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RESPOSTAS FISIOLÓGICAS E BIOQUÍMICAS AO ESTRESSE DE ALUMÍNIO E FÓSFORO EM GENÓTIPOS DE BATATA (*Solanum tuberosum*)

TESE DE DOUTORADO

Liana Veronica Rossato

Santa Maria, RS, Brasil

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E-mail: liana.rossato@gmail.com

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COMISSÃO EXAMINADORA:

Fernando Teixeira Nicoloso, Dr.
(Presidente/Orientador)

Carla Andrea Delatorre, Drª. (UFRGS)

Arthur Germano Fett Neto, Dr. (UFRGS)

Dilson Antonio Bisognin, Dr. (UFSM)

Maria Rosa Chitolina Schetinger, Drª. (UFSM)

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RESUMO

Tese de Doutorado
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AUTOR: LIANA VERONICA ROSSATO
ORIENTADOR: FERNANDO TEIXEIRA NICOLOSO
Local da Defesa e Data: Santa Maria, 14 de Março de 2014.

A toxicidade do alumínio (Al) e a deficiência de fósforo (P) frequentemente coexistem em solos ácidos, sendo fatores limitantes para o crescimento e produção das plantas, incluindo a batata (*Solanum tuberosum*). A compreensão dos mecanismos fisiológicos relacionados à interação entre Al e P pode facilitar a obtenção de genótipos mais tolerantes ao Al e/ou eficientes no uso de P. O objetivo deste estudo foi verificar se genótipos eficientes no uso de P são tolerantes ao Al e se essa eficiência está vinculada à atividade de fosfatases ácidas. Oito genótipos de batata (SMIC148-A, Dakota Rose, Solanum microdontum, SMINIA793101-3, SMIB106-7, SMIF212-3, SMIJ319-7 e SMIG145-1), demostrando diferentes respostas ao P e/ou eficiência no uso de P foram cultivados em solução nutritiva (pH 4,0) com 0 e 200 mg de Al L^{-1} na ausência de P. Através da avaliação de diferentes parâmetros de crescimento, como comprimento da parte aérea, consumo de solução nutritiva, e massa fresca e seca total, os genótipos de batata foram classificados como tolerantes (SMIF212-3 (mais tolerante), SMIC148-A e S. microdontum), intermédiarios (SMINIA793101-3 e SMIB106-7) e sensíveis (Dakota Rose , SMIJ319-7 (mais sensível) e SMIG145-1) ao Al. A tolerância ao Al nos genótipos de batata parece estar relacionada com o aumento da concentração de P nos tecidos. Nos genótipos tolerantes ao Al (SMIC148-A e *S. microdontum*) foi verificado um aumento na concentração de P com o aumento da concentração de Al, principalmente nas folhas. A sensibilidade ao Al em genótipos de batata sob deficiência de P pode estar associada ao decréscimo na eficiência de utilização e translocação do P. Além disso, o aumento da concentração de Al afetou a taxa de absorção e distribuição dos nutrientes nas diferentes partes das plantas (raízes, caule, folhas, estolões e tubérculos). A tolerância ao Al nos genótipos SMIC148-A, *S. microdontum* e SMIF212-3 pode estar relacionada aos maiores níveis de nutrientes nas raízes e folhas. Entre os oito genótipos analisados anteriormente, quatro genótipos contrastantes quanto à tolerância ao Al e eficiência ao P (tolerante ao Al: SMIC148-A [NER] e SMIF212-3 [ENR]; sensível ao Al: Dakota Rose [ER] e SMIJ319-7 [NENR]) foram selecionados e utilizados para verificar os efeitos da interação entre Al e P. Os genótipos de batata foram cultivados em solução nutritiva (pH 4,0) com 0, 25 e 125 uM P e 0 ou 200 mg de Al L^{-1}. Em geral, o aumento da concentração de P não influenciou na tolerância ao Al. Em ambos os experimentos, a atividade da fosfatase ácida não foi correlacionada à eficiência no uso do P. Com o objetivo de checar se o estresse oxidativo provocado pelo Al difere entre os genótipos Dakota Rose (sensível ao Al) e SMIC148-A (tolerante ao Al), os quais apresentam distinto grau de escape ao Al, foram cultivados em sistema de raízes divididas por sete dias, com cinco tratamentos de variação de concentração e localização de Al. De modo geral, a exposição ao Al causou uma redução nos parâmetros de crescimento tanto no genótipo tolerante quanto no sensível. Além disso, foi observado aumento na concentração de Al tanto na metade da raiz exposta quanto na metade da raiz não exposta ao Al. Em ambos os genótipos foi observado decréscimo na concentração de P na metade da raiz tratada com

Al, contudo na metade da raiz não exposta ao Al foi observado aumento na concentração de P, principalmente no genótipo tolerante. Tanto no genótipo sensível quanto no genótipo tolerante ocorreu aumento da concentração de P no caule em plantas expostas ao Al, contudo, somente no genótipo tolerante observou-se aumento na concentração de P na folha. Além disso, no genótipo tolerante, os parâmetros bioquímicos avaliados foram menos afetados pelo Al do que no genótipo sensível. No genótipo tolerante foi observado aumento da concentração de clorofilas e carotenóides, enquanto que, no genótipo sensível foi observado decréscimo com a exposição ao Al. No genótipo sensível foi observado aumento da peroxidação lipídica nas raízes e folhas de plantas expostas às maiores doses de Al. Entretanto, no genótipo tolerante a mesma resposta não foi observada. Essas diferenças entre os genótipos não poderam ser associadas à atividade das enzimas antioxidantes. Tanto no genótipo tolerante quanto no sensível a exposição ao Al aumentou a atividade da POD e, de modo geral, promoveu um ligeiro aumento na atividade da CAT e diminuiu a atividade da APX na raiz. Por outro lado, a tolerância ao Al no genótipo SMIC148-A pode estar associada à menor translocação de Al para as folhas principalmente em plantas onde somente metade da raiz foi exposta ao Al e à maior habilidade de remobilização do P de raízes expostas ao Al para as raízes não expostas.

Palavras-chave: Batata. alumínio. fósforo. fosfatases ácidas.

ABSTRACT

Doctoral Thesis
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BIOCHEMICAL AND PHYSIOLOGICAL RESPONSE OF POTATO GENOTYPES IN RELATION TO ALUMINUM AND PHOSPHORUS STRESS

AUTHOR: LIANA VERONICA ROSSATO
ADVISOR: FERNANDO TEIXEIRA NICOLOSO
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Aluminum (Al) toxicity and phosphorus (P) deficiency often coexist in acid soils that severely limit crop growth and production, including potato (*Solanum tuberosum*). Understanding the physiological mechanisms relating to plant Al and P interactions should facilitate the development of more Al-tolerant and/or P-efficient crops. The objective of this study was to investigate if P- efficiency were related to Al-tolerance and if P- efficiency was related to acid phosphatase activity. Eight potato genotypes (SMIC148-A, Dakota Rose, *S. microdontum*, SMINIA793101-3, SMIB106-7, SMIF212-3, SMIG145-1 and SMIJ319-7) showing different responses and/or efficiency to P were grown in a nutrient solution (pH 4.0) with 0 and 200 mg Al L^{-1} and P-starvation. Based on shoot length, nutrient solution consumption, and total fresh and dry weight, the potato genotypes were classified as Al-tolerant (SMIF212-3 (more tolerant), SMIC148-A and *S. microdontum*), Al-intermediate (SMINIA793101-3 and SMIB106-7) and Al-sensitive (Dakota Rose, SMIJ319-7 (more sensitive) and SMIG145-1). The Al-tolerance in potato genotypes appears to be related to the increase in P concentration in the tissues. The Al tolerance in genotypes (SMIC148-A and S. microdontum) might be associated with higher tissue Al immobilization due to the higher tissue P content, mainly in the leaves. The Al sensitivity in the potato genotypes under P-starvation condition was associated with decreasing P utilization and translocation efficiencies. Furthermore, the increase of Al accumulation affected the rate of uptake and distribution of nutrients in the different plant parts (roots, stem, leaf, stolon and tuber) of potato genotypes. The Al-tolerance in the SMIC148-A, *S. microdontum* and SMIF212-3 genotypes may be connected with highest levels of nutrients in the roots and leaves. Among the eight previously analyzed genotypes, four genotypes with contrasting Al-tolerance and P-efficiency/or responsive (Al-tolerant: SMIC148-A [NER] and SMIF212-3 [ENR]; Al-sensitive: Dakota Rose [ER] and SMIG145-1 [NENR]) were utilized to investigate the effects of Al-P interactions. Potato genotypes were grown in a nutrient solution (pH 4.0) with 0, 25 and 125 μM P and 0 or 200 mg Al L^{-1}. In this second experiment the P supply did not influence on Al tolerance response. In both experiments, it was not observed a straight relationship between tissues APase activities and P utilization efficiency (PUE). With the objective of checking whether Al oxidative stress differs in potato genotypes, Dakota Rose (Al-sensitive) and SMIC148-A (Al-tolerant), which present distinct degrees of Al- avoidance, were cultivated in a split root system for seven days with five treatments of varying concentrations and locations of Al. In general, the Al exposure caused a reduction in growth parameters in both Al-tolerant and Al-sensitive genotypes. Furthermore, it was observed an increase in Al concentration in both Al-treated and Al-untreated root half. In both genotypes was observed decrease in the P concentration in the Al-treated root half, however, in the Al-untreated root half was observed an increased in the P concentration, mainly in the Al-tolerant genotype. In both genotypes was observed an increase in the P concentration in stem in all Al treatments, however, only in the

Al-tolerant genotype was observed an increased in the leaf P concentration. In addition, in the Al-tolerant genotype the biochemistry parameters were lower affected than Al-sensitive genotype. In Al-tolerant genotype was observed an increase in the total chlorophyll and carotenoids concentration whereas in the Al-sensitive genotype was observed a decrease with Al exposure. In Al-sensitive genotype was observed an increased in the leaf and root lipid peroxidation in plants exposed at higher Al treatments. On the other hand, in the Al-tolerant genotype was not observed increase in the plants exposed at higher Al treatments. However, this difference between potato genotypes can be not related to antioxidant enzymes activities. In both genotypes, in general, the Al exposure caused a decreased in the root APX activity, an increased in the GPX activity and a slight increased in CAT activity. On the other hand, the Al-tolerance in the SMIC148-A can be associated to lower Al translocation for leaf mainly in the plants only one root half was exposed at Al and the higher ability this genotype in the remobilization P from Al-treated to Al-untreated root half.

Keywords: Potato, aluminum, phosphorus, acid phosphatases.

LISTA DE FIGURAS

SUMÁRIO

1 INTRODUÇÃO

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 subespécie *tuberosum* (PEREIRA; DANIELS, 2003), ocupa o quarto lugar em volume de produção mundial de alimentos (323 milhões de toneladas), sendo superada somente pelo trigo, milho e arroz (FAO, 2012).

Essa cultura é plantada em, pelo menos, 140 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 (SALLES, 1997). É um dos alimentos mais consumidos no mundo como fonte de energia, devido à composição, à versatilidade gastronômica e tecnológica e ao baixo custo de comercialização dos tubérculos (COELHO; VILELA; CHAGAS, 1999), sendo a hortaliça de maior importância econômica no Brasil (BISOGNIN, 1996).

A batata se desenvolve sob uma variedade de altitudes, latitudes e condições climáticas, desde o nível do mar até 4000 metros de altitude (DAVIES JR et al., 2005). A batata possui uma distribuição radicular superficial e pouco densa no campo quando comparada a outras culturas. Além disso, foi observado que a batata apresenta o menor comprimento radicular tanto na camada superficial quanto nas camadas mais profundas em relação a outras culturas (IWAMA, 2008).

Essa espécie vegetal tolera acidez moderada no solo, produzindo bem na faixa de pH 5,0 a 6,5 (FILGUEIRA, 2003). Por outro lado, nos solos excessivamente ácidos (pH abaixo de 5,0) ocorrem decréscimos de produção, uma vez que o pH baixo prejudica o crescimento da planta pela própria ação da acidez (H^+), além de diminuir a disponibilidade de nutrientes e aumentar a concentração de alumínio trocável (Al^{3+}) no solo (ROSSIELLO; JACOB, 2006).

Em solos tropicais e subtropicais úmidos, com altas precipitações pluviométricas, os nutrientes solúveis como o cálcio, o magnésio, o potássio e outros elementos básicos são lixiviados. Quando a remoção de cátions básicos é maior que sua taxa de liberação pelas intempéries, o pH do solo diminui. A mineralização da matéria orgânica por microrganismos do solo resulta na liberação de nitrato e hidrogênio, ocasionando a diminuição do pH. Em pH baixo, o hidrogênio (H^+) atua sobre os minerais liberando íons alumínio (Al^{3+}) que ficam predominantemente retidos pelas cargas negativas das partículas de argila do solo, em equilíbrio com o Al^{3+} em solução. Assim, a quantidade de Al^{3+} em solução aumenta com a acidez do solo (BOHNEN,

1995).

Em pH baixo (pH<5,0) a solubilidade do Al aumenta, de tal modo que a espécie de Al trivalente, Al^{3+}, predomina, enquanto que as espécies $Al(OH)^{2+}$ e $Al(OH)_2{}^{+}$ são formadas quando o pH aumenta. Em pH próximos da neutralidade ocorre a fase sólida $Al(OH)_3$ e o $Al(OH)_4{}^{-}$ predomina em condições alcalinas (Figura 1.1). Muitos destes cátions de Al monoméricos ligam-se a ligantes orgânicos e inorgânicos como $PO_4{}^{3-}$, $SO_4{}^{2-}$, F^{-}, ácidos orgânicos, proteínas e lipídios (DELHAIZE; RYAN, 1995).

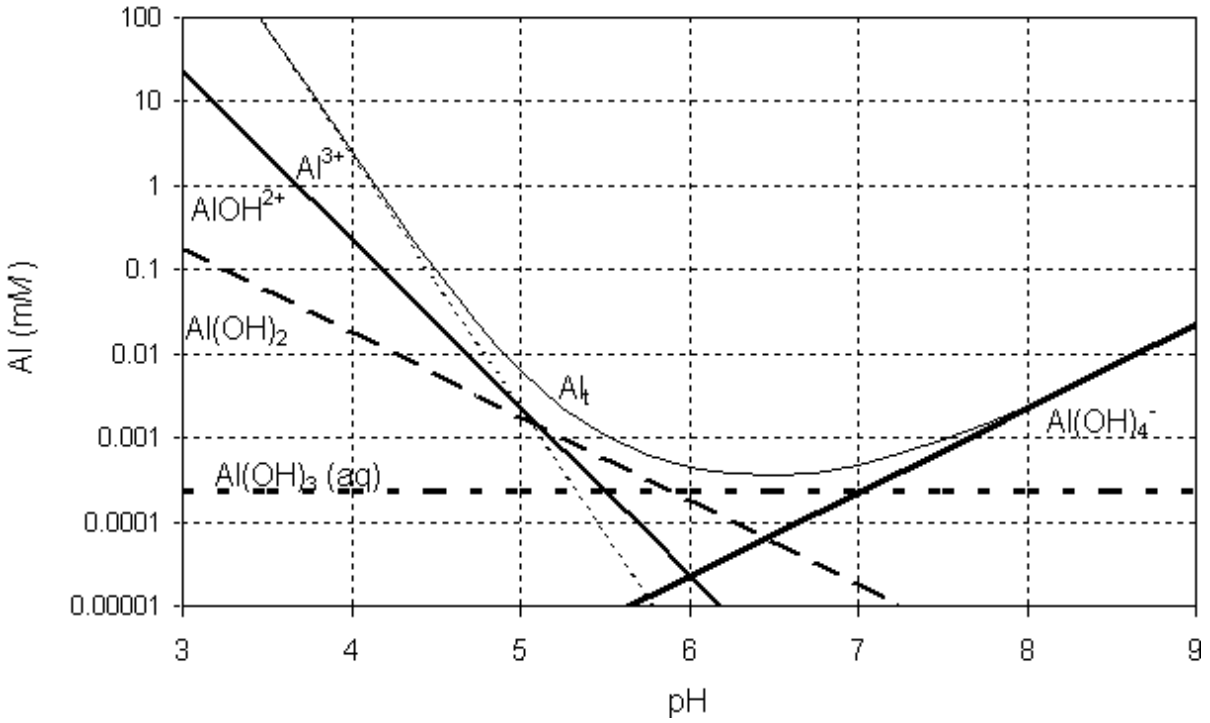

Figura 1.1 – Distribuição das atividades relativas de Al^{3+} e das espécies mononucleares de Al-OH em função do pH (Fonte: KINRAIDE; PARKER, 1989).

O alumínio (Al) é o mais abundante metal na crosta terrestre e é altamente tóxico para animais e plantas (SILVA et al., 2002). A toxidez do Al é um dos principais fatores limitantes da produtividade agrícola em solos ácidos (KOCHIAN; HOEKENGA; PIÑEROS, 2004). Sendo que o Al se apresenta em níveis elevados em aproximadamente 40% das terras aráveis do mundo que são potencialmente usadas para a produção de biomassa e alimentos (MA; RYAN; DELHAIZE, 2001). No Brasil, a ocorrência de solos ácidos com problemas de toxidez de Al é da ordem de 60%, considerando-se as terras com potencial para a atividade agrícola (ABREU JR; MURAOKA; LAVORANTE, 2003).

O Al^{3+} afeta inúmeros processos citológicos, bioquímicos e fisiológicos da maioria das espécies cultivadas. Sendo que, o primeiro e, talvez, o principal efeito do Al seja a inibição do

crescimento radicular através da inibição do alongamento celular mais do que pela divisão celular (RENGEL; ZHANG, 2003; KOCHIAN; PINEROS; HOEKENGA, 2005). O Al^{3+} acumula-se predominantemente nas células localizadas dentro da zona de divisão e alongamento celular (ápice radicular) podendo alterar as propriedades da parede e da plasmalema, inibindo o alongamento celular, tornando as raízes mais grossas e pouco funcionais, bem como afeta o sistema de carregadores de nutrientes (AHN et al., 2001). Em concentrações baixas (μM), o Al^{3+} pode inibir o crescimento da raiz dentro de minutos ou horas (FORTUNATO; NICOLOSO, 2004). Como o maior efeito do Al^{3+} está na redução do crescimento do sistema radicular, sua influência sobre a absorção de nutrientes nas condições naturais poderá manifestar-se principalmente para aqueles íons cujo suprimento à raiz é na maior parte representado pelo processo de difusão, como é o caso do P e do K (CANAL; MIELNICZUK, 1983). Esses efeitos promovem uma diminuição tanto na captação de água quanto de nutrientes resultando em redução do crescimento e da produtividade (MA; RYAN; DELHAIZE, 2001; TABALDI et al., 2009).

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 do desbalanço ou deficiência nutricional, especialmente do fósforo (ROSSIELLO; JACOB, 2006). PEREIRA et al. (2006) verificaram que a presença desse metal no substrato causou inibição da enzima δ-ALA-D em pepino, fato atribuído ao Al^{3+} ter competido com Mg^{2+} ou reduzido a expressão da δ-ALA-D. YAMAMOTO et al. (2002) relataram que o Al^{3+} acumulado em células de tabaco causou a redução da atividade mitocondrial, inibindo a respiração e reduzindo a produção de ATP, afetando o crescimento das células. Esses danos em nível mitocondrial foram relacionados com o aumento na produção de espécies reativas de oxigênio (EROs). A exposição das plantas a uma condição de estresse pode intensificar a produção de EROs a ponto de o sistema antioxidante não ser capaz de destoxificar essa quantidade excessiva, o que pode causar oxidações a componentes celulares (FOYER; LELANDAIS; KUNERT, 1994). 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), a catalase (CAT) e a guaicol peroxidase (POD) (BOSCOLO; MENOSSI; JORGE, 2003). Tem sido demonstrado que o aumento da atividade das enzimas antioxidantes e da produção de antioxidantes não enzimáticos está relacionada a um aumento da tolerância ao Al em várias espécies (DARKÓ et al., 2004;

DIPIERRO et al., 2005; TABALDI et al., 2009).

