RD 0	4,11 a	4,13 a	4,26 a	4,26 a	4,41 ab	4,45 a	4,88 a	4,89 a
RE 50, RD 50	3,78 c	3,78 c	3,90 c	3,87 c	3,80 c	3,79 c	4,03 b	3,97 b
RE 0, RD 100	4,0 ab	3,84 bc	4,3 a	3,87 c	4,1 bc	3,84 c	4,58 a	3,97 b
RE 100, RD 100	3,84 bc	3,83 bc	3,88 c	3,85 c	3,84 c	3,82 c	3,96 b	3,96 b
RE 0, RD 200	3,96 abc	3,95 abc	4,1 b	3,93 c	4,07 bc	3,91 c	4,0 b	3,97 b

Tratamentos	3º dia (24 h)				4º dia (48 h)			
	Macaca		SMIC148-A		Macaca		SMIC148-A	
(mg Al L^{-1})	RE	RD	RE	RD	RE	RD	RE	RD
RE 0, RD 0	4,16 ab	4,18 a	4,12 n.s.	4,12 n.s.	4,46 a	4,46 a	4,23 ab	4,32 a
RE 50, RD 50	3,94 ab	3,94 ab	4,15 n.s.	4,10 n.s.	4,18 ab	4,16 ab	4,01 cd	3,96 cd
RE 0, RD 100	3,91 ab	3,95 ab	4,13 n.s.	4,12 n.s.	4,14 ab	4,06 b	4,11 bc	3,93 cd
RE 100, RD 100	3,89 ab	3,88 b	4,13 n.s.	4,09 n.s.	4,07 b	4,06 b	3,96 cd	3,93 cd
RE 0, RD 200	3,93 ab	3,96 ab	4,09 n.s.	4,14 n.s.	4,04 b	4,14 ab	3,95 cd	3,92 d

Tratamentos	5º dia (24 h)				6º dia (48 h)			
	Macaca		SMIC148-A		Macaca		SMIC148-A	
(mg Al L^{-1})	RE	RD	RE	RD	RE	RD	RE	RD
RE 0, RD 0	4,21 a	4,22 a	4,23 ab	4,27 a	4,34 a	4,35 a	4,21 ab	4,25 a
RE 50, RD 50	3,98 ab	4,0 ab	3,95 d	3,95 d	3,95 b	3,95 b	3,91 bc	3,91 bc
RE 0, RD 100	4,07 ab	3,97 ab	4,21 abc	3,98 cd	3,97 b	3,91 b	4,14 abc	3,9 bc
RE 100, RD 100	4,01 ab	4,01 ab	4,0 bcd	4,0 bcd	3,93 b	3,91 b	3,9 bc	3,88 c
RE 0, RD 200	3,94 b	4,0 ab	4,04 bcd	3,96 d	3,74 b	3,87 b	3,87 c	3,83 c

Tratamentos (mg Al L^{-1})	7º dia (24 h)				8º dia (48 h)			
	Macaca		SMIC148-A		Macaca		SMIC148-A	
	RE	RD	RE	RD	RE	RD	RE	RD
RE 0, RD 0	4,2 a	4,21 a	4,21 a	4,22 a	4,29 a	4,39 a	4,3 a	4,44 a
RE 50, RD 50	4,0 b	4,03 b	4,04 b	4,01 b	4,13 b	4,13 b	3,95 b	3,91 b
RE 0, RD 100	3,97 b	3,96 b	4,09 ab	4,0 b	4,02 b	4,04 b	4,0 b	3,88 b
RE 100, RD 100	4,0 b	4,0 b	4,02 b	3,99 b	4,09 b	4,07 b	3,89 b	3,87 b
RE 0, RD 200	3,8 c	3,94 b	4,0 b	4,02 b	3,86 c	4,0 bc	3,87 b	3,85 b

Tratamentos (mg Al L^{-1})	9º dia (24 h)				10º dia (48 h)			
	Macaca		SMIC148-A		Macaca		SMIC148-A	
	RE	RD	RE	RD	RE	RD	RE	RD
RE 0, RD 0	4,08 a	4,11 a	4,09 a	4,09 a	4,28 a	4,29 a	4,23 ab	4,28 a
RE 50, RD 50	3,93 cd	3,91 d	3,87 bc	3,84 c	3,95 cd	3,95 cd	4,02 abc	3,98 bc
RE 0, RD 100	3,75 e	3,89 d	3,99 ab	3,87 bc	4,16 ab	3,95 cd	4,07 abc	4,02 abc
RE 100, RD 100	3,96 bc	3,95 bc	3,9 bc	3,88 bc	3,92 d	3,87 d	4,04 abc	4,0 bc
RE 0, RD 200	3,73 e	3,98 b	3,86 bc	3,91 bc	4,07 bc	3,91 d	3,91 c	4,01 bc

*Médias seguidas de mesma letra não diferem entre si pelo teste de Tukey em nível de 5% de probabilidade de erro.

n.s. – Não significativo.

RE: metade esquerda da raiz; RD: metade direita da raiz.

APÊNDICE C – Cultivo *in vitro* de clones de batata em câmara climatizada (A); Sistema hidropônico em câmara climatizada (B); Clones de batata crescendo em areia em casa de vegetação (C); Clones de batata em sistema de raízes divididas em casa de vegetação (D).