Além disso, as plantas apresentam estratégias que impedem a entrada do Al na célula como a exsudação de compostos fenólicos, de ácidos orgânicos e elevação do pH da rizosfera para valores maiores que 5,5 (SILVA et al., 2002). Outro potencial mecanismo de tolerância ao Al é através da formação de complexos entre o Al e o P na rizosfera, no apoplasto e no vacúolo (PIETRASZEWSKA, 2001).

Nos solos tropicais e subtropicais, a baixa disponibilidade de fósforo tem sido considerada uma das principais limitações agrícolas. O fósforo é um dos seis macronutrientes necessários para o desenvolvimento e crescimento das plantas (FRITSCHE-NETO; BORÉM, 2011). Apesar de ser o quinto nutriente em ordem decrescente de absorção, o P é o elemento que promove aumentos mais significativos na produtividade da batata (PREZOTTI; CARMO; ANDRADE NETO, 1986; EKELÖF, 2007). O P não somente é um componente de inúmeras macromoléculas tais como ácidos nucléicos, fosfolipídios e açúcares fosfatados, mas também é parte integrante do metabolismo energético e em processos biológicos como a fotossíntese, a respiração e o transporte transmembrana (RAGHOTHAMA; KARTHIKEYAN, 2005). Podendo atuar na planta como condicionador da produção, estimulando a formação de tubérculos, apressando a maturação, reduzindo o ciclo cultural e aumentando a incidência de tubérculos graúdos (PREZOTTI; CARMO; ANDRADE NETO, 1986; EKELÖF, 2007).

O estresse causado pela restrição de P às plantas é um dos principais fatores limitantes à produtividade das culturas (RAMAEKERS et al., 2010). O processo de aquisição de P pela planta é dificultado em função da sua concentração na solução do solo ser baixa (HINSINGER, 2001). Muitos solos ao redor do mundo são deficientes em P e, por isso, até em solos férteis a sua disponibilidade raramente excede 0,31 mg L^{-1} (BIELESKI, 1973). Frequentemente, a concentração (0.06 mg L^{-1}) de P inorgânico (Pi) disponível na solução do solo está várias ordens de magnitude abaixo daquela presente nos tecidos de plantas (155-620 mg L^{-1}) (RAGHOTHAMA, 1999).

Devido as suas fortes interações com os componentes do solo, a principal forma de aquisição do Pi pelas raízes é a difusão, e não o fluxo de massa (HINSINGER, 2001; EKELÖF, 2007; FANG et al., 2009).

O fósforo pode estar presente no solo em duas formas, inorgânico e orgânico. Em muitos solos, 30-60% do P está presente na forma inorgânica, embora esta fração possa variar de 5-95%. A disponibilidade do P é controlada através da solubilização e precipitação do fosfato em

formas inorgânicas e através da mineralização da fração orgânica (EKELÖF, 2007). A adsorção do P inorgânico depende do pH do solo. Em solos com pH menor que 7, aumenta a concentração do ânion $H_2PO_4^-$, forma preferencialmente absorvida pelas plantas (FRITSCHE-NETO; BORÉM, 2011; COVARRUBIAS-RAMIREZ; CASTILHO-AGUILAR; VERA-NUNEZ, 2005), entretanto em solos ácidos o fósforo solúvel rapidamente precipita com o Fe e Al diminuindo a sua disponibilidade.

Outra fonte de P para as plantas pode ser através do P orgânico existente no solo, o qual deve ser hidrolisado para que possa ocorrer a sua absorção (RAGHOTHAMA, 1999). O P orgânico pode constituir de 5 a 80% do total de P do solo (FRITSCHE-NETO; BORÉM, 2011). A importância do P orgânico do solo como uma fonte de P disponível às plantas depende, dessa forma, de sua taxa de solubilização e da taxa de P inorgânico liberado. Nesse sentido, vários tipos de enzimas do tipo fosfatases são capazes de aumentar a taxa de hidrólise do P orgânico no solo, liberando Pi às plantas (YADAV; TARAFDAR, 2003).

As fosfatases ácidas ou ortofosfato monoéster fosfoidrolases são um grupo de enzimas que catalisam a hidrólise de uma variedade de ésteres de fosfato em meio ácido liberando o Pi (YONEYAMA et al., 2007). As fosfatases ácidas são ubíquas e abundantes em plantas, animais, fungos e bactérias, e exibem baixa especificidade a substratos (DUFF; SARATH; PLAXTON, 1994).

Essas enzimas são amplamente distribuídas nas plantas (LUHOVÁ et al., 2006), e podem ser encontradas na mitocôndria, no vacúolo, na parede celular e ainda serem secretadas (YONEYAMA et al., 2007; TRAN; HURLEY; PLAXTON, 2010), sugerindo que essas enzimas estão envolvidas em vários processos metabólicos.

Muitas funções têm sido descritas para as fosfatases ácidas em plantas, incluindo a participação na transdução de sinal (RAGHOTHAMA, 1999), na regulação do metabolismo por desfosforilação de proteínas (DUFF; SARATH; PLAXTON, 1994) e na liberação de fosfato inorgânico a partir de fosfato orgânico (RAGHOTHAMA, 1999). TRAN; HURLEY; PLAXTON (2010) relataram que várias fosfatases ácidas púrpuras que exibiram significante atividade como fosfatases ácidas também apresentaram atividade como alcalinas e peroxidases, não sendo afetadas por inibidores da fosfatase ácida. Além disso, estas enzimas estão envolvidas em situações de estresse oxidativo, atuando no metabolismo de espécies reativas de oxigênio (EROs) (DEL POZO et al., 1999). LI; SHAO; LAM (2008) verificaram em soja que a expressão de fosfatase ácida púrpura (GmPAP3) aumentou a tolerância ao dano oxidativo causado por es-

tresse salino. TRAN; HURLEY; PLAXTON (2010) também relataram que as fosfatases ácidas podem estar envolvidas na biossíntese do ascorbato e na homeostase de Fe/Mn.

O controle da expressão de fosfatases ácidas é mediado por uma variedade de fatores ambientais e de desenvolvimento (DUFF; SARATH; PLAXTON, 1994). As fosfatases ácidas são induzidas sob a ação de diferentes agentes estressantes, incluindo a deficiência de água, a salinidade e o ataque de patógenos (BOZZO; RAGHOTHAMA; PLAXTON, 2002). Além disso, a ativação das fosfatases ácidas em resposta à deficiência de Pi é bem documentada (DUFF; SARATH; PLAXTON, 1994; TRAN; HURLEY; PLAXTON, 2010; MISSON et al., 2005; WU et al., 2003; LI et al., 2002; BOZZO; DUNN; PLAXTON, 2006; ZIMMERMANN et al., 2004). Estas enzimas estão envolvidas na produção, no transporte e na reciclagem de Pi, o qual é crucial para o metabolismo celular e para os processos de transdução de energia (DUFF; SARATH; PLAXTON, 1994). As fosfatases ácidas intracelulares normalmente controlam a homeostase interna de Pi enquanto as fosfatases ácidas secretadas controlam a aquisição externa de Pi (DUFF; SARATH; PLAXTON, 1994). A toxicidade do Al e a deficiência de P muitas vezes coexistem em solos ácidos e não podem ser considerados como fatores independentes, já que ambos interagem fortemente através de reações químicas e bioquímicas (MIMMO et al., 2009).

Um mecanismo bem conhecido utilizado por plantas tolerantes ao Al para prevenir a entrada do Al nas células radiculares é o aumento da produção e a exsudação de ácidos orgânicos, os quais formam complexos com o Al (DELHAIZE; RYAN; RANDALL, 1993; MA et al., 1997). Da mesma forma, a exsudação de ácidos orgânicos é utilizada pelas plantas para liberar o P adsorvido a partículas de argila e complexado a óxidos de Fe a Al (HINSINGER, 2001). Em soja (*Glycine max*) a toxicidade por Al^{3+} promoveu um aumento da exsudação de citrato (NIAN et al., 2003; DONG; PENG; YAN, 2004; LIAO et al., 2006), enquanto que na deficiência de P ocorreu um aumento da exsudação de malato e oxalato (DONG; PENG; YAN, 2004; LIAO et al., 2006). Contudo, quando as plantas de soja foram expostas a ambos os estresseres, a deficiência de P induziu maior exsudação de citrato durante a toxicidade de Al^{3+} (NIAN et al., 2003).

WARD et al. (2011) demonstraram que durante a toxicidade de Al, a deficiência de P aumentou a incorporação e metabolismo de CO_2 via PEPC (fosfoenolpiruvato carboxilase), levando a uma maior produção e exsudação de ácidos orgânicos derivados da PEPC durante a toxicidade por Al. Estes resultados indicam que o status do P pode influenciar a resposta ao Al

induzindo maior utilização de ácidos orgânicos derivados da PEPC para a destoxificação do Al.

Entretanto, em plantas expostas concomitantemente ao P e Al a massa seca tanto da parte aérea quanta das raízes aumentou com a suplementação de P sobre estresse de Al, demonstrando que a aplicação de P aliviou o efeito tóxico do Al sobre o crescimento de plantas de *Citrus grandis* (CHEN et al., 2009), arroz (NAKAGAWA; MORI; YOSHIMURA, 2003) e *Lespedeza bicolor* (SUN et al., 2008). Esta resposta pode ser devido à formação de complexos poliméricos entre o Al e o P tanto na solução externa quanto dentro das raízes (parede celular e vacúolo) (DELHAIZE; RYAN, 1995; NIAN et al., 2003). CRAWFORD; MARSHALL; WILKENS (1998) observou em plantas expostas ao Al um aumento da concentraçao de P em sítios de acumulação de Al ou externamente, devido o efluxo de P para sítios extracelulares (MARIENFELD; STELZER, 1993; OWNBY, 1993). Portanto, em plantas expostas ao Al a presença de altos níveis de P interno ou externo pode promover maior quantidade de P como substrato para a formação de complexos poliméricos Al - fosfato, que em tratamentos com deficiência de P (WARD et al., 2011).

Em vista disto, o estudo do potencial genético-adaptativo de espécies tolerantes ao Al^{3+} e a deficiência de P em solos é um aspecto relevante para países em desenvolvimento, como é o caso do Brasil. A co-ocorrência dos estresses de Al e P indica a possibilidade das espécies terem desenvolvido mecanismos similares de adaptação. Tendo em vista a característica ácida dos solos do Rio Grande do Sul e sendo a batata cultivada em grande área nesse Estado, torna-se relevante analisar o comportamento de diferentes genótipos de batata com relação ao Al^{3+} e ao P, tanto nos aspectos nutricionais (disponibilidade de nutrientes e crescimento) quanto nos bioquímicos (atividade de enzimas envolvidas no metabolismo da aquisição de nutrientes e no sistema protetor das plantas).

1.1 Objetivos

Objetivo geral

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

Objetivos específicos

1. Classificar os genótipos de batata quanto à tolerância e sensibilidade ao Al^{3+} e a eficiência de utilização do P.

2. Verificar se a atividade da enzima fosfatase ácida está relacionada a tolerância ao Al e a eficiência de utilização do P em genótipos de batata.

3. Investigar e comparar respostas fisiológicas e de estresse oxidativo de genótipos de batata diferindo quanto a tolerância e a sensibilidade ao Al^{3+} em um sistema de cultivo hidropônico.

4. Analisar a influência do estresse de alumínio no conteúdo de nutrientes em genótipos de batata.

5. Examinar os efeitos locais e/ou sistêmicos do alumínio em parâmetros bioquímicos de genótipos de batata expostos ao alumínio crescendo em sistema de raízes divididas.

1.2 Organização da Tese

Capítulo 2 – Manuscrito I: Aluminum tolerance and phosphorus utilization efficiency are not strictly correlated to acid phosphatase activity in potato genotypes

O objetivo desse trabalho foi classificar os genótipos quanto a sua tolerância ou sensibilidade ao Al. Posteriormente verificar se a tolerância ao Al estava associada a eficiência de utilização do P e a atividade da enzima fosfatase ácida. Esse manuscrito foi submetido a revista Plant Physiology and Biochemistry.

Capítulo 3 – Manuscrito II: Mineral nutrition of potato genotypes with distinct physiological sensitivity to Al stress

O objetivo desse estudo foi verificar se a tolerância ao Al está relacionada ao aumento da concentração de macro e micronutrientes em genótipos de batata. Esse manuscrito não foi submetido.

Capítulo 4 – Manuscrito III: Nutrient uptake and translocation in Al-sensitive and Al-tolerant potato genotypes as affected by localized supply of aluminum in a split-root system

O objetivo desse trabalho foi investigar se a captação e translocação de nutrientes é mais pronunciada no genótipo tolerante do que no genótipo sensível ao Al ambos expostos a uma suplementação heterogênea de Al. Esse manuscrito não foi submetido.

Capítulo 5 – Manuscrito IV: Biochemical and physiological responses in two potato genotypes (*Solanum tuberosum*) that differ in Al-avoidance by localized supply of aluminum in a split-root system

Com o objetivo de verificar as respostas fisiológicas e bioquímicas induzidas pela suplementação localizada de Al, nesse trabalho foram utilizados dois genótipos de batata com distinta resposta ao Al. Um genótipo tolerante ao Al (SMIC148-A) e um genótipo sensível ao Al (Dakota Rose). Esse manuscrito não foi submetido.

Capítulo 7 – Conclusão

Este capítulo descreve as conclusões gerais.

2 ALUMINUM TOLERANCE AND PHOSPHORUS UTILIZATION EFFICIENCY ARE NOT STRICTLY CORRELATED TO ACID PHOSPHATASE ACTIVITY IN POTATO GENOTYPES

Liana Veronica Rossato, Luciane Almeri Tabaldi, Bibiana Silveira Moraes, Vanessa Menezes Ocom, Dilson Antonio Bisognin, Fabiane Goldschmidt Antes, Valderi Luiz Dressler, Fernando Teixeira Nicoloso,

Abstract

The objective of this study was to evaluate whether the potato genotypes that are Al-tolerant show also high P utilization efficiency (PUE), as well as evaluate whether this interaction is related to a higher tissues acid phosphatase (EC 3.1.3.2) (APase) activities. In experiment one, eight potato genotypes (SMIC148-A, Dakota Rose, S. microdontum, SMINIA793101-3, SMIB106-7, SMIF212-3, SMIG145-1 and SMIJ319-7), showing different PUE, were grown in nutrient solution (pH 4.0) with 0 and 200 mg Al L^{-1} and without P for 12 days. Based on growth parameters, the genotypes were classified as Al-tolerant (SMIF212-3, SMIC148-A and S. microdontum), Al-intermediate (SMINIA793101-3 and SMIB106-7) and Al-sensitive (SMIJ319-7, Dakota Rose and SMIG145-1). In experiment two, four genotypes selected in the experiment one that showed contrasting Al response and PUE (no efficient: SMIC148-A and SMIG145-1; and efficient SMIF212-3 and Dakota Rose) were grown in a nutrient solution (pH 4.0) with 0, 25 and 125 μM P and with 0 or 200 mg Al L^{-1} for 10 days. The results show that Al tolerance in potato might be associated with higher tissue Al immobilization due to the higher tissue P content, mainly in the leaves. Furthermore, Al sensitivity in the potato genotypes under P-starvation condition was associated with decreasing P utilization and translocation efficiencies. Based on plant fresh and dry weight and consumption of nutrient solution per plant, P supply did not influence the Al tolerance response. In both experiments, it was not observed a straight relationship between tissues APase activities and PUE.

Keywords: acid phosphatase, aluminum, phosphorus, potato, P utilization efficiency, tolerance

2.1 Introduction

Potato (*Solanum tuberosum* L.) is grown worldwide under a wider range of altitudes, latitudes, and climatic conditions than any other major food crop – from sea level to over 4000 m elevation (SIECZKA; THORNTON; CHASE, 1992). 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 grow under unfavorable conditions such as drought, cold, salinity and high irradiation (LI; 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. TABALDI et al. (2007) proposed that the reduced growth in aluminum (Al)-sensitive genotypes of potato exposed to toxic levels of Al might be induced by an enhanced production of toxic oxygen species (ROS) and subsequent lipid peroxidation. Moreover, it was shown that Al-tolerant genotypes developed some defense mechanisms against oxidative stress. TABALDI et al. (2009) observed that Al-tolerant genotype (SMIC148-A) had a more efficient antioxidant system, which resulted in higher tolerance to Al. Thus, the finding that *Solanum* species is a suitable plant for studying other aspects of abiotic stress resistance mechanisms.

Acidic tropical soils, which constitute about 40% of world arable soil, are often characterized by high concentration of Al, low total and available phosphorus (P) content and high P retention capacity (LENOBLE et al., 1996). It has been generally accepted that Al toxicity and P deficiency are the primary factors limiting crop growth and production in acid soils (KOCHIAN; HOEKENGA; PIÑEROS, 2004). Ionic Al is highly toxic to plant growth and appears to interfere with a number of physiological and biochemical processes (KOCHIAN; PINEROS; HOEKENGA, 2005). Aluminum is reported to increase cell wall rigidity by crosslinking pectins, reduce DNA replication, interfere with cell division, fix P in less available forms in soils and on plant root surfaces, decrease root respiration (PIETRASZEWSKA, 2001), interfere in enzyme activity (PEREIRA et al., 2006; TABALDI et al., 2011), induce oxidative stress (TABALDI et al., 2007, 2009), modify structure and function of plasma membranes, reduce water uptake, and interfere with the uptake, transport and metabolism of several nutrients (PIETRASZEWSKA, 2001; TABALDI et al., 2009).

Although many studies have been conducted on plant Al tolerance and P efficiency, Al toxicity and P deficiency are almost always studied separately as independent factors (JEMO

et al., 2007; YAN; LYNCH; BEEBE, 1995). Since P deficiency and Al toxicity commonly coexist in acid soils, it is assumed that plants with good performance on acid soils might be both P efficient and Al tolerant. Consistent with this assumption, recent studies showed that P-efficient genotypes of soybean and buckwheat had increased Al tolerance, possibly through precipitating or chelating toxic Al around roots (LIAO et al., 2006; ZHENG et al., 2005). Plants have evolved a number of adaptive mechanisms for growth on low-P and high Al soils, and these include the exudation of several solutes from roots, including organic acids, phosphatases, and other compounds that may mobilize P from bound P pools in the soil (Fe-P, Al-P compounds, and organic phosphate esters) and thus contribute to P efficiency and Al tolerance in plants (LIAO et al., 2006).

Relatively few studies have been done to investigate Al and P interactions in plants. CHEN et al. (2009) found that increasing P supply might have a role in ameliorating Al phytotoxicity, possibly due to the increased P concentration in roots and leaves. DONG; PENG; YAN (2004) provided evidence for root Al and P interactions that had an influence on soybean growth. ZHENG et al. (2005) found that the P content of the root apex of buckwheat was significantly correlated with the immobilization and detoxification of Al, indicating that there can be a significant P-Al interaction in roots.

Acid phosphatases (APases) are a group of enzymes that catalyze the hydrolysis of a variety of phosphate esters releasing Pi from phosphorylated substrates in acidic environments (YONEYAMA et al., 2007). They are widely distributed in plants (LUHOVÁ et al., 2006), and are present in the apoplast and in different cell compartments (YONEYAMA et al., 2007), suggesting that these enzymes are involved in various metabolic pathways. They appear to be important in the production, transport and recycling of Pi (GARCIA et al., 2004). A number of studies have reported that secretion and expression of APases are enhanced under P and Al stress (BOZZO; RAGHOTHAMA; PLAXTON, 2004; CIERESZKO; ŻEBROWSKA; RUMINOWICZ, 2011; HUTTOVÁ; TAMÁS; MISTRIK, 2002). However, a negative relationship was also observed between root APase activity and P uptake by wheat under phosphorus stress (MCLACHLAN et al., 1987). In addition, HUNTER; MCMANUS (1999) found no significant difference in root surface APase among white clover genotypes with contrasting P-efficiency. In common bean, the induction of a major leaf APase did not confer adaptation to low P availability in P-efficient genotype (YAN et al., 2001). Therefore, the role of APase in plant adaptation to low P availability is unclear. Thus, the objective of this study was to evaluate whether the

potato genotypes that are Al-tolerant show also high P utilization efficiency, as well as evaluate whether this interaction is related to a higher acid phosphatase (APase) activity in the plant tissues.

2.2 Materials and methods

Experiment 1 - Screening for Al tolerance

Plant materials and growth conditions

Seven adapted (2n=4x=48) potato (*Solanum tuberosum L.*) genotypes [SMIC148-A (C), Dakota Rose (D), SMINIA793101-3 (F), SMIB106-7 (J), SMIF212-3 (M), SMIG145-1 (O) and SMIJ319-7 (S)] and one wild species (2n=2x=24) genotype [PI595511-5/ *Solanum microdontum* Bitter (E)] were evaluated in this study. These genotypes were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, RS, Brazil. In previous studies, based on the total biomass of output (e.g., tuber yield) under two P levels (4.64 and 46.46 mg L^{-1} in nutrient solution), these genotypes were classified as: P utilization efficient and responsive [ER] (D and S), P utilization efficient and nonresponsive [ENR] (F and M), no efficient and responsive [NER] (C), and no efficient and nonresponsive [NENR] (E, J and O) (Nicoloso, personal communication).

Fourteen-day-old plants (shoot length of five centimeters) grown in pots containing sand were transferred into plastic vessels (1.5 L) containing 1300 g sand as substrate and grown in greenhouse. Each vessel received one plantlet. Each experimental unit consisted of five plants, totalizing three replicates per treatment. Throughout cultivation, sand was maintained at 80% of field capacity (195 ml), determined with a sample altered on a tension table. Irrigation was performed daily by replacement of both transpired and evaporated water, calculated by weighing the vessels. In total, 125 vessels were prepared, where 120 vessels received one plant which were used to calculate the water lost by transpiration, and the remaining 5 vessels, containing only sand, were used to measure water evaporation. Evaporated and transpired water was daily replaced with nutrient solution, which had the following composition (mg L^{-1}): 85.31 N; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 176.76 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe. The pH solution was adjusted to 4.0 ± 0.1 with a 1 M solution of HCl or NaOH.

After four days of plant acclimatization, Al in the form of $AlCl_3$ was added to a nutrient solution at concentrations of 0 (control) and 200 mg Al L^{-1}, which did not contain P. The treatments were applied daily for 12 days. For biochemical analysis, at the end of the experiments the plants were gently washed with distilled water dried with towel paper and then divided into leaves, stem, root, stolon and tubers, which were frozen immediately in liquid N_2 and stored at -86 ^{o}C. To obtain the fresh weight, excess water was removed with a paper towel after root washing. To obtain dry weight, the plants were left at 65^{o}C to a constant weight.

Experiment 2 - Effect of P supply on Al tolerance

Plant materials and growth conditions

From experiment two, four potato genotypes contrasting in Al tolerance (C and M, Al-tolerant; D and O, Al-sensitive) were selected to investigate the effect of P on Al tolerance. The experimental design used and the growth conditions were the same of experiment one.

After four days of plant acclimatization, treatments were initiated and applied daily for 10 days. There were six treatments in total, including three P levels (0, 25 and 125 μM P as KH_2PO_4) and two Al levels (0 and 200 mg Al L^{-1} as $AlCl_3$) in the nutrient solution. The pH solution was adjusted to 4.0$\pm$0.1 with a 1 M solution of HCl or NaOH. At the end of the experiments, the growth and biochemical parameters were determined as in experiment 1.

Al and P concentration and P utilization and translocation efficiency

Al and P concentration was determined in roots, leaves and stems. Dried plant tissues, between 0.01 and 0.25 g, were ground and digested with 5 ml of concentrated HNO_3. Sample digestion was carried out in an open digestion system, using a heating block Velp Scientific (Milano, Italy). Heating was set at 130^{o}C for 2 h. Plastic caps were fitted to the vessels to prevent losses by volatilization. The Al and P content was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-EOS), using a PerkinElmer Optima 4300 DV (Shelton, USA) equipped with a cyclonic spray chamber and a concentric nebulizer. The P utilization efficiency (PUE) in the plant was calculated as follows: (mg total dry weight)^2/(μg total P accumulated in plant) (SIDDIQI; GLASS, 1981). The P translocation efficiency (PTE) in the plant was calculated as follows: (total P accumulated in aboveground tissues)/(total P accumulated in plant) (SIDDIQI; GLASS, 1981).

Growth parameters and consumption of nutrient solution

Growth of potato genotypes was determined by measuring the fresh and dry weight of leaves, stem, roots, stolon and tuber, and shoot length. The plant materials were oven-dried at 65°C to a constant weight for the determination of biomass. The consumption of nutrient solution was calculated based on the water that was absorbed by a plant, corresponding to the weight of water lost by each vessel containing one plant minus the average water lost by a vessel without a plant.

Acid phosphatases assay

Frozen roots and leaves were centrifuged at 20,000 g for 30 min and the resulting supernatant was used for enzyme assay. Acid phosphatase activity was determined according to TABALDI et al. (2007) 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 was added to the reaction mixture, except in controls, and pre-incubated 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% TCA to a final concentration of 5%. Inorganic phosphate (Pi) was quantified at 630 nm using malachite green as the colorimetric reagent and KH_2PO_4 as standard for the calibration curve. All assays were performed using PPi as substrate at a final concentration of 3.0 mM.

Soluble phosphorus content (Pi)

The same material utilized in the acid phosphatases assay was utilized to quantify the soluble phosphorus content. An aliquot of the diluted sample (800 μL) was incubated at 45°C for 45 min in a medium containing 2.5 N sulfuric acid, 4.8 mM ammonium molybtate and 35 mM ascorbic acid in a total volume of 1 mL. A standard curve was constructed using KH_2PO_4. After cooling at room temperature the samples were read at 650 nm.

Statistical analysis

The analyses of variance were computed for statistically significant differences determined based on the appropriate F-tests. The results are the means $\pm$ SD of at least three independent replicates. The mean differences were compared utilizing Tukey test at $P<0.05$.

2.3 Results and Discussion

Experiment 1 - Screening for Al tolerance

Growth parameters and consumption of nutrient solution

The Al toxicity and P deficiency have many effects on the growth and physiological processes in many plants (JEMO et al., 2007; JIANG et al., 2009; HE et al., 2011). In this study, shoot length, nutrient solution consumption and total fresh and dry weight demonstrated to be suitable parameters for screening potato genotypes for Al tolerance (Fig. 2.1). Significant differences were observed in shoot length among the potato genotypes under Al stress (Fig. 2.1I). The shoot length in potato genotypes D, O and S were significantly decreased by Al exposure, with inhibitions of 17%, 21% and 14%, respectively. On the other hand, C, E, F, J and M genotypes did not show any alteration in shoot length at 200 mg Al L^{-1} (Fig. 2.1I). The consumption of nutrient solution by plant per day decreased with Al exposure in the potato genotypes D, F, O and S (25%, 10%, 14% and 8%, respectively), whereas it increased in M genotype at 200 mg Al L^{-1} (Fig. 2.1II). The Al exposure caused a decrease of 34%, 17% and 31% in total fresh weight, respectively, for D, J and S potato genotypes (Fig. 1III). On the other hand, the M genotype showed increase in fresh weight at 200 mg Al L^{-1} (Fig. 2.1III). In addition, at the same concentration, it was observed a decrease in total dry weight of D and S genotypes (19% and 13%, respectively) (Fig. 2.1IV). Furthermore, the O genotype showed increase of 53% in its dry weight at 200 mg Al L^{-1} (Fig. 2.1IV). Aluminum apparently interacts directly and/or indirectly with factors that influence shoot length, primarily affecting the root tips (PIETRASZEWSKA, 2001). Effects of Al 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; HORST, 2001). Furthermore, Al can act directly on the shoot length due to cellular and ultrastructural changes in leaves, reduction of stomatal aperture, decreased photosynthetic activity, increase of lipid peroxidation and decrease of enzyme activity (PIETRASZEWSKA, 2001; TABALDI et al., 2007). The remarkable reduction in nutrient solution consumption in the D, F, O and S genotypes (Fig. 2.1II), which might be related to a decrease in leaf area that can per se reduce the transpiration rate (KOCHIAN; PINEROS; HOEKENGA, 2005). Moreover, it was reported that Al caused reduction of stomatal aperture (ÖZYIĞIT; AKINCI, 2009; PIETRASZEWSKA, 2001). On the other hand, it was observed an increase in nutrient solution

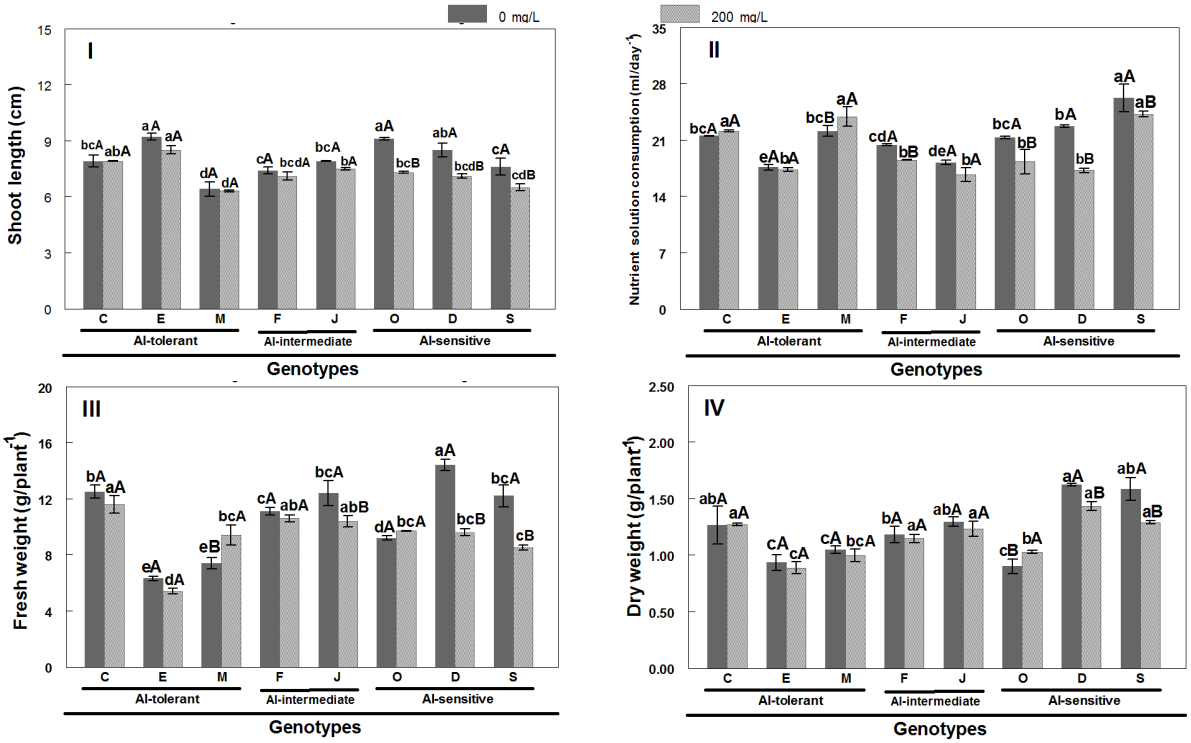

Figure 2.1 – Effect of increasing Al level on shoot length (I), nutrient solution consumption (II), total fresh (III) and dry weight (IV) in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean ± S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

consumption in the M genotype at 200 mg Al L^{-1}. This may be related with increase in total fresh weight (Fig. 2.1III). TANG et al. (2002) suggested that Al-tolerant genotype used more water when grown in acid soil due to its higher root depth and root length, and greater shoot growth than the Al-sensitive genotype. Therefore, Al-tolerant plants would be expected to be better able to exploit water and nutrient reserves (TANG et al., 2002). Based on our results, the potato genotypes were classified as Al-tolerant (M (more tolerant), C and E), Al-intermediated (F and J) and Al-sensitive (D, S (more sensitive) and O).

Al and P concentration

The concentration of Al in both the roots and leaves of all potato genotypes studied increased with the Al exposure, except for S genotype in the leaves (Fig. 2.2I, II). Interestingly, in the leaves, the higher increases in Al concentration were observed in the Al-tolerant genotypes

[C (121%) and E (84%)] which showed higher increases in the tissue P concentration (7% and 6%, respectively) with the Al exposure (Fig. 2.2I, III). On the other hand, in the D, O (Al-sensitive) and F, J (Al-intermediate) genotypes, the Al exposure caused an increase in tissue Al concentration (Fig. 2.2I) and decreased P concentration (8%, 14%, 4% and 11%, respectively) (Fig. 2.2III). These data suggest that the immobilization of Al in leaves by precipitation with P might contribute to the genotypic differences in potato. The formation of Al-P complexes like Al_4 $(PO_4)_3$, may be helpful by retarding the uptake of Al into the cytosol (ZHENG et al., 2005). Furthermore, VÁZQUEZ et al. (1999) reported that Al resistance in maize relied on the active transport of Al-P complex from the cell wall to vacuoles. In the roots, the increase of Al concentration caused an increase in the P concentration of 15%, 7% and 5%, respectively, in the S (Al-sensitive), E (Al-tolerant) and J (Al-intermediate) genotypes by Al exposure (Fig. 2.2II, IV). GAUME; MÄCHLER; FROSSARD (2001) proposed that the Al tolerance of maize was associated with the immobilization of Al by P in the root tissues. On the other hand, in the present study, it was observed a decrease in P concentration of 38%, 37%, 31% and 18%, respectively, in the M (Al-tolerant), O, D (Al-sensitive) and F (Al-intermediate) genotypes (Fig. 2.2IV). In both organs (leaves and roots) the O, D (Al-sensitive) and F (Al-intermediate) genotypes demonstrated the same response with the Al exposure. Both genotypes showed decrease in P concentration by Al exposure (Fig. 2.2I, II, III and IV).

Aluminum fixes phosphorus in less available forms in soils and on plant root surfaces. Once within the cell, Al may react with P compounds, and negatively affects the plant P metabolism. Furthermore, Al interferes with plasma membrane and cell wall which interfere with their properties and architecture interfering with uptake of nutrients (PIETRASZEWSKA, 2001; SILVA et al., 2010).

The inhibition of tissue phosphorus accumulation by Al was already shown by PIETRASZEWSKA (2001); SILVA et al. (2010). Interestingly, this effects was more observed in Al-sensitive (D and O), except for S, and Al-tolerant intermediate genotypes (J and F). In contrast, the Al-tolerant genotype (M) demonstrated the higher decrease in P concentration and the second higher Al concentration in the root, which was not accompanied with alteration in the growth parameters (Fig. 2.1 and 2.2). GAUME; MÄCHLER; FROSSARD (2001) observed that the superior Al-tolerant cultivars had a higher capability to utilize P.

Higher Al accumulation was observed in the Al-tolerant genotypes (C and E (leaf), and E and M (root) than in Al-sensitive genotypes (Fig. 2.2I, II) corroborating findings by other

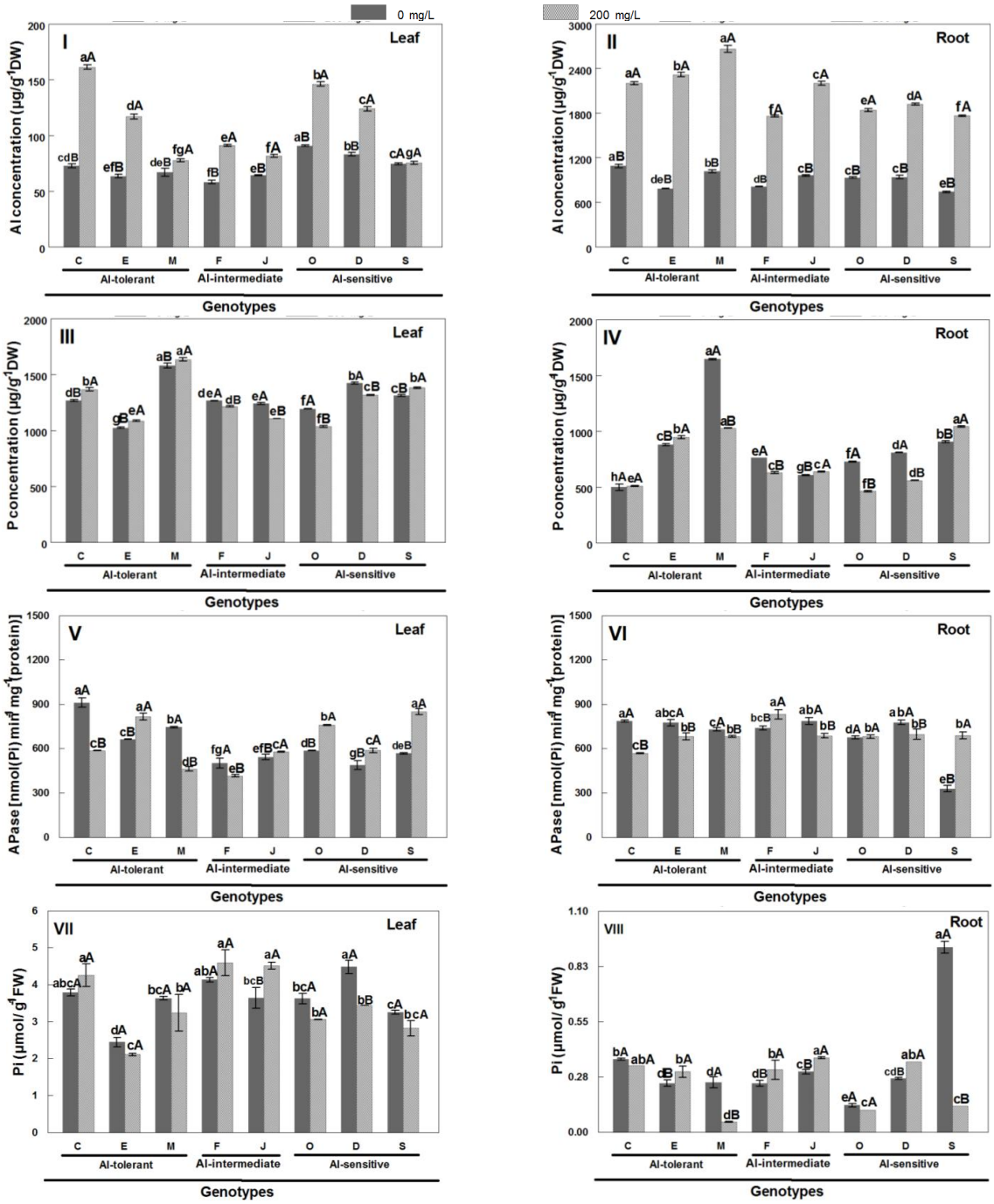

Figure 2.2 – Effect of increasing Al level on Al concentration in leaf and root (I, II), P concentration in leaf and root (III, IV), acid phosphatase activity in leaf and root (V, VI) and soluble P concentration in leaf and root (VII, VIII) in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean ± S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

authors (HORST; PÜSCHEL; SCHMOHL, 1997; JEMO et al., 2007). In addition, WANG; STASS; HORST (2004) suggested that the total Al concentration in the root tip may not be the main factor for Al toxicity. The higher Al concentration in the leaves upon addition of Al levels in the Al-tolerant genotypes (C and E) seemed to indicate that exclusion of Al from uptake and translocation are not the main mechanisms of Al tolerance in these potato genotypes.

Acid phosphatase activities (APases) and soluble phosphorus (Pi) concentration

APases function in the production, transport and recycling of Pi, a crucial macronutrient for cellular metabolism and bioenergetics (VELJANOVSKI et al., 2006). Intracellular APases are believed to remobilize and scavenge Pi from intracellular P monoesters and anhydrides in Pi-deficient plants. This is accompanied by marked reductions in cytoplasmic P-metabolic pools during extended P stress (VELJANOVSKI et al., 2006). Extracellular and intracellular APases activities increase under Pi deficiency in many plant species. However, the level of APases activity (and secretion from roots) quite often may differ between plant species and between varieties (CIERESZKO; ŻEBROWSKA; RUMINOWICZ, 2011).

Al stress produced significant effects on APase activity in all potato genotypes. In the leaves, APase activity was activated in the D, E, J, O and S genotypes and inhibited in the C, F and M genotypes (Fig. 2.2V). The APase activity in the leaves was positively correlated with Al concentration in the Al-sensitive (D and O) and Al-intermediate (E and J) genotypes (r = 0.67, 0.93, 0.77 and 0.81, respectively) and negatively correlated in the Al-tolerant (C and M) and Al-Intermediate (F) genotypes (r = -0.97, -0.87 and -0.90, respectively). In the roots, the APase activity was increased in the Al-intemediate (F) and Al-sensitive (S) genotypes (r = 0.85 and 0.70, respectively) and inhibited in the Al-sensitive (D), Al-intemediate (E and J) and Al-tolerant (C and M) genotypes at 200 mg Al L^{-1} (Fig. 2.2VI). The Al-intermediate (J) and Al-tolerant (C and E) genotypes showed negative correlation (r = -0.93, -0.98 and -0.94, respectively) with APase activity.

The increase in APase activity could simply be the result of decreasing P concentration leading to activation of enzyme. HUTTOVÁ; TAMÁS; MISTRIK (2002) showed that different behavior of APase enzyme in barley cultivars during Al stress may play an important function in coping by the plants with Al induced P deficiency syndrome. In the leaves, the increase in the APase activity in the D and O (Al-sensitive) genotypes might be due the decrease P concentration with increase Al concentration (r = -0.98 and -0.87, respectively). On the other

hand, the decrease in the APase activity in the C and M (Al-tolerant) genotypes might be due the increase P concentration with increase Al concentration (r = 0.94 and 0.60, respectively).

The Al exposure led to either an enhancement of leaf soluble phosphorus (Pi) concentration only in J genotype or decreased in the D genotype. On the other hand, the root Pi concentration was increased in the D, E, F and J genotypes by Al exposure. In the M and S genotypes, the increase in the Al level caused a decrease in Pi concentration at 200 mg Al L^{-1}. Furthermore, plants accumulated higher Pi concentration in leaves than in roots (Fig. 2.2VII, VIII). In many plants, Al toxicity resembles P, Ca or Fe deficiency syndrome (PIETRASZEWSKA, 2001). Aluminum forms insoluble and stable complexes with inorganic and organic phosphates, therefore their solubilization is a prerequisite for P uptake by plants (MA; RYAN; DELHAIZE, 2001). Some studies correlate the increase in APase activity with Al-tolerance. HUTTOVÁ; TAMÁS; MISTRIK (2002) observed significant difference in the APase activity between Al-tolerant and Al-sensitive barley cultivars, being that the APase activity increased linearly with increasing Al concentration in the Al-tolerant, the same was not observed for Al-sensitive. Similar results were described by PATRA; LENKA; PANDA (1994) where higher activation of APase was observed in tolerant grass than in non-tolerant during mercury and cadmium treatment. However, our results did not demonstrate a straight relationship between Al-tolerance and APase activity. In general, the leaf and root APase activity decreased in the Al-tolerant genotypes (C, E and M), whereas, in the Al-sensitive (D, O and S) it was observed an increase of APase activity mainly in the leaf (Fig. 2.2).

Phosphorus deficiency usually has a significant impact on root-associated APase activities (CIERESZKO; ŻEBROWSKA; RUMINOWICZ, 2011). However, some studies showed negative relationship between APase activity and the Pi uptake efficiency under Pi starvation (YAN et al., 2001; YUN; KAEPPLER, 2001). Experiments on maize varieties with contrasting Pi uptake efficiency suggested that APase might not be the main mechanism for acquiring Pi (YUN; KAEPPLER, 2001). In this study, we did not observe clear relationship between APase activity and the P efficiency in the P-efficient genotypes (D, S, F and M) in both leaves and roots (Fig. 2.2V, VI). However, it has been demonstrate that increased APase is often associated with P deficiency symptoms in the plant (LI et al., 2011; WASAKI et al., 2003). Therefore, our data suggest a minor role for APase activity in relation to P utilization efficiency. Because exist generally an inverse relationship between APase and P concentration in some plants, it has been suggested that APase could be used as diagnostic criterion for P deficiency (MCLACHLAN

et al., 1987).

P utilization and translocation efficiency

Phosphorus utilization efficiency (PUE) is defined as the ability of the genotype to produce higher dry matter yield per unit of P in the tissue compared to other genotypes under P limiting condition (BLAIR, 1993). Based on the total plant biomass production, in the control treatment (0 mg Al L^{-1}), the genotypes C, J, D and S had higher PUE compared to genotypes E, M and O, indicating that the former genotypes were P-efficient while the latter were P-inefficient (Fig. 2.3I). Furthermore, the F genotype was classified as intermediate in PUE. These results were not consistent with our previous results (NICOLOSO, F.T., personal communication). However, the previous classification was based on the total biomass of output (e.g., tuber yield). In addition, these genotypes differed effectively in the partitioning of dry weight into tubers with values reaching 40% (S genotype) to 70% (D genotype) of the total plant at mature harvest. Nonetheless, the PUE decreased by Al exposure in the D and S (Al-sensitive) genotypes (Fig. 2.3I). In contrast, in the O (Al-sensitive) genotype it was observed an increase by Al exposure (Fig. 2.3I). On the other hand, C, E, M, F and J genotypes did not show any alteration in PUE at 200 mg Al L^{-1} (Fig. 2.3I).

At 0 mg Al L^{-1}, the P translocation efficiency (PTE) was higher in the C, F and O and lower in genotypes E, M and D (Fig. 2.3II). The PTE decreased by Al exposure in the O and S (Al-sensitive) genotypes, whereas it increased in the E and M (Al-tolerant) genotypes.

The Al sensitivity observed in the D, O and S genotypes, based on growth parameters, might be due to the decrease in both PUE (D and S genotypes) and PTE (O and S genotypes) (Fig. 2.3). CHEN et al. (2009) showed that Al decreased the root and leaf P concentration. Once within the cell, Al may react with P compounds, and interfere with the plant P metabolism. QUARTIN; AZINHEIRA; NUNES (2001) observed that P deficiency is considered to be the key cause of growth reduction in Al-stressed plants. In the present study, the D genotype showed reduction in shoot length and total fresh and dry weight with increasing Al levels. The decrease in the growth parameters in the Al-sensitive D genotype may be related to decrease in the PUE with increasing Al levels (Fig. 2.3). However, this was not the case in the Al-sensitive O genotype. Although it was observed a decline in P concentration in all organs analyzed, it was observed an increase in the total dry weight. This response may be due to the increase in the PUE with increasing Al levels (Fig. 2.3I).

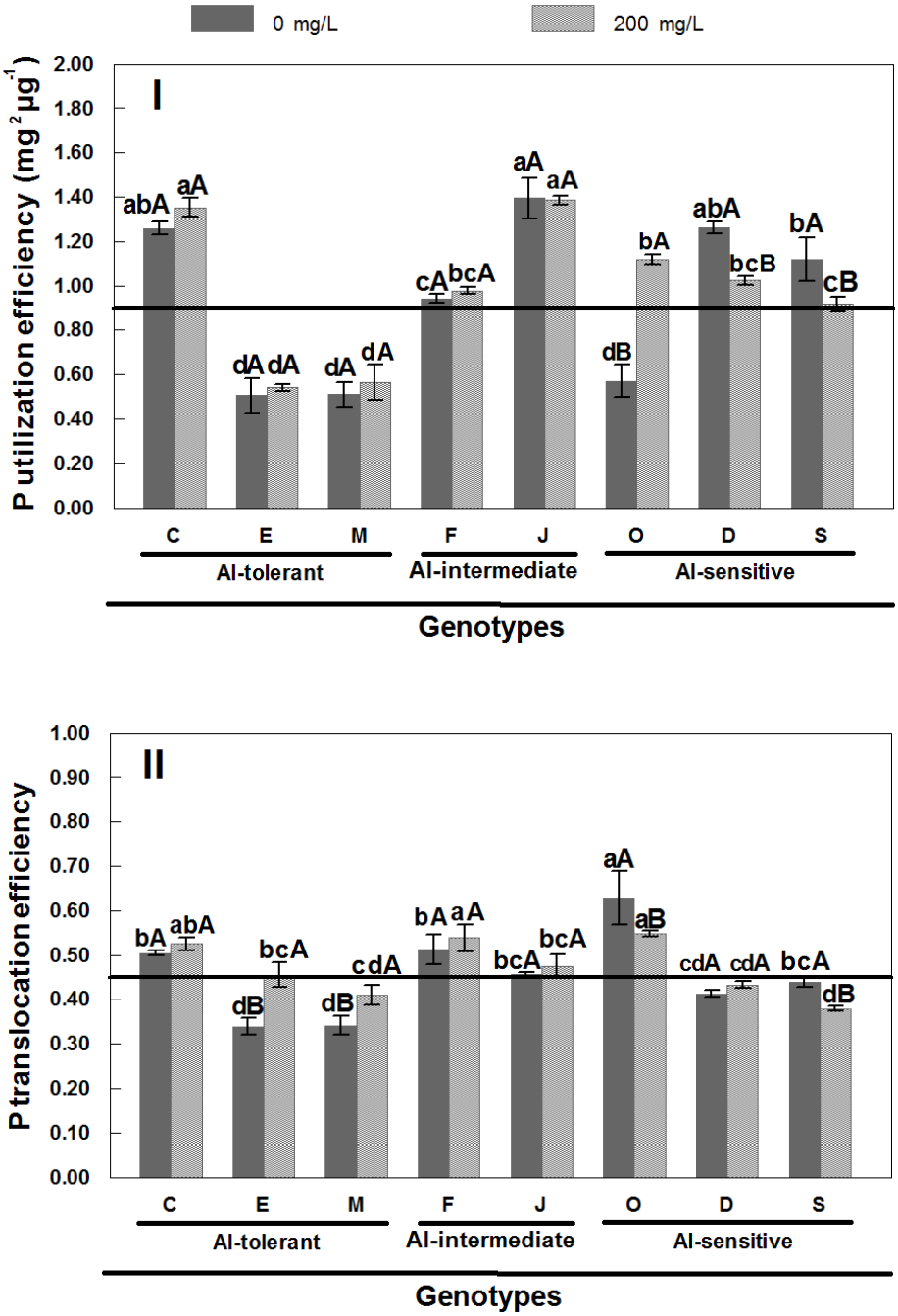

Figure 2.3 – Effect of increasing Al level on P utilization (A) and translocation (B) efficiency in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), Dakota Rose (D), SMIG145-1 (O) AND SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean ± S.D. of three replicates.The horizontal line represents the average of the P utilization (A) and translocation (B) efficiency among the genotypes in the 0 mg l^{-1}. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

Experiment 2. Effect of P supply on Al-tolerance

Growth parameters and consumption of nutrient solution

Al toxicity and P deficiency often coexist in acid soils, and therefore the relative ranking of Al tolerance in plants may be affected by P and Al interactions (LIAO et al., 2006). In soybean, Al tolerance was influenced by varying the P concentrations in the nutrient solution, under conditions where the solution Al^{3+} activity was held constant (LIAO et al., 2006). In the present study, four potato genotypes differing in Al tolerance (C and M (Al-tolerant) and D and O (Al-sensitive)) and P utilization and responsiveness efficiency (C (low PUE and high P responsiveness (NER)), D (high PUE and high P responsiveness (ER)), M (high PUE and low P responsiveness (ENR)) and O (low PUE and low P responsiveness (NENR)) were used (NICOLOSO, F.T, personal communication).

The shoot length of C, D, M and O potato genotypes increased (20%, 46%, 19% and 19%, respectively) in response to P supply in the absence of Al (Fig. 2.4I). Moreover, the two P-responsive potato genotypes (C and D) exhibited higher response to P supply than did the P-unresponsive (M and O). Under Al stress, shoot length increased with increasing P levels in the D and M genotypes (29% and 8%, respectively), whereas it decreased in the C genotype (Fig. 2.4I). In the absence of Al, the consumption of nutrient solution in C, D and O genotypes increased (20%, 12% and 13%, respectively) in response to P supply, whereas it did not change in the M genotype. On the other hand, it was observed a decreased in the consumption of nutrient solution in the D genotype (17%) with increasing P levels under Al stress (Fig. 2.4II).

The increase in P supply in the absence of Al caused an increase in the total fresh weight in all genotypes (48%, 46%, 36% and 29% in the D, C, M and O genotypes, respectively) (Fig. 2.4III). The two P-responsive potato genotypes (C and D) exhibited higher response to P supply. On the other hand, it was observed a decreased in the fresh weight in the C genotype (11%) with increasing P supply under Al stress (Fig. 2.4III). Furthermore, the increase in P supply in the absence of Al caused an increase in the dry weight in the C, D and M genotypes (26%, 69% and 24%, respectively) (Fig. 2.4IV). In addition, the P-responsive potato genotypes (C and D) exhibited higher response to P supply. On the other hand, the C and D genotypes showed decrease in dry weight with increasing P levels under Al stress (42% and 14%, respectively) (Fig. 2.4IV).

In most growth parameters evaluated, the four potato genotypes differing in Al tolerance

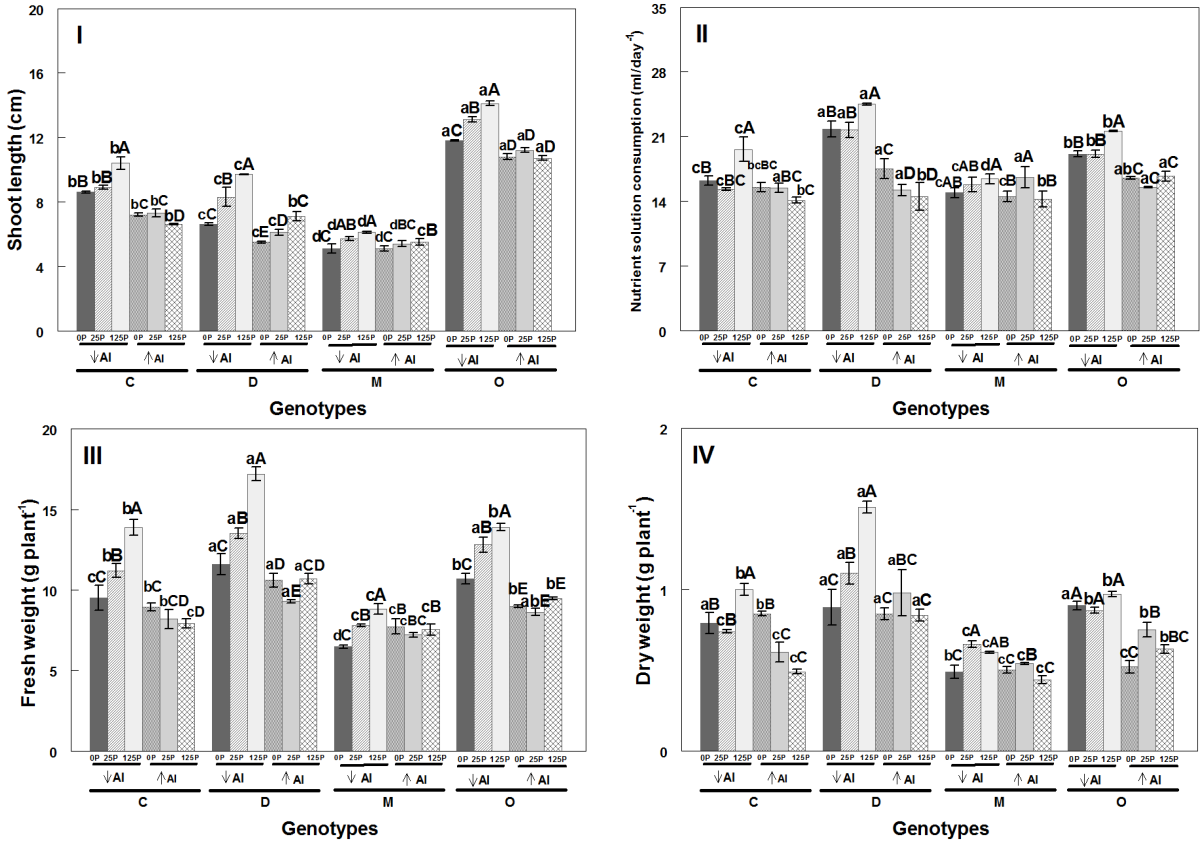

Figure 2.4 – Effect of Al-P interaction on shoot length (I), nutrient solution consumption (II), total fresh (III) and dry weight (IV) in SMIC148-A (C), Dakota Rose (D), SMIF212-3 (M) and SMIG145-1 (O) potato genotypes grown greenhouse. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between P/Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same P/Al level ($p < 0.05$).

and P efficiency did not significantly differ in Al tolerance under P-starvation and low level P (25 μM) conditions at 200 mg Al L^{-1} (Fig. 2.4). However, at higher level of P (125μM) in the nutrient solution with Al, the Al tolerance of two potato genotypes (D and M) was significantly increased, when based only on shoot length (Fig. 2.4I). Under these conditions, evidence for some differential Al tolerance response was noticed, as the two PUE genotypes (D and M) appeared to be more Al-tolerant than the P-inefficient genotypes (C and O). This agrees with some previous findings suggesting that P supply had positive effects with regard to ameliorating Al tolerance (GAUME; MÄCHLER; FROSSARD, 2001; LIAO et al., 2006; ZHENG et al., 2005). In many plant species, Al tolerance appears to be closely associated with PUE. Aluminum markedly increases the redox potential of root tissues and decrease the contents of high energy bond P (SLASKI et al., 1996). LIAO et al. (2006) demonstrated that P efficiency may

play a role in Al tolerance in soybean due to P-efficient genotypes may be able to enhance Al tolerance not only through direct Al-P interactions but also through indirect interactions associated with stimulated exudation of different Al-chelating organic acids, which in turn enhances plant tolerance to Al toxicity. Aluminum binding by organic acids prevents the formation of P-Al complexes, which results in an increased availability of P in the root cell.

In contrast, when the C potato genotype, which has low PUE, was exposed at higher level of P (125 μM) in the presence of Al, the shoot length and total dry and fresh weight were significantly decreased than that when it was exposed only at Al stress (Fig. 2.4). However, the same was not observed in O potato genotype. Furthermore, the exposition at higher level of P (125 μM) in the presence of Al caused a decrease in all growth parameters in the C genotype, when compared to the control treatment (0 mg Al L^{-1} and 0 μM P) (Fig. 2.4).

In the experiment 1, it was observed a positive correlation between the increase in tissue P concentration and plant Al tolerance in the Al-tolerant genotypes. The C genotype showed an increase in the P concentration in the leaf under Al-stress and P-starvation. The same occurred in the roots under Al-stress with P supply. CHEN et al. (2009) observed that root Al concentration increased with P supply in *Citrus grandis* due to increase in insoluble and non-toxic Al-P precipitates at the root surface and/ or in the root tissues. However, LIAO et al. (2006) observed that the exudation of organic acids was strongly induced by Al toxicity and P-starvation. However, Al-induced organic acids exudation was greatly decrease when roots were also grown on high-P levels, indicating that there is a clear Al and P interaction regarding root organic acids exudation. These organic acid anions can desorb P from mineral surfaces and solubilize P from Al-, Fe- and Ca-phosphates by chelating the metals (RYAN; DELHAIZE; JONES, 2001). The Al-P interaction in the C potato genotype did not affect the nutrient solution consumption; however it decreased the shoot length and total fresh and dry weights (Fig. 2.4). In addition, the increase of shoot length, nutrient solution consumption and total fresh and dry weight in response to P supply in the absence of Al was higher in the P-responsive potato genotypes (C and D) (Fig. 2.4).

Effects of P-Al interactions on acid phosphatase activities and soluble phosphorus (Pi) concentration

Increase of extracellular and intracellular APase activity under P deficiency is a common phenomenon in various plants. However, the level of APases activity (and secretion from

roots) quite often may differ between plant species and between varieties (CIERESZKO; ŻEBROWSKA; RUMINOWICZ, 2011). In the present study, in general, leaf APase activity in D, M and O genotypes decreased with increased availability P in the absence of Al (Fig. 2.5I). This response might be due to the increase in the P availability, which can be evidenced either by an increase or no change in the Pi concentration (Fig. 2.5III). Interestingly, in the C genotype it was observed an increase the APase activity concomitantly with decrease in Pi concentration. However, under Al stress, APase activity either decreased in the D and O (Al-sensitive) potato genotypes or increased in the C and M (Al-tolerant) potato genotypes with increasing P supply. DEL POZO et al. (1999) proposed that APase could be involved in P mobilization and in the metabolism of reactive oxygen species (ROS) in stressed or senescent parts of the plant. In kidney bean it was observed that purple acid phosphatase (PAP) showed an antioxidant role to prevent the formation of oxygen radicals in the seed (KLABUNDE et al., 1995). LI; SHAO; LAM (2008) observed that expression of PAP (*GmPAP3*) in transgenic tobacco may play a role in stress tolerance by enhancing ROS scavenging. The activities and gene expression of most plant PAPs were frequently found to be P-regulated (induced under P starvation) (LI et al., 2002; DEL POZO et al., 1999), consistent with their roles in P metabolism. However, a study of the PAP gene family in *A. thaliana* showed that some of the gene members are unresponsive to P status (LI et al., 2002), suggesting that some members may be involved in other physiological functions.

In the root, APase activity of D and O genotypes decreased with increasing P levels in the absence of Al. In the C and M genotypes the root APase activity was not altered with P supply. In addition, under Al stress, APase activity decreased in the C, M and O genotypes with P supply (Fig. 2.5II). This response of APase activity in the M and O genotypes may be related to increase in the Pi concentration in these genotypes. However, APase activity was not related to Pi concentration in C and D genotypes (Fig. 2.5II, IV).

Intracellular APases are important mainly for remobilization of internal Pi source, e.g. from vacuole (TOMSCHA et al., 2004; XIAO et al., 2006). CIERESZKO; ŻEBROWSKA; RUMINOWICZ (2011) observed that P-sufficient plants decrease internal APase activities in extracts from barley shoots and roots as compared to P deficient plants. In potato, TABALDI et al. (2011) showed that the effects of Al toxicity on in vitro APase activity depends not only on Al availability but also on the plant organ, genetic background, and the growth system evaluated, suggesting that acid phosphatase activity assessed in vitro might not be a good parameter to

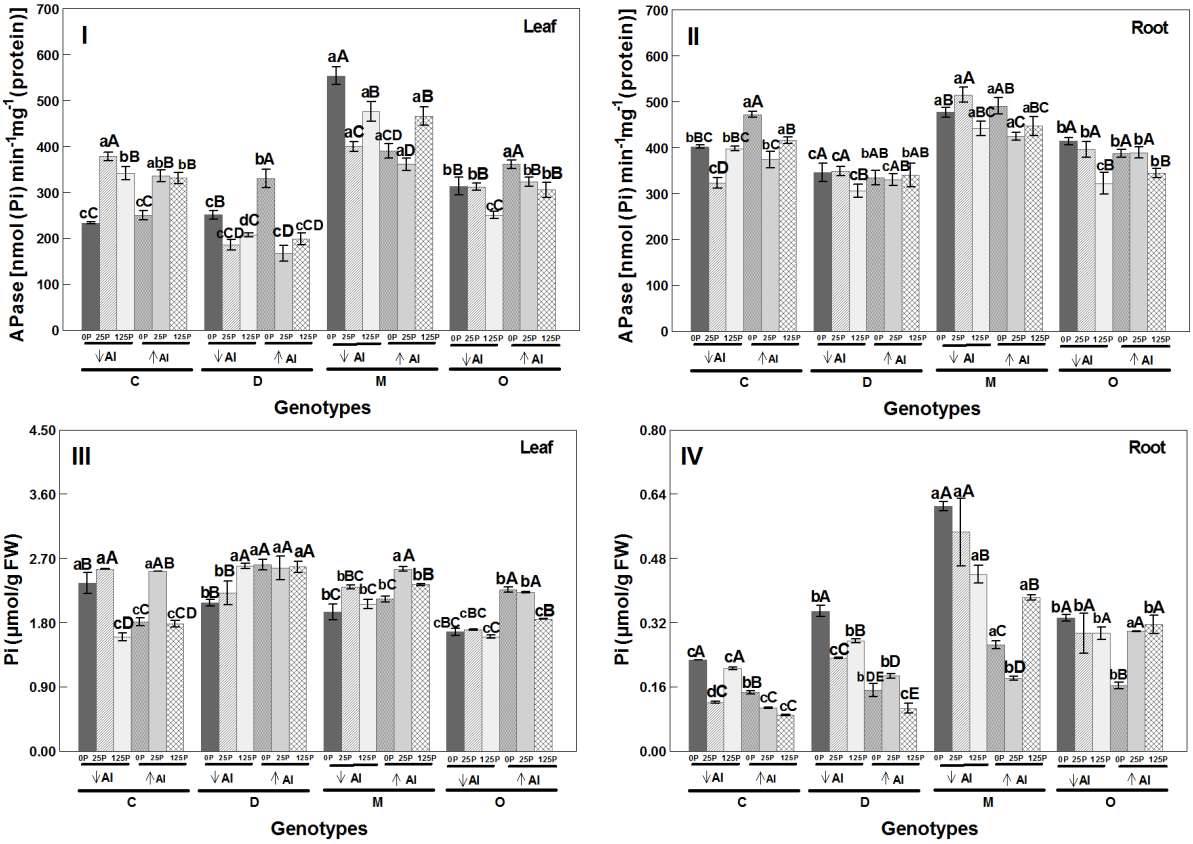

Figure 2.5 – Effect of Al- P interaction on acid phosphatase activity in leaf and root (I, II) and soluble P concentration in leaf and root (III, IV) in SMIC148-A (C), Dakota Rose (D), SMIF212-3 (M) and SMIG145-1 (O) potato genotypes grown in greenhouse. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between P/Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same P/Al level ($p < 0.05$).

validate the screening for adaptation of potato genotypes to Al toxicity.

2.4 Conclusion

In the present study, the results show that Al tolerance in potato might be associated with higher tissue Al immobilization due to the higher tissue P content, mainly in the leaves. Furthermore, under Al stress condition, it was evidenced that the Al sensitivity in the potato genotypes under P-starvation condition is associated with the decrease in P utilization and translocation efficiencies. Moreover, the APase activity was not associated with P utilization efficiency.

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3 MINERAL NUTRITION OF POTATO GENOTYPES WITH DISTINCT PHYSIOLOGICAL SENSITIVITY TO AL STRESS

Abstract

The objective of this study was to investigate if nutrient concentration is related with aluminum (Al) response in potato genotypes. Eight potato genotypes showing different response to Al were grown in a nutrient solution (pH 4.0) with 0 and 200 mg Al L^{-1} and P-starvation. Based on growth parameters, the potato genotypes were classified as Al-tolerant (SMIF212-3, SMIC148-A, *Solanum microdontum*, SMINIA793101-3, SMIB106-7 and SMIG145-1) and Al-sensitive (Dakota Rose and SMIJ319-7). The concentration of Al in the roots, stem, leaves, stolon and tuber of all potato genotypes increased by Al exposure, except for SMIJ319-7 in the leaves. Moreover, Al supply affected the rate of uptake and distribution of nutrients in the different plant parts (roots, stem, leaf, stolon and tuber) of potato genotypes. The Al-tolerance in the SMIC148-A, S. microdontum and SMIF212-3 genotypes may be connected with greater levels of nutrients in the roots and leaves. The other way around, the Al-tolerant genotypes may be related to P concentration. The Al-tolerant genotypes showed increase in the concentration of P with increasing in the Al levels, mainly in the leaves.

Keywords: *Solanum tuberosum*, aluminum tolerance, nutrient partition

3.1 Introduction

Acid soils comprise up to 40% of the world's potentially arable lands. In many acid soils through the tropics and subtropics, aluminum (Al) toxicity is considered one of the most important factors limiting crop productivity (KOCHIAN; HOEKENGA; PIÑEROS, 2004; POSCHENRIEDER et al., 2008). The primary Al-toxicity symptom observed in plants is inhibition of root growth, followed by less nutrient and water absorption, resulting in poor growth and production (KOCHIAN; HOEKENGA; PIÑEROS, 2004; POSCHENRIEDER et al., 2008).

A further characteristic of Al-toxicity syndrome is the disturbance of plant ion homeostasis. Aluminum interferes with the uptake, transport, and utilization of essential elements such as P, Ca, K, Mg, Fe, Mn and Zn (TAYLOR; BLARNEY; EDWARDS, 1998; LIDON; AZINHEIRA; BARREIRO, 2000; SCHöLL et al., 2005; GUO; ZHANG; ZHANG, 2007; OLIVARES et al., 2009; TABALDI et al., 2009; ALI et al., 2011; GARZóN et al., 2011). It is

assumed that Al interferes with uptake of nutrients by replacing them at the binding sites of the cell wall and plasma membrane of cells, by blocking ion channels over the plasma membrane and by reducing the negative charge associated with the plasma membrane phospholipids and proteins by binding to these charged groups or shielding the surface potential (KOCHIAN, 1995; KINRAIDE, 2001; KINRAIDE; PEDLER; PARKER, 2004).

Some plant species and genotypes within a species have developed strategies to tolerate aluminum toxicity. Mechanisms of Al-tolerance are commonly classified into two categories: avoidance or exclusion of Al from the roots, and internal or protoplastic tolerance (KOCHIAN; HOEKENGA; PIÑEROS, 2004; POSCHENRIEDER et al., 2008). Several mechanisms for Al plant exclusion have been proposed including, Al efflux, selective permeability, plant induced changes in pH rhizosphere, and exudation of chelate ligands (HOSSAIN; KOYAMA; HARA, 2006; PIñEROS; CANÇADO; KOCHIAN, 2008). Furthermore, Al tolerance in some species was stated to be due to their ability to maintain in their roots and/or shoots adequate levels of some macro- and micronutrients (GIANNAKOULA et al., 2008). In maize and rice was observed that Al-tolerant plants retained higher concentrations of Ca, Mg and K than Al-sensitive (SIVAGURU; PALIWAL, 1993; GIANNAKOULA et al., 2008). Furthermore, evidence has shown that Al-toxicity can be alleviated by P in some plants, including maize (SILVA et al., 2010), *Citrus* spp (YANG et al., 2011), *Lepedeza bicolor* (YAN et al., 2001) and buckwheat (ZHENG et al., 2005).

Potato (*Solanum tuberosum*) is grown worldwide under a wide range of altitudes, latitudes, and climatic conditions than any other major food crop – from sea level to over 4000 m elevation (SIECZKA; THORNTON; CHASE, 1992). However, the potato plant has a relatively small nutrient exploration area because of its shallow root system and low root/foliage ratio (EKELÖF, 2007). Moreover, potato is considered to have shallower and less dense root distribution in the field compared with other field crops (IWAMA, 2008). TABALDI et al. (2007) observed reduced growth (length of roots and shoots) in Al sensitive genotypes exposed to toxic levels of Al. TABALDI et al. (2009) analyzed the influence of Al exposure in nutrient solution on the micronutrients (Zn, Fe, Mn and Cu) concentration in roots and shoot of the Al-sensitive (Macaca and Dakota Rose) and Al-tolerant (SMIC148-A and *S. microdontum*) potato genotypes obtained from *in vitro* culture. In the same work, the authors observed that excessive Al accumulation affected the uptake and distribution of Zn, Fe, Mn and Cu in roots and shoots of potato genotypes and that Al tolerance in *S. microdontum* might be connected with greater levels of

Zn, Fe and Mn in the roots of potato genotypes. GONçALVES et al. (2009) evaluated the effect of cadmium (Cd^{2+}) toxicity on mineral nutrient accumulation in two potato genotypes (Asterix and Macaca) cultivated both *in vitro* and in hydroponic experiments. The authors observed that the influence of Cd^{2+} on nutrient content in potato was related to the level of Cd^{2+} in the substrate, potato cultivar, plant organ, essential element, growth medium and exposure time.

In this study, we utilized eight potato genotypes with contrasting response to aluminum and phosphorus. The experiment was conducted in a greenhouse with plants obtained from seed tubers. The objective of this study is verify if Al tolerance is related to macro and micronutrient accumulation in potato genotypes.

3.2 Materials and methods

Plant materials and growth conditions

Seven adapted (2n=4x=48) potato (*Solanum tuberosum L.*) genotypes [SMIC148-A (C), Dakota Rose (D), SMINIA793101-3 (F), SMIB106-7 (J), SMIF212-3 (M), SMIG145-1 (O) and SMIJ319-7 (S)] and one wild species (2n=2x=24) genotype [PI595511-5/ *Solanum microdontum* Bitter (E)] were evaluated in this study. These genotypes were obtained from the Potato Breeding and Genetics Program, Federal University of Santa Maria, RS, Brazil. In previous studies, based on the total biomass of output (e.g., tuber yield) under two P levels (4.64 and 46.46 mg L^{-1} in nutrient solution), these genotypes were classified as: P utilization efficient and responsive [ER] (D and S), P utilization efficient and nonresponsive [ENR] (F and M), no efficient and responsive [NER] (C), and no efficient and nonresponsive [NENR] (E, J and O) (Nicoloso, personal communication).

Fourteen-day-old plants (shoot length of five centimeters) grown in pots containing sand were transferred into plastic vessels (1.5 L) containing 1300 g sand as substrate and grown in greenhouse. Each vessel received one plantlet. Each experimental unit consisted of five plants, totalizing three replicates per treatment. Throughout cultivation, sand was maintained at 80% of field capacity (195 ml), determined with a sample altered on a tension table. Irrigation was performed daily by replacement of both transpired and evaporated water, calculated by weighing the vessels. In total, 125 vessels were prepared, where 120 vessels received one plant which were used to calculate the water lost by transpiration, and the remaining 5 vessels, containing only sand, were used to measure water evaporation. Evaporated and transpired water was daily

replaced with nutrient solution, which had the following composition (mg L^{-1}): 7.54 P; 85.31 N; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 176.76 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe. The pH solution was adjusted to 4.0 $\pm$ 0.1 with a 1 M solution of HCl or NaOH.

After four days of plant acclimatization, Al in the form of $AlCl_3$, was added to nutrient solution at concentrations of 0 (control) and 200 mg Al L^{-1}. This solution did not contain P. The treatments were applied daily for 12 days. At the end of the experiments, the plants were gently washed with distilled water and then divided into leaves, stem, root, stolon and tuber. To obtain the fresh weight, excess water was removed with a paper towel after root washing. To obtain dry weight, the plants were left at 65°C to a constant weight.

Growth parameters and transpiration ratio

Growth of potato genotypes was determined by measuring the fresh and dry weight of leaves, stem, roots, stolon and tuber, and shoot length. The plant materials were oven-dried at 65°C to a constant weight for the determination of biomass. The transpiration ratio was calculated base on the ratio between the water that was absorbed by a plant (corresponding to the weight of water lost by each vessel containing one plant minus the average water lost by a vessel without a plant) and dry matter produced for it at the end of experiment.

Al and nutrient determination

Al, P, K, Ca, Mg, Fe, Mn, Cu and Zn concentration was determined in roots, stem, leaves, stolon and tuber. Dried plant tissues, between 0.01 and 0.25 g, were ground and digested with 5 mL of concentrated HNO_3. Sample digested was carried out in an open digestion system, using a heating block Velp Scientific (Milano, Italy). Heating was set at 130°C for 2 h. Plastic caps were fitted to the vessels to prevent losses by volatilization. The Al and nutrient concentration was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-EOS), using a PerkinElmer Optima 4300 DV (Shelton, USA) equipped with a cyclonic spray chamber and a concentric nebulizer.

Statistical analysis

The analyses of variance were computed for statistically significant differences determined based on the appropriate F-tests. The results are the means±SD of at least three independent replicates. The mean differences were compared utilizing Tukey test at P<0.05.

3.3 Results and Discussion

Growth parameters and transpiration ratio

The primary symptom of Al toxicity is an inhibition of root growth due to inhibition root cell expansion and cell division (MATSUMOTO, 2000; BARCELO; POSCHENRIEDER, 2002). However, VÁZQUEZ et al. (1999) observed that maize seedlings exposed to Al after 24 h had the root growth recovered to the control values of plants before the start of the Al treatment. In the present work, the potato genotypes were exposed to Al during long time (12 d), which can justify the increase in root dry weight of C and D potato genotypes of 22% and 50%, respectively, compared to the control (Fig. 3.1I).

Interestingly, the Al toxicity effects were observed on the shoots (stem and leaf) and below ground parts (stolon and tuber). The D genotype showed decrease of 24% in stem dry weight with increasing Al levels (Fig. 3.1II). In the leaf, it was observed a decrease in dry weight of D and S genotypes (19% and 43%, respectively) (Fig. 3.1III). None genotype showed any alteration in stolon dry weight at 200 mg Al L^{-1}, compared to the control (Fig. 3.1IV).

In the tuber, it was observed a decrease in dry weight at 200 mg Al L^{-1} of D genotype (38%), compared to the control (Fig. 3.1V). In addition, it was observed a decrease in total dry weight of D and S genotypes (19% and 13%, respectively). The O genotype increased 53% in total dry weight at 200 mg Al L^{-1} (Fig. 3.1VI). In contrast, O genotype decreased 35% in transpiration ratio at 200 mg Al L^{-1}, compared to the control (Fig. 3.1VII). Effects of Al on shoot development may be expressed only at later stages as a result of altered water and nutrient uptake as well as phytohormone production (BARCELO; POSCHENRIEDER, 2002). Furthermore, Al can act directly on the shoot length due to cellular and ultrastructural changes in leaves, reduction of stomatal aperture, decreased photosynthetic activity, increase of lipid peroxidation and decrease of enzyme activity (MATSUMOTO, 2000; BARCELO; POSCHENRIEDER, 2002). Based on growth parameters, it was demonstrated that C, E, M, F, J and O are

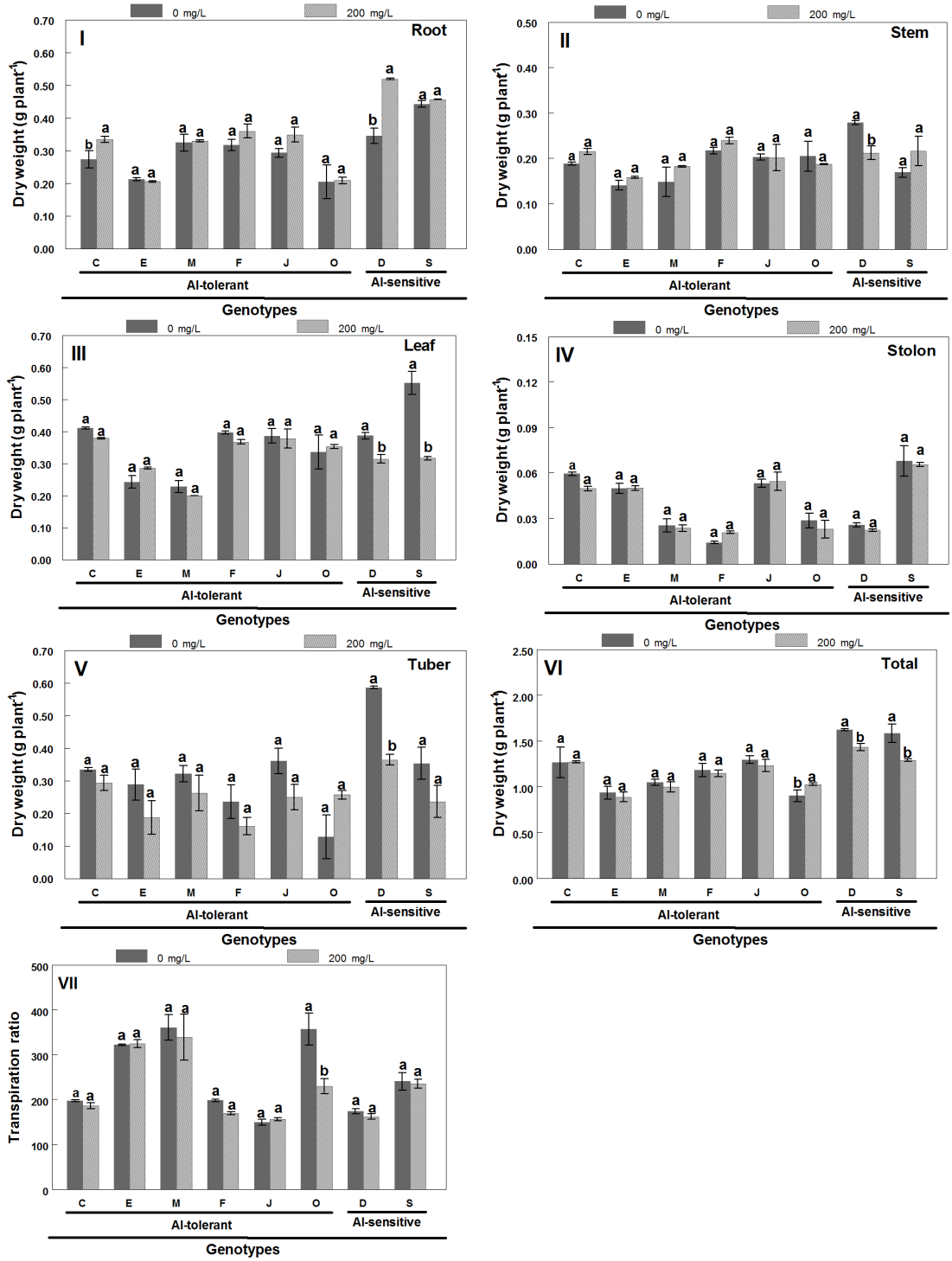

Figure 3.1 – Effect of increasing Al level on root (I), stem (II), leaf (III), stolon (IV), tuber (V) and total (VI) dry weight and transpiration ratio (VII) in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. . Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

Al-tolerant genotypes, whereas D and S are Al-sensitive genotypes.

Al and nutrient accumulation

The concentration of Al in the leaves, stem, roots, stolon and tuber of all potato genotypes studied increased with increasing Al levels, except for S genotype in the leaves. In addition, the increase in tissue Al concentration was much steeper for C and E (121% and 84%, respectively) in the leaves, E and M (195% and 162%, respectively) in the roots, M and D (1254% and 539%, respectively) in the stem, J and D (868% and 756%, respectively) in the tuber and E and C (776% and 489%, respectively) in the stolon (Fig. 3.2). The higher increase in the tissue Al concentration upon addition of Al levels was observed in the Al-tolerant genotypes E, M, C, E and J, respectively, in the root, stem, leaf, stolon and tuber (Fig. 3.2). Furthermore, the maximum concentration of Al in roots, stem, leaves, stolon and tuber was respectively found in M, D, C, E and C genotypes at 200 mg Al L^{-1} (Fig. 3.2). In relation to plant parts, the higher tissue Al concentration was observed in root > stolon > stem> tuber> leaf in all genotypes, except in E (Fig. 3.2). The higher Al concentration in roots and stolon may be due to direct contact with the nutrient solution. In contrast, this behaviour was not observed for tubers, but this response may be due to the low exposure time.

Aluminum interferes with the uptake, transport, and utilization of essential mineral elements including P, K, Ca, Mg, Mn, Cu, Zn and Fe (GUO et al., 2004; GUO; ZHANG; ZHANG, 2007; SCHöLL et al., 2005). The decrease in the uptake of nutrients by Al can be due to poor root growth, which induces less uptake of water and nutrients which disrupts metabolic processes (MATSUMOTO, 2000). Aluminum may bind to the phospholipids heads of the plasma membrane, reduce the negative charge associated with the plasma membrane phospholipids and proteins by binding to these charged groups or shielding the surface potential (KINRAIDE, 2001), and modify the activity of nutrients transporters (KOCHIAN, 1995).

In the present study the phosphorus concentration in the roots either increased in the E, J and S genotypes (7%, 5% and 15%, respectively) or decreased in M, F, D and O genotypes (38%, 18%, 31% and 37%, respectively) by Al exposure. The K, Mn and Zn concentration either decreased in the M, D and O genotypes or increased in the other genotypes, with exception of K concentration in F genotype, that was not affected by Al exposure. Calcium concentration decreased in the C, E, M, F, D and S genotypes by Al exposure. In addition, Mg concentration either decreased in the E, M, F, J, D, O genotypes or increased in the C genotypes at by Al

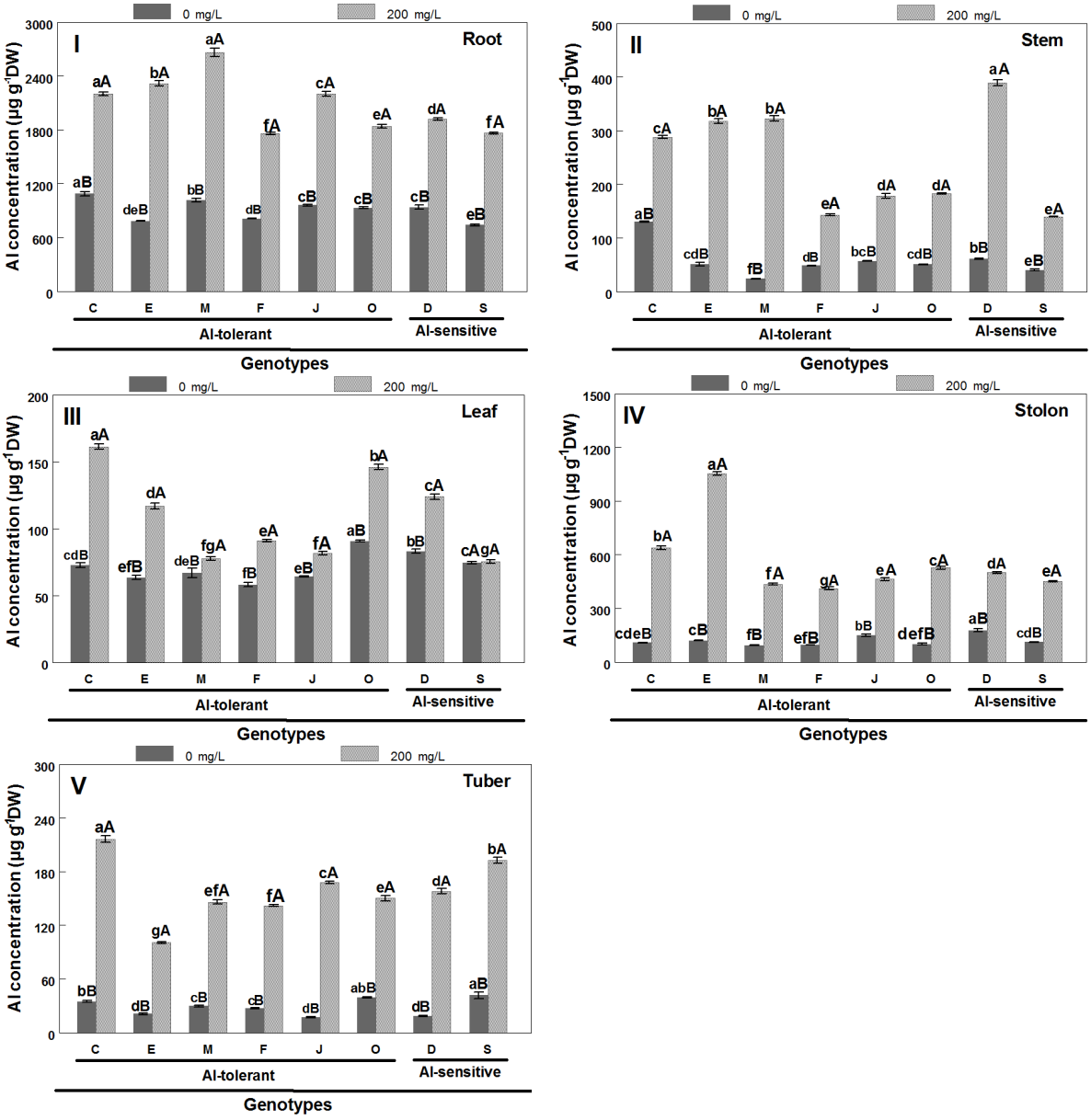

Figure 3.2 – Effect of increasing Al level on Al concentration in root (I), stem (II), leaf (III), stolon (IV) and tuber (V) in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

exposure. In S genotype no significant change in tissue Mg concentration was observed. In the E, M, D, O and S genotypes it was observed a decrease in the Cu concentration by Al exposure. However, in the C genotype, it was observed increase in the Cu concentration (Fig. 3.3).

Aluminum toxicity is considered closely associated with the P availability on plant grown in acid soils (FOY, 1988). Through precipitation/adsorption of aluminum phosphate,

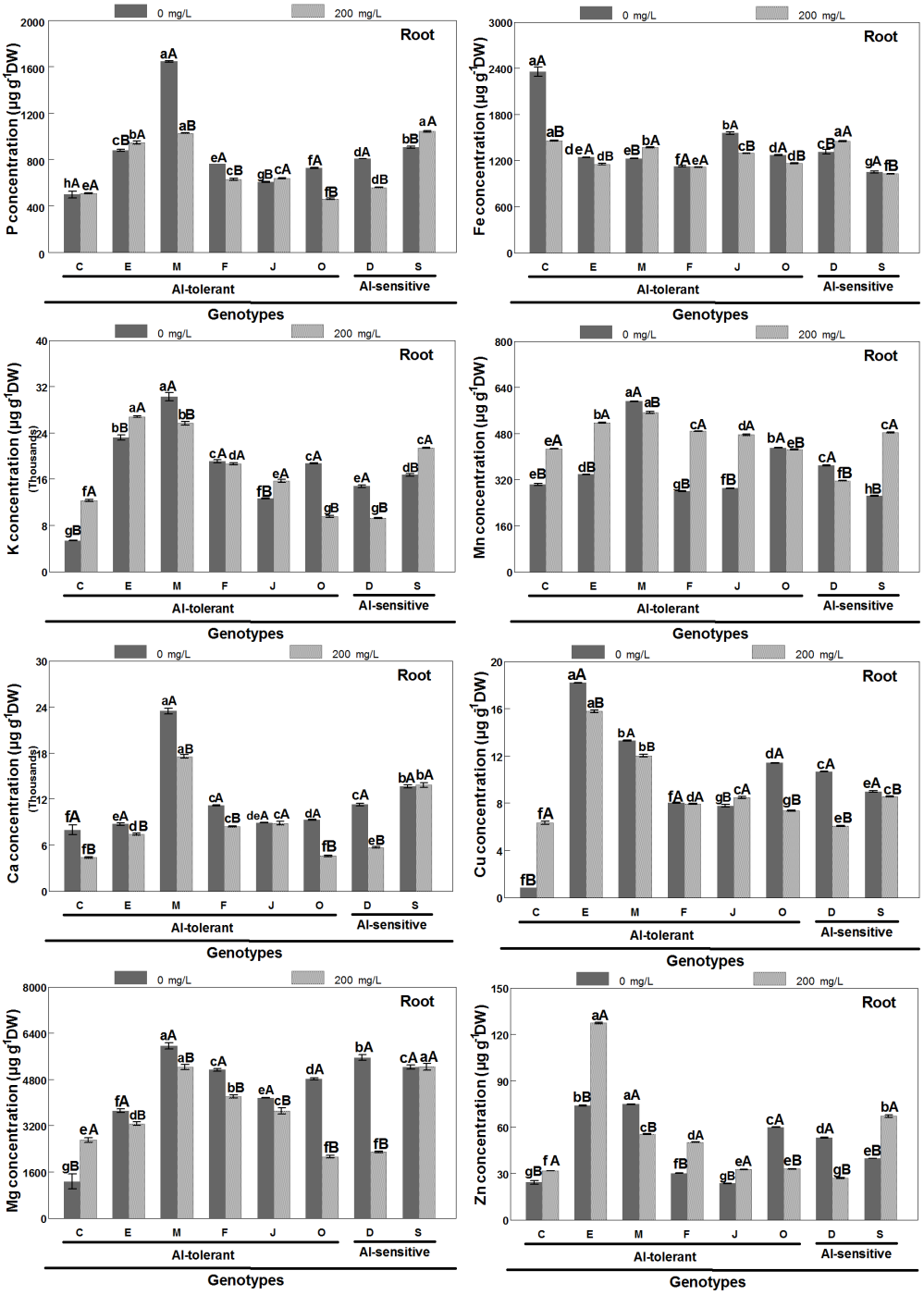

Figure 3.3 – Effect of increasing Al level on P, K, Ca, Mg, Fe, Mn, Cu and Zn concentration in root in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

both in the soil and in the plant roots, P bioavailability can be reduced (MACKLON; LUMSDON; SIM, 1994). There are a number of potential adaptive mechanisms that P-efficient plants can employ for better growth on the low-P soils (WANG; YAN; LIAO, 2010). The main strategy for P acquisition used by plants consists of maximal and continued soil exploration through proliferation and extension of all root types (FANG et al., 2009; RAMAEKERS et al., 2010; WANG; YAN; LIAO, 2010). In the C and D genotypes it was observed an increase the root dry weight with increasing Al levels (Fig. 1A). The increase in the Al levels might be caused a decrease in the P availability in the nutrient solution.

In the stem, the P concentration either increased in the F and D (5% and 3%, respectively) or decreased in C, E, J and O genotypes (4%, 7%, 12% and 6%, respectively) by Al exposure. The stem K concentration either decreased in the C, E, M, F, J and O or increased in the S by Al exposure. In the C, E, F, D and O genotypes was observed a decrease in the stem Ca concentration by Al exposure. In contrast, in the M genotype was observed an increase in the Ca concentration. The stem Mg concentration either decreased in the C, J, D and S or increased in the M, F and O genotypes by Al exposure. The tissue micronutrient concentrations (Fe, Mn, Cu and Zn) increased in all genotypes by Al exposure, except in the C genotype, where it was observed a decrease in the Fe concentration (Fig. 3.4).

In leaves, an increase in the P concentration with increasing of Al levels in the C, E, M and S (7%, 6%, 3%, 5%, respectively) genotypes was observed. However, in the F, J, D and O genotypes a decrease in P concentration (4%, 11%, 8% and 14%, respectively) was observed. The K concentration either increased in the C and S or decreased in the E, F and J genotypes by Al exposure. The Ca concentration either increased in the C, M and D or decreased in the E, F, J and S genotypes by Al exposure. The Mg concentration decreased in all genotypes, with exception in C and D by Al exposure. Ca concentration increased in C genotype by Al exposure. The Fe concentration either increased in the C or decreased in the E, M, J and S genotypes by Al exposure. The Mn concentration increased in all genotypes by Al exposure. The Cu concentration either increased in the C and F or decreased in the E, J, D and S genotypes by Al exposure. The Zn concentration either increased in the C and M or decreased in the F and J genotypes by Al exposure (Fig. 3.5).

Stolon P concentration increased in all genotypes by Al exposure, with exception in the J and O genotypes, where a decrease was observed (Fig. 3.6). The K concentration either increased in the E, M and D or decreased in the S genotype by Al exposure (Fig. 3.6). The

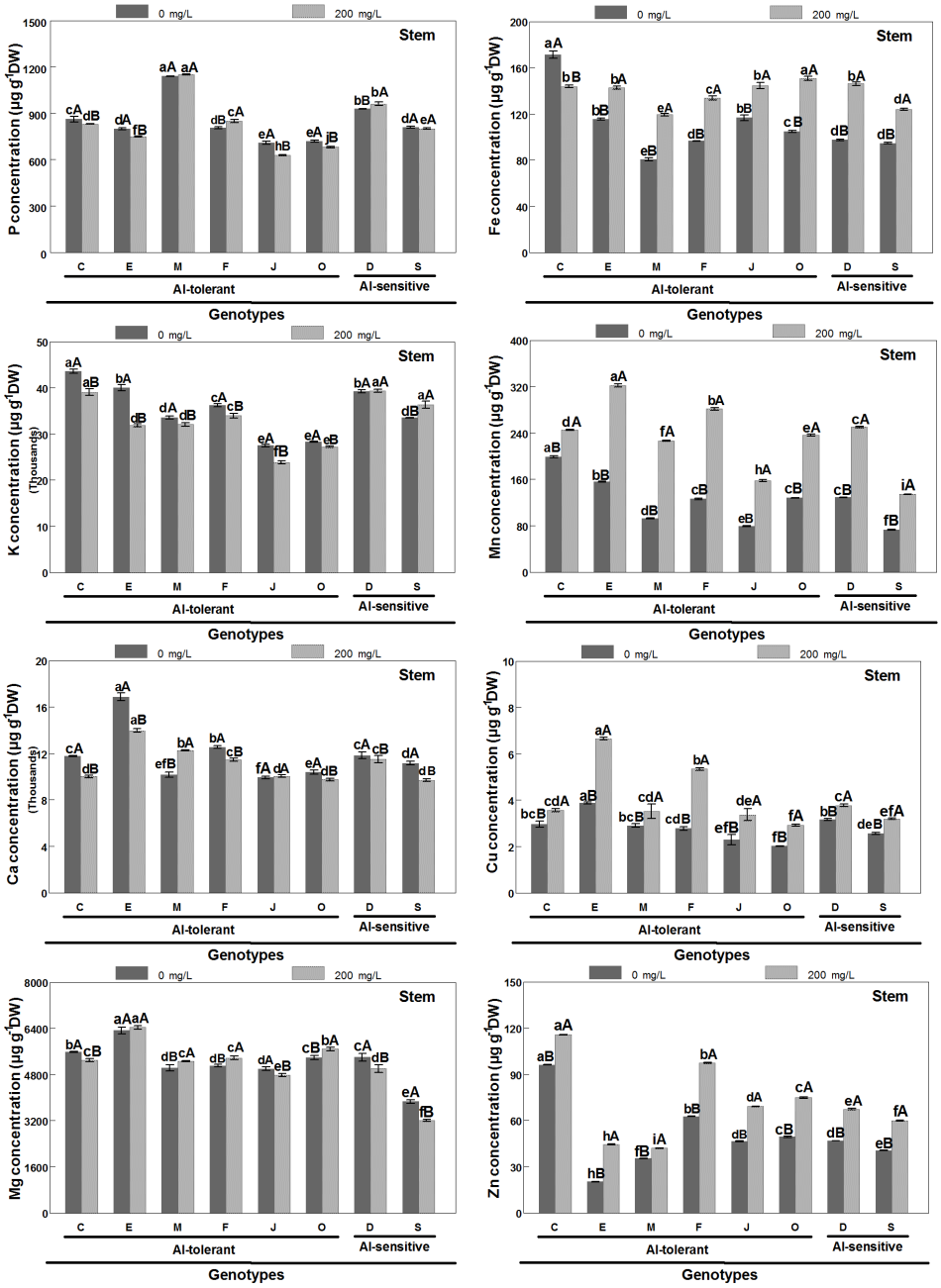

Figure 3.4 – Effect of increasing Al level on P, K, Ca, Mg, Fe, Mn, Cu and Zn concentration in stem in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

Ca concentration either increased in the E and M or decreased in the C, F and O genotypes by Al exposure (Fig. 3.6). The Mg concentration decreased in the C, F, J and O genotypes by Al exposure (Fig. 3.6). The Fe concentration either increased in the M, O and S or decreased in the

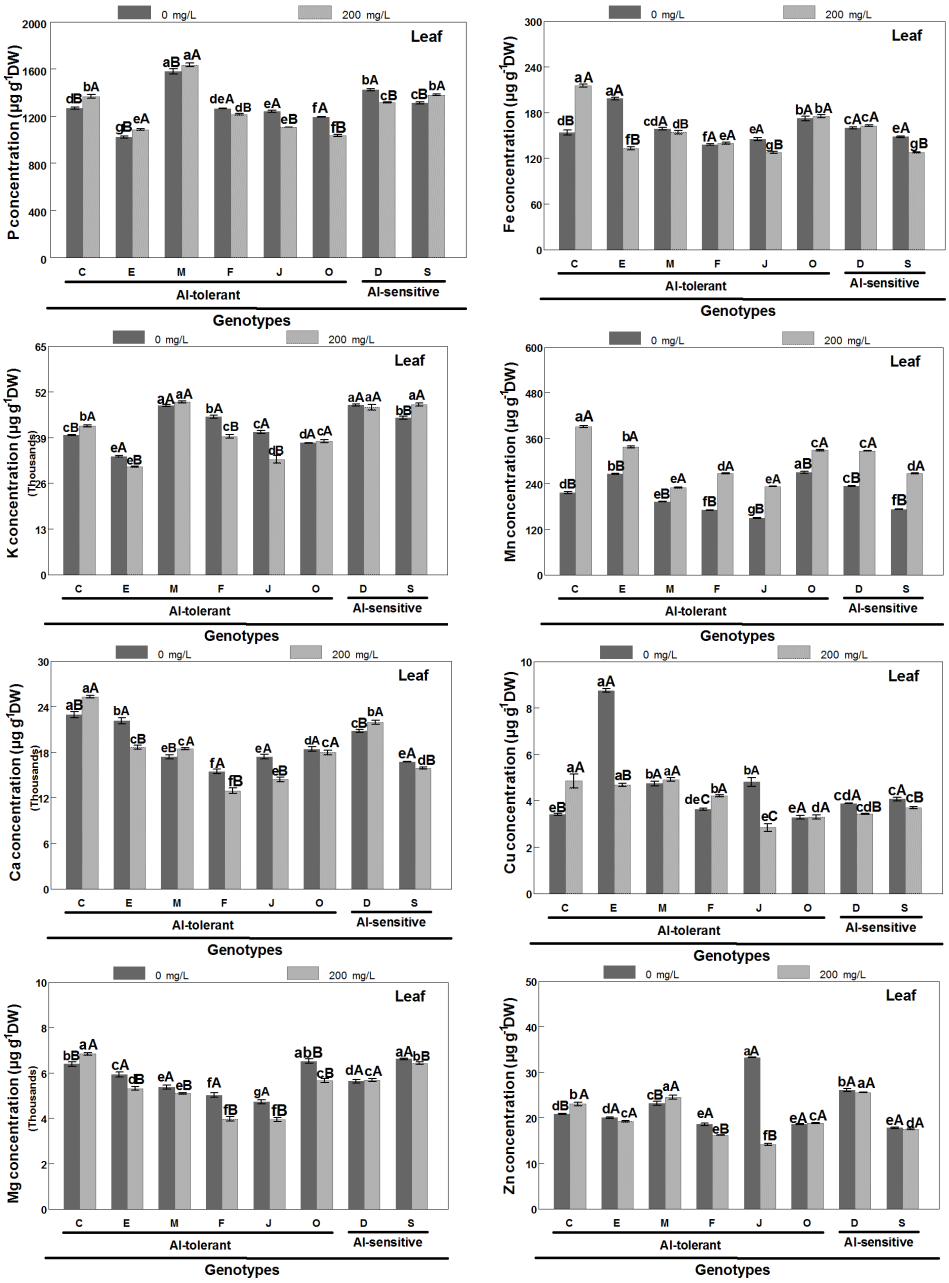

Figure 3.5 – Effect of increasing Al level on P, K, Ca, Mg, Fe, Mn, Cu and Zn concentration in leaf in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

E, F, J and D genotypes by Al exposure (Fig. 3.6). In stolon it was observed an increase in Mn concentration in all genotypes by Al exposure (Fig. 3.6). The same was observed for Cu concentration, with exception in the M genotype. In the M genotype was observed a decrease in the

Cu concentration by Al exposure (Fig. 3.6). The stolon Zn concentration increased in all genotypes, with exception in the J genotype, where no significant difference in the Zn concentration by Al exposure was observed (Fig. 3.6)

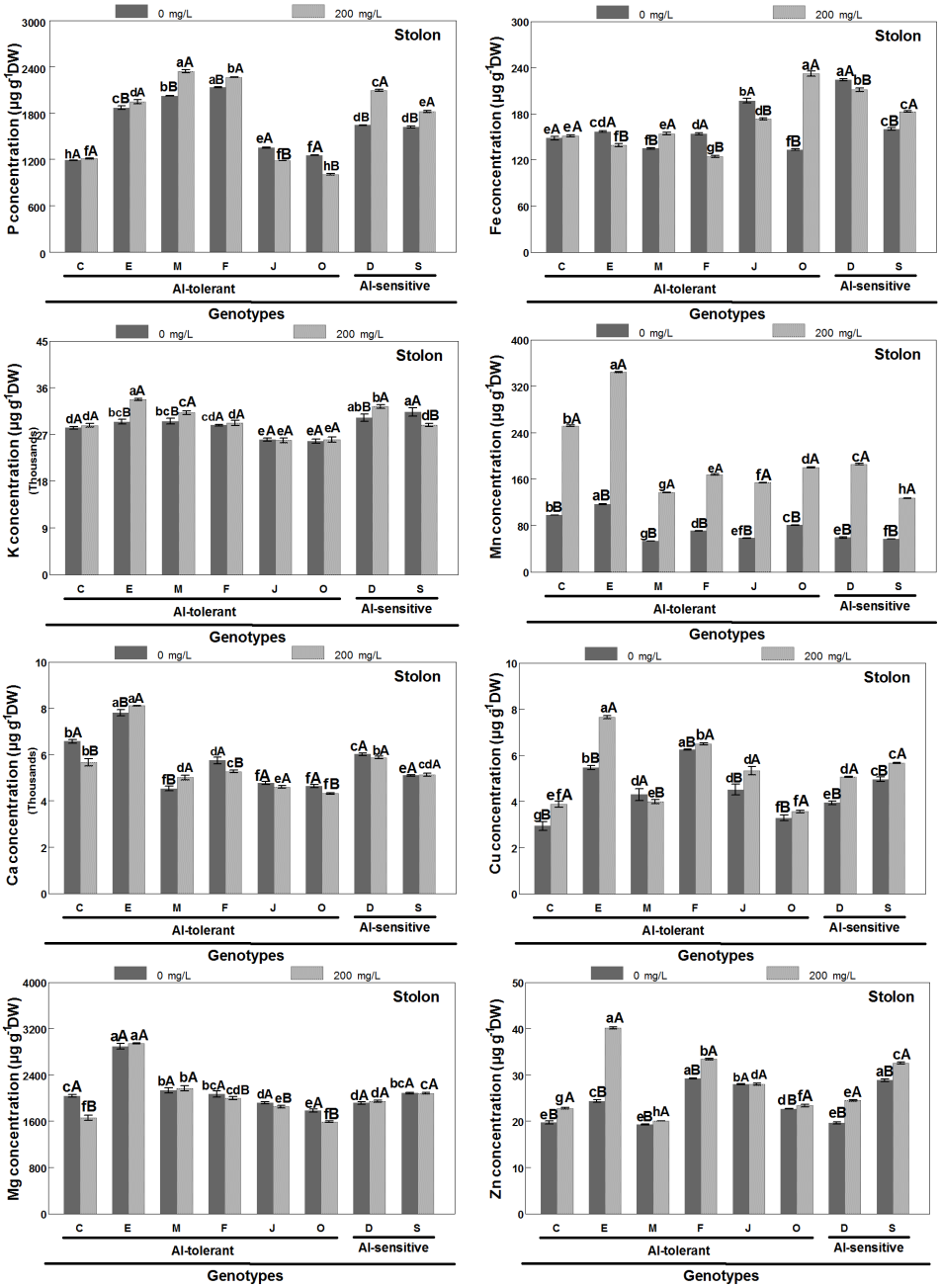

Figure 3.6 – Effect of increasing Al level on P, K, Ca, Mg, Fe, Mn, Cu and Zn concentration in stolon in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

Tuber P concentration either increased in the M, F and S genotypes or decreased in the C, E, J, D and O genotypes by Al exposure (Fig. 3.7). The K concentration either increased in the F and S genotypes or decreased in the E and D genotypes by Al exposure (Fig. 3.7). Furthermore, Ca concentration in tuber either increased in the M, F and J genotypes or decreased in the C, E and O genotypes by Al exposure (Fig. 3.7). The Mg concentration either increased in the M or decreased in the C, E, J, D and O genotypes by Al exposure (Fig. 3.7). In addition, tuber Fe concentration decreased in all genotypes by Al exposure. Furthermore, tuber Mn concentration increased in all genotypes at Al treatment compared to the control (Fig. 3.7). The same was observed for Cu concentration, with exception in the E genotype. In the E genotype it was observed a decrease in the Cu concentration in the tuber by Al exposure (Fig. 3.7). The Zn concentration either increased in the C, E, M and F or decreased in the J and D genotypes by Al exposure (Fig. 3.7).

There have been many reports about the effect of Al toxicity on mineral uptake, distribution and accumulation in plants, such as in barley (GUO; ZHANG; ZHANG, 2007), *Pinus sylvestrys* (SCHöLL et al., 2005), *Zea mays* (GARZóN et al., 2011) and *Solanum tuberosum* (TABALDI et al., 2009). ALI et al. (2011) showed that Al stress inhibited P, Ca, Mg, S, Cu, Mn, Zn and B uptake and restrained K and Fe from being translocated into stem and leaves. In contrast, the present study showed that Al stress did not inhibit the translocation of most nutrients to the shoot (stem and leaves). However, the P concentration was decreased in the D and O (Al-sensitive) genotypes. The inhibition of P accumulation by Al had been already observed by PIETRASZEWSKA (2001) and SILVA et al. (2010). CHEN et al. (2009) showed that Al decreased root and leaf P concentration. The Al fixes P in less available forms in soils and on plant root surfaces. Once within the cell, Al may react with P compounds, and upset the plant P metabolism. QUARTIN; AZINHEIRA; NUNES (2001) observed that P deficiency is considered to be the key cause of growth reduction in Al-stressed plants.

In the C, E, F and J (Al-tolerant) and S (Al-sensitive) genotypes the exposure at Al caused either an increase or no alteration in the concentration of most nutrients analyzed in the roots (Fig. 3.8). Interestingly, either the increase or no alteration in the nutrients concentrations in the roots was observed mainly in Al-tolerant genotypes (Fig. 3.8). TANG; BARTON; MCLAY (1997) established a relationship between H^+ extrusion and excess cation uptake in legume species grown in nutrient solution. SAS; RENGEL; TANG (2001) observed that the ratio of H^+ extrusion to excess cation uptake increased when P supply was decreased. In the

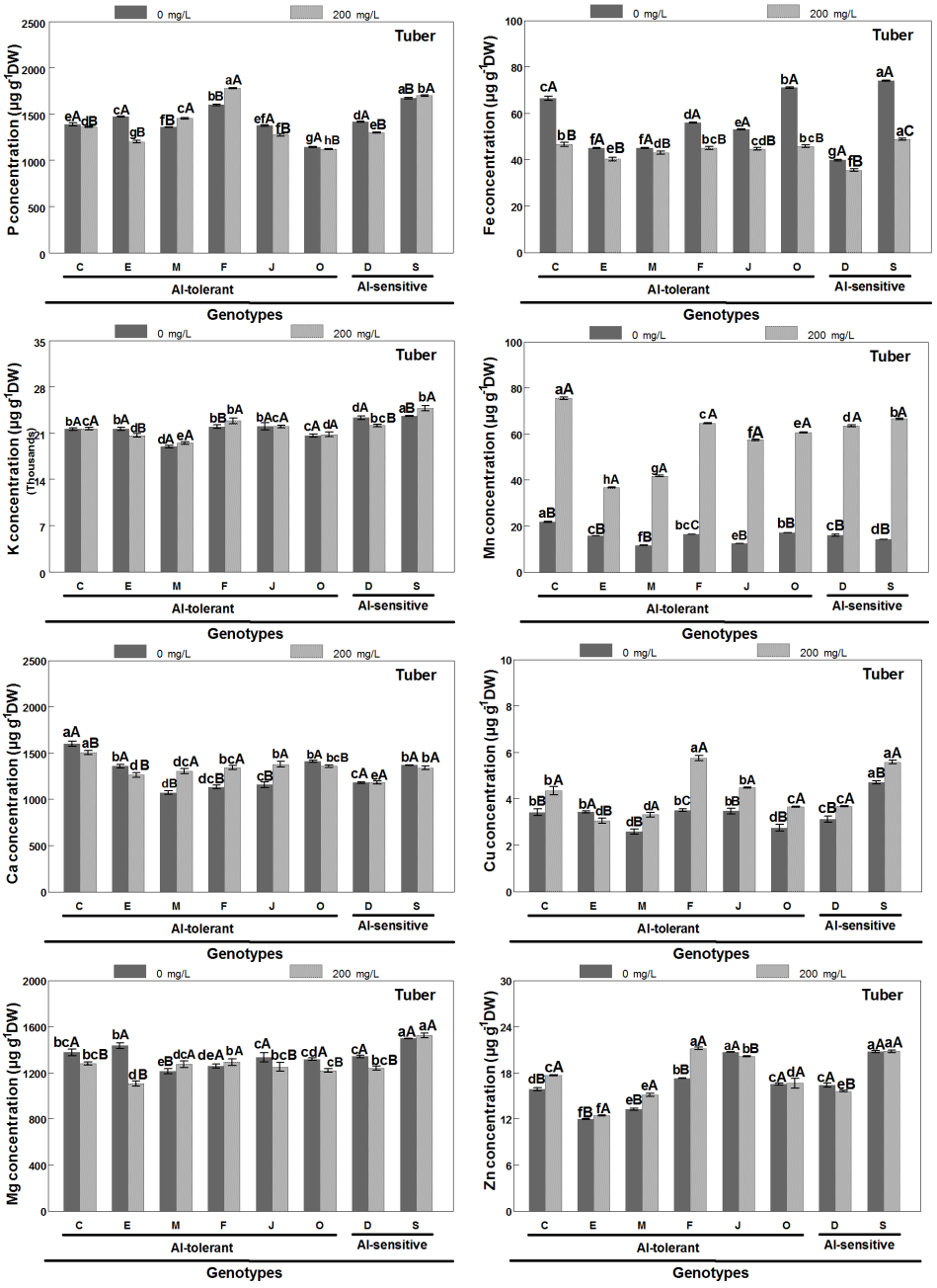

Figure 3.7 – Effect of increasing Al level on P, K, Ca, Mg, Fe, Mn, Cu and Zn concentration in tuber in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between Al levels in the same potato genotype ($p < 0.05$). Different lowercase letters indicate significant differences between potato genotypes at the same Al level ($p < 0.05$).

present study, it was observed a reduction in the nutrient solution pH with the increase of Al concentration in all genotypes (data not showed). Furthermore, as Al availability increases under acid soils, the accumulation of other elements as Cu, Fe, Mn and B also may increase and

eventually reach toxic levels (SCHROTH; LEHMANN; BARRIOS, 2003).

Parts		Genotypes							
		C	E	M	F	J	D	O	S
Root	↑	Al Mg Mn Cu K	Al P K Mn Zn	Al Fe	Al Mn Zn	Al P K Mn Cu Zn	Al Fe	Al	Al P K Mn Zn
	↓	Ca Fe	Ca Mg Fe Cu	P K Ca Mg Mn Cu Zn	P Ca Mg	Mg Fe	P K Ca Mg Mn Cu Zn	P K Ca Mg Fe Mn Cu Zn	Fe Cu
Stem	↑	Al Mn Cu e Zn	Al Fe Mn Cu Zn	Al Ca Mg Fe Mn Cu Zn	Al P Mg Fe Mn Cu Zn	Al Fe Mn Cu e Zn	Al P Fe Mn Cu Zn	Al Mg Fe Mn Cu Zn	Al K Fe Mn Cu Zn
	↓	P K Ca Mg e Fe	P K Ca	K	K Ca	P Mg	Ca Mg	P K Ca	Ca Mg
Leaf	↑	Al P K Ca Mg Fe Mn Cu Zn	Al P Mn	Al P Ca Zn Mn	Al Mn Cu	Al Mn	Al Ca Mn	Al Mn	P K Mn
	↓		K Ca Mg Fe e Cu	Mg Fe	P K Ca Mg Zn	P K Ca Mg Fe Cu Zn	P Cu	P Mg	Ca Mg Fe Cu
Stolon	↑	Al Mn Cu Zn	Al P K Ca Mn Cu Zn	Al P K Ca Fe Mn Zn	Al Mn Cu	Al Mn Cu	Al P K Mn Cu Zn	Al Fe Mn Cu Zn	Al P Fe Mn Cu Zn
	↓	Ca Mg	Fe	Cu	P Mg Fe	P Mg Fe	Fe	P Ca Mg	K
Tuber	↑	Al Mn Cu Zn	Al Mn Zn	Al P Ca Mg Mn Cu Zn	Al P K Ca Mn Cu Zn	Al Ca Mn Cu Zn	Al Mn Cu Zn	Al Mn Cu	Al P K Mn Cu
	↓	P Mg Fe Ca	P K Ca Mg Fe Cu	Fe	Fe	P Mg Fe	P Mg Fe	P Mg Fe Ca	Fe

Figure 3.8 – Representation of nutrients variation in roots, stem, leaves, stolon and tuber in SMIC148-A (C), *Solanum microdontum* (E), SMIF212-3 (M), SMINIA793101-3 (F), SMIB106-7 (J), SMIG145-1 (O), Dakota Rose (D) and SMIJ319-7 (S) potato genotypes grown in greenhouse. Arrows represents an increase (up arrow) or decrease (down arrow) in nutrient values.

In contrast, a decrease in the concentration of most nutrients analyzed (P, K, Ca, Mg and Fe) in stem of the C genotype with Al exposure was observed (Fig. 3.8). Interestingly, in the leaves of the C genotype, it was observed an increase in the concentration of all nutrients (Fig. 3.8). Furthermore, the C genotype showed the higher increases in the P (7%), Ca (10%), Mg (6%), Fe (39%), Mn (80%), Cu (42%) and Zn (10%) concentrations in the leaves. The Al-tolerance in C genotype can be associated with its higher capacity in uptake, transport and accumulation of nutrients to the leaves. TABALDI et al. (2009) observed that Al-tolerant potato genotype *Solanum microdontum* had greater concentrations of most micronutrients analyzed (Mn, Zn and Fe). Furthermore, the maximum concentration of the nutrients in the all plant parts was observed, in general, in the Al tolerant C, E and M genotypes. GIANNAKOULA et al. (2008) observed that energy supply is essential for plants to absorb nutrients, especially under stress conditions. In their study, it was observed a higher energy supply in tolerant maize line than in sensitive maize inbred line, since the tolerant line retained larger concentrations of Ca^{2+}, Mg^{2+}, and K^{+}, measured in roots and in shoots/leaves, compared with the sensitive inbred line, suggesting that an efficient metabolism system exists in the tolerant maize line under Al stress. Thus, higher levels of mineral nutrients may be connected with Al tolerance. Interestingly, in the C (Al-tolerant) genotype it was also observed the greater increase in the Al concentration in the leaves (121%). There are many proposed mechanisms of Al tolerance in plants that involve external avoidance or internal tolerance (KOCHIAN, 1995; BARCELO; POSCHENRIEDER, 2002; KOCHIAN; HOEKENGA; PIÑEROS, 2004). Probably, in the C genotype the main defense mechanism is internal. TABALDI et al. (2009) observed that the Al-tolerant SMIC148-A potato genotype had more efficient antioxidant system, which resulted in higher tolerance to Al. WANG; STASS; HORST (2004) suggested that the total Al content in the root tip may not be the main factor to Al toxicity. These authors demonstrated that the accumulation of hydroxyl-Al silicates in the root apoplast reduced the expression of Al toxicity. Furthermore, Al can form insoluble complexes with P, precipitating and accumulating at the surface or inside the root cells, reducing Al toxicity (PELLET; GRUNES; KOCHIAN, 1995; PELLET et al., 1997). The formation of less toxic organic Al-complexes seems also a prerequisite for the tolerance to high internal Al concentrations that have been observed in plants able to accumulate high shoot Al concentrations such as tea, buckwheat or Hydrangea (BARCELO; POSCHENRIEDER, 2002). Among the ligands that form stable complexes with Al, organic acid anions, phenolic substances, and silicon may be implied in Al detoxification

inside shoot tissues (BARCELO; POSCHENRIEDER, 2002). YANG et al. (2011) observed that higher Al-tolerance may be related to the production of organic acids in *C. sinensis*. However, TABALDI et al. (2007) observed that oxidative stress caused by Al in potato may harm several components of the cell, mainly in Al-sensitive genotypes. Moreover, it was observed that Al-tolerant plants developed some defense mechanisms against oxidative stress. The Al-tolerant SMIC148-A genotype had more efficient antioxidant system, which resulted in higher tolerance to Al (TABALDI et al., 2009).

In the stem, it was observed an increase in the concentration of most nutrients analyzed with the Al exposure, mainly the micronutrients concentrations, in the E, F, J (Al-tolerant) and S (Al-sensitive) genotypes (Fig. 3.8). In contrast, in the leaves, the Al exposure decreased the concentration of K, Ca, Mg, Fe and Cu in E genotype, P, K, Ca, Mg and Zn in F genotype, P, K, Ca, Mg, Fe, Cu and Zn in J genotype and Ca, Mg, Fe and Cu in S genotype (Fig. 3.5). In the E and F genotypes, the Ca concentration decreased in the root. The same was observed for the Mg concentration in the J genotype with Al exposure (Fig. 3.3, 3.4 and 3.5). Aluminum interferes with the uptake of Ca and Mg by replacing them at the binding sites of the cell wall and plasma membrane of the root cortex cells, by blocking ion channels over the plasma membrane and by reducing the electrostatic attraction of the plasma membrane and cell wall for Ca and Mg (KINRAIDE, 2001; KINRAIDE; PEDLER; PARKER, 2004).

Furthermore, in the leaves, it was observed an increase in the P concentration in C, E and M (Al-tolerant), and S (Al-sensitive) genotypes with increasing Al exposure. In addition, in the C and E (Al-tolerant) genotypes, it was observed the higher increase in the Al concentration by Al exposure (Fig. 3.2). However, it was not observed a decrease in the leaf dry weight with increasing Al levels in the Al-tolerant C, E and M genotypes (Fig. 3.1).

Insoluble Al-P precipitates can accumulate on the root surface, in the cell wall, or in the cell vacuole (TAYLOR, 1991) and generally considered nontoxic to plants. We suggest that the immobilization of Al in leaves by precipitation with P might contribute to the fenotypic differences in potato. The formation of Al-P complexes like Al_4 $(PO_4)_3$, may be helpful by retarding the uptake of Al into the citosol (ZHENG et al., 2005). Furthermore, VÁZQUEZ et al. (1999) reported that the resistance in maize relied on the active transport of Al-P complex from the cell wall to vacuoles.

Interestingly, in the Al-sensitive S genotype it was not observed a significant difference in the Al concentration in the leaves at the higher Al level in relation to control (Fig.

3.1 and 3.3). However, a decrease in the leaf dry weight with increasing Al levels was observed (Fig. 3.1), although the P concentration increased in all plant parts, except in the stem. A plant that is supplied with sufficient Pi may still suffer from what is known as Pi limitation of photosynthesis, due to the fact that most of the Pi present in photosynthetically active cells is sequestered in the vacuole and not metabolically available during short-term limitations (MIMURA, 1995, 1999).

In the present study, the results show that the increase of Al accumulation affected the rate of uptake and distribution of nutrients in the different plant parts (roots, stem, leaf, stolon and tuber) of potato genotypes tested. Aluminum tolerance in the C, E and M genotypes may be connected with greater levels of nutrients in the roots and leaves. In addition, the Al-tolerance may be related to P concentration and metabolism. The Al-tolerant genotypes showed increase in the concentration of P with increasing Al levels, mainly in the leaves.

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4 NUTRIENT UPTAKE AND TRANSLOCATION IN POTATO GENOTYPES IS AFFECTED BY LOCALIZED SUPPLY OF ALUMINUM IN A SPLIT-ROOT SYSTEM

Abstract

The objective of this study was to avaluate if nutrient uptake and translocation is more pronounced in the Al-tolerant than Al-sensitive genotypes in plants exposed to heterogeneous supply of Al. Contrasting Al-tolerant (SMIC148-A) and Al-sensitive (Dakota Rose) potato genotypes were cultivated in a split-root system for 7 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. In both Al-sensitive and Al-tolerant, all root halves Al exposed showed lower dry root weight, when compared to the control (0/0). However, in the Al-tolerant genotype the Al-untreated root half did not differ from the control and dry stem weight was not altered. In the Al-sensitive genotype, the half of the root system untreated to Al (0/200) showed lower dry root weight, when compared to control (0/0). Furthermore, plants treated with 100 mg Al L^{-1} in only one root half showed higher stem and leaf dry weight. In both Al-sensitive and Al-tolerant genotype Al was visualized by hematoxilyn staining after short exposure time (6h) in the root half exposed to Al. In the long exposure time (168 h) in the Al-tolerant genotype was not observed Al in the Al-untreated root half, while in the Al-sensitive genotype Al was visualized in this condition. Interestingly, in both Al-tolerant and Al-sensitive genotype was observed an increase in the Al concentration in the Al-untreated root half, when compared to the control. In the Al-sensitive genotype, stem and leaf Al concentration increased when the plants were exposed to Al. In the Al-tolerant genotype was not observed difference in the stem and leaf Al concentration in the plants with only root half exposed to 200 mg Al L^{-1}. In both Al-tolerant and Al-sensitive genotype was observed a decrease in the P, K, Mg, Cu and Zn and an increased in the Mn and Fe concentration in the root exposed to Al. In the Al-untreated root of Al-tolerant genotype was observed a decrease in the Cu, Fe and Zn root concentration. However, in the Al-sensitive genotype was observed a decrease only in Zn concentration. Both in the Al-tolerant and Al-sensitive genotype was observed an increased in the P concentration in the stem, however in the Al-tolerant genotype was observed an increase in K and Ca in the plants where the root half was exposed to 100 mg Al L^{-1}. In the Al-sensitive genotype was observed a decrease in the all

nutrient concentration in the leaf. On the other hand, in the Al-tolerant genotype was observed an increase in the leaf Zn concentration in all treatments and in the P, Ca and Mg concentration in the plants where the root half was exposed to 100 mg Al L^{-1}. These results show that aluminum tolerance in the SMIC148-A genotype can be correlated to lower Al translocation from root to shoot and a higher nutrient translocation.

Keywords: *Solanum tuberosum*, aluminum, compensatory growth, nutrient uptake

4.1 Introduction

Potatoes (*Solanum tuberosum* L.) rate fourth in world production among various agricultural products, following wheat, rice and maize (**?**), with an overall annual production of nearly 327 million tons and about 19 million ha cultivated. 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; FENNELL, 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 pH of 5.0 to 6.5. But, 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; CAPALDI; STEFANUTO, 2005) are a limiting factor to crop growth and are usually associated to low levels of plant-available phosphorus (P) (JEMO et al., 2007) and high levels of aluminum (Al) (VON UEXKÜLL; MUTERT, 1995), which is solubilized in acidic pH into the toxic cation Al^{3+}.

Cultivated soil frequently exhibits agronomic or environmental risks such as higher supply of pesticides and fertilizers that can increase pollution, or compaction and erosion of soil due to heavy machines and repeated operations (LIMOUSIN; TESSIER, 2007). No-tillage system is considered as an alternative to avoid some of these problems. During 1990s, the no-tillage practices increased more than of 200% in the United States of American (CTIC, 2000). In this practice, the soil exhibited strong pH vertical gradients. Acidification is higher in the upper layers where the fertilizers were supplied. Furthermore, under this system a high amounts of exchangeable Al in the upper layers of soils has been observed (LIMOUSIN; TESSIER, 2007; HOUX III; WIEBOLD; FRITSCHI, 2011).

Aluminum is known to inhibit plant growth (ČIAMPOROVÁ, 2002), mainly that of the

root (BALESTRASSE; GALLEGO; TOMARO, 2006; TABALDI et al., 2007). Symptoms of Al toxicity are also manifested in the shoot and are regarded as a consequence of injuries to the root system (VITORELLO; CAPALDI; STEFANUTO, 2005). In addition, Al also alters water relations (BARCELO; POSCHENRIEDER, 2002), reduces stomatal opening, decreases photosynthetic activity and causes chlorosis and necrosis of leaves, decreasing carbon sequestration and biomass formation (VITORELLO; CAPALDI; STEFANUTO, 2005). Potential alternatives to the direct amelioration of subsoil acidity include the use of Al-tolerant germplasm (FOY, 1988).

Plant roots are characterized by very high adaptability. Their growth and development involve complex interactions with both the soil environment and the shoot (MARSCHNER, 1995). 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.

Utilizing a homogeneous supply of Al to the roots of potato genotypes grown in a hydroponic growth system, TABALDI et al. (2007) demonstrated that the SMIC148-A genotype was Al-tolerant, whereas the Macaca genotype was Al-sensitive. Moreover, it was observed that Al supply induced oxidative stress, mainly in the Al-sensitive genotype. Therefore, we formulated the hypothesis that potato genotypes 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 tolerance and nutrient uptake should be more pronounced for the Al-tolerant genotype. The aim of the present paper is to test this hypothesis.

4.2 Material and Methods

Plant materials and growth conditions

Microtubers of potato genotypes (*Solanum tuberosum* L.) Dakota Rose (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 (mg L^{-1}): 7.54 P; 85.31 N; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 176.76 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe. The pH solution was adjusted to 4.0 ± 0.1 with a 1 M solution of HCl or NaOH.

Fourteen-day-old plants (shoot length of five centimeters) were 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 three days. After that acclimatization period, these plants with split-roots were cultivated for 7 days in a new nutrient solution (without P and pH 4.0 ± 0.1) with five treatments (ten 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. At harvest, the plants of both genotypes were divided into shoot (leaf and stem), left root and right root to evaluate nutrient and Al concentration, and growth parameters.

Growth parameters

Growth of potato genotypes was determined by measuring the dry weight of leaves, stem and roots and shoot length. The plant materials were oven-dried at 65°C to a constant weight for the determination of biomass.

Al and nutrient determination

Al, P, K, Ca, Mg, Fe, Mn, Cu and Zn concentration was determined in roots, stem and leaves. Dried plant tissues, between 0.01 and 0.25 g, were ground and digested with 5 mL of concentrated HNO_3. Sample digestion was carried out in an open digestion system, using a heating block Velp Scientific (Milano, Italy). Heating was set at 130°C for 2 h. Plastic caps were fitted to the vessels to prevent losses by volatilization. The Al and nutrient concentration was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-EOS), using a PerkinElmer Optima 4300 DV (Shelton, USA) equipped with a cyclonic spray chamber and a concentric nebulizer.

Aluminium localization by hematoxylin

For visualizing Al accumulation in the root tips was utilized hematoxylin staining. The roots were treated as described by POLLE; KONZAK; KATTRICK (1978). After 6, 12, 24 and 168 h (end of the experiment) exposure to treatments, the roots were rinsed in deionized water and stained by hematoxylin (2 g L^{-1} hematoxylin and 0.2 g L^{-1} $NaIO_3$) for 45 min. The roots were washed again for 10 min in deionized water to remove excess of stain. The root tips were then excised and photographed using stereomicroscopy.

Statistical analysis

The analyses of variance were computed for statistically significant differences determined based on the appropriate F-tests. The results are the means$\pm$SD of at least three independent replicates. The mean differences were compared utilizing Tukey test at $P<0.05$.

4.3 Results and Discussion

Growth parameters

After 7d of growth in a split-root system, all root halves Al exposed showed lower root dry weight (Fig. 4.1A, B), when compared to the control (treatment: 0/0). Interestingly, in the Al-sensitive genotype, the half of the root system untreated (treatment: 0/200) also showed lower dry root weigth, when compared to the control (treatment: 0/0). This result suggests that the Al may have been translocated from Al-treated root to the Al-untreated root causing a

decrease in the root dry weight. On the other hand, in the Al-tolerant genotype (SMIC148-A), no significant difference was observed in root dry weight of the Al-untreated root (treatments: 0/100 and 0/200) when compared to the control (Fig. 4.1A).

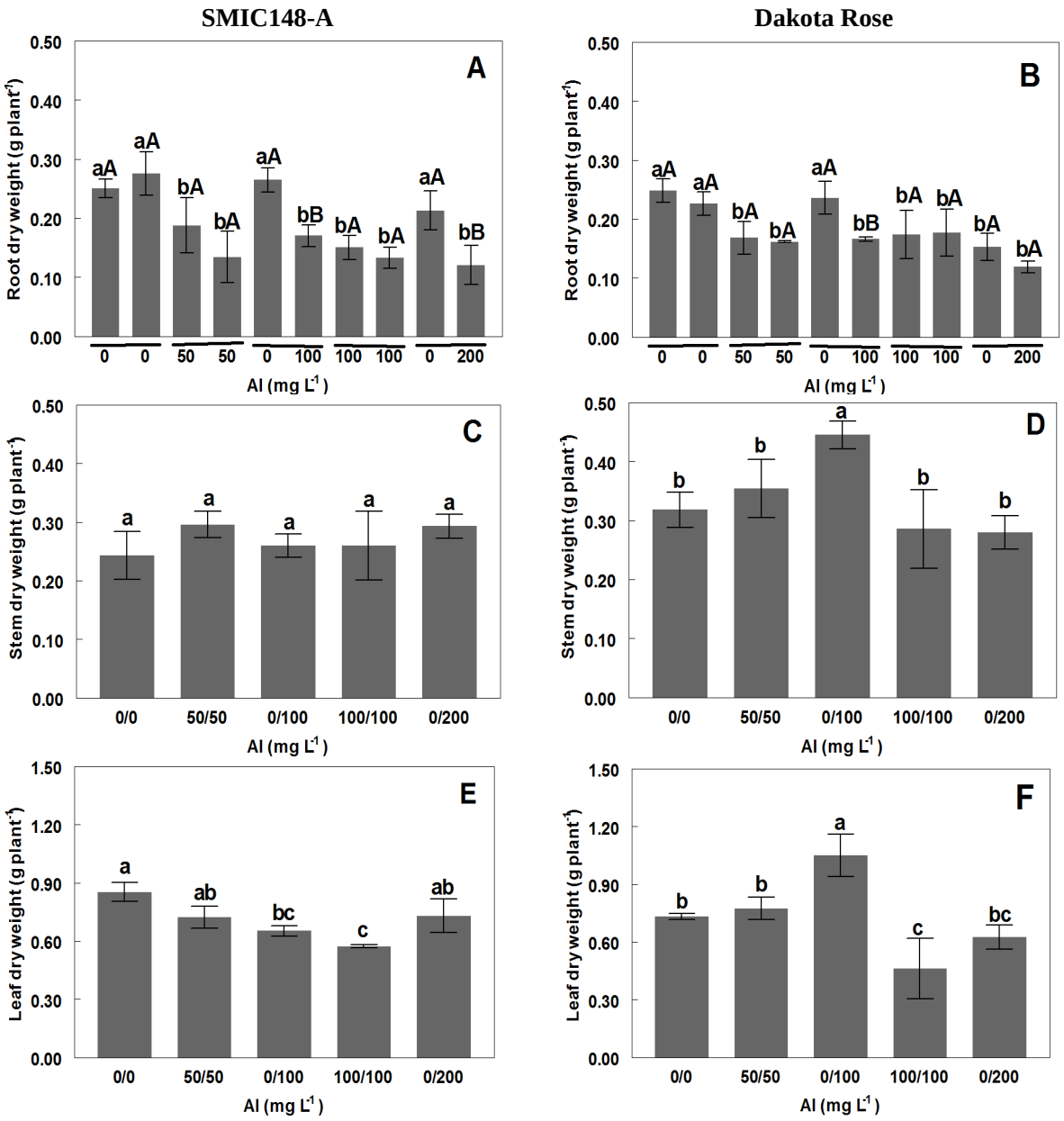

Figure 4.1 – Effect of Al concentrations on root, stem and leaf dry weight in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

The typical symptom observed in plants exposed to Al toxicity is the reduction in the

root growth. This symptom can be due to increased production of H_2O_2 in the roots exposed to Al stress. ŠIMONOVIČOVÁ et al. (2004) observed that H_2O_2 produced in barley roots during Al stress might play an active role in inducing cell death. Furthermore, Al induces changes in root cytoskeleton (SIVAGURU et al., 1999), rapid callose formation causing a increase cell wall rigidity (HORST; PÜSCHEL; SCHMOHL, 1997; AHN et al., 2002), and decrease in H^+ - pumping activity (AHN et al., 2001). Al could neutralize the surface charge of the plasma membrane and cause a surface potential shift from -120 to +1mV. Such Al-related shift in plasma membrane surface potential causes disturbance in ion transport processes (AHN et al., 2002).

In the Al-tolerant genotype despite that occurred a reduction in the root dry weight under Al stress, it was not observed reduction in stem dry weight in any Al treatments, when compared to the control (Fig. 4.1C). On the other hand, in the Al-sensitive genotype was observed an increase in stem dry weight and leaf dry weight when only one root half was exposed to 100 mg Al L^{-1} (treatment: 0/100), when compared to the control (Fig. 4.1D, F).

In the Al-tolerant genotype was observed reduction in the leaf dry weight in plants where both root halves were exposed to 100 mg Al L^{-1} (treatment:100/100) and in plants with only one root half exposed to 100 mg Al L^{-1} (treatment: 0/100) (Fig. 4.1E). However, in plants where both root halves were exposed to 50 mg Al L^{-1} (treatment:50/50) and in plants with only one root half exposed to 200 mg Al L^{-1} (treatment:0/200) was not observed decrease in the leaf dry weight, when compared to the control (Fig. 4.1E). Besides the increase of dry leaf weight when only one root half was exposed to 100 mg Al L^{-1}, in the Al-sensitive genotype was observed decrease in the leaf dry weight only in plants where both root halves were exposed to 100 mg Al L^{-1} (treatment:100/100), compared to the control (Fig. 4.1F). The increase in both stem and leaf dry weight of Al-sensitive genotype (Fig. 4.1D, F) may be due to the hormetic effect. Growth hormesis represents an over compensation due to a disruption in homeostasis that has been described in relation to different factors (POSCHENRIEDER et al., 2013). Hormetic growth stimulation has frequently been observed in plants exposed to low concentrations of non-essential, toxic metal ions (CALABRESE; BLAIN, 2009). Metal ions can act as elicitors of defense responses that in turn can stimulate the growth of plants, particularly under stress conditions (POSCHENRIEDER et al., 2013). OSAKI; WATANABE; TADANO (1997) observed that the growth of most plants adapted to low pH soils in the tropical and temperate regions was stimulated by Al application, which is assumed to be caused by the stimulation of

N, P, and K uptake by Al application. Growth stimulation by Al is considered to alleviate H^+ toxicity at low pH (KINRAIDE, 1993). GHANATI; MORITA; YOKOTA (2005) observed that Al-induced increase in the activities of antioxidant enzymes, resulting in increased membrane integrity and delayed lignification and aging, that can be considered as a possible reason for the stimulatory effects of Al on the growth of the tea plants (*Camellia sinensis*) and this is irrespect of the presence of other nutrients and their interaction with Al.

Tissue Al concentration

In both Al-sensitive and Al-tolerant genotypes was observed an increase in root Al concentration when plants were supplied either in only half of the root system (treatments: 0/100 and 0/200) or when both halves of the root were treated with Al (treatments: 50/50 and 100/100), when compared to the control (treatment:0/0) (Fig. 4.2). However, this increase was more pronounced in Al-sensitive than in Al-tolerant genotype. In addition, in plants supplied with Al at 100 or 200 mg Al L^{-1} to only one half of the root system (treatments: 0/100 and 0/200), the root Al concentration in the Al-untreated root half was lower than in root half treated with Al. However, it was observed a higher increase in the root Al concentration, when compared to the control plants (treatment:0/0). This response can be due to the translocation of the Al from Al-treated root half through the shoot to the Al-untreated root half (Fig. 4.2). Furthermore, in Al-tolerant genotype the percentage of translocation was lower than in the Al-sensitive genotype [Al-tolerant (0/100, increase 80%; 0/200, increase 203%) and Al-sensitive (0/100, increase 209%; 0/200, increase 258%)] (Fig. 4.2).

In wheat root apices, Al accumulation was considered indicative of Al sensitivity (SAMUELS; KUCUKAKYUZ; RINCÓN-ZACHARY, 1997). Roots take up Al in the form of ionic Al (Al^{3+}) due to a large inwardly directed electrochemical gradient for this ion. Following the uptake, Al is chelated with the internal organic acids (oxalate and citrate) in the root cells, forming a stable, non-phytotoxic Al complex (MA; HIRADATE, 2000). In buckwheat (MA; HIRADATE, 2000), Melastoma (WATANABE; OSAKI, 2001) and *Camellia sinensis* (MORITA et al., 2004), Al has been shown to be transported from the root to shoot as an Al–citrate (1:1) complex. When Al-citrate moves from the xylem to the leaf cells, the Al- organic acid complex is then sequestered in vacuoles of the leaves (SHEN et al., 2002). The present work showed a significant increase in the root Al concentration in Al-untreated root half (treatments: 0/100 or 0/200), when compared to the control plants (treatment: 0/0) (Fig. 4.2). This results can be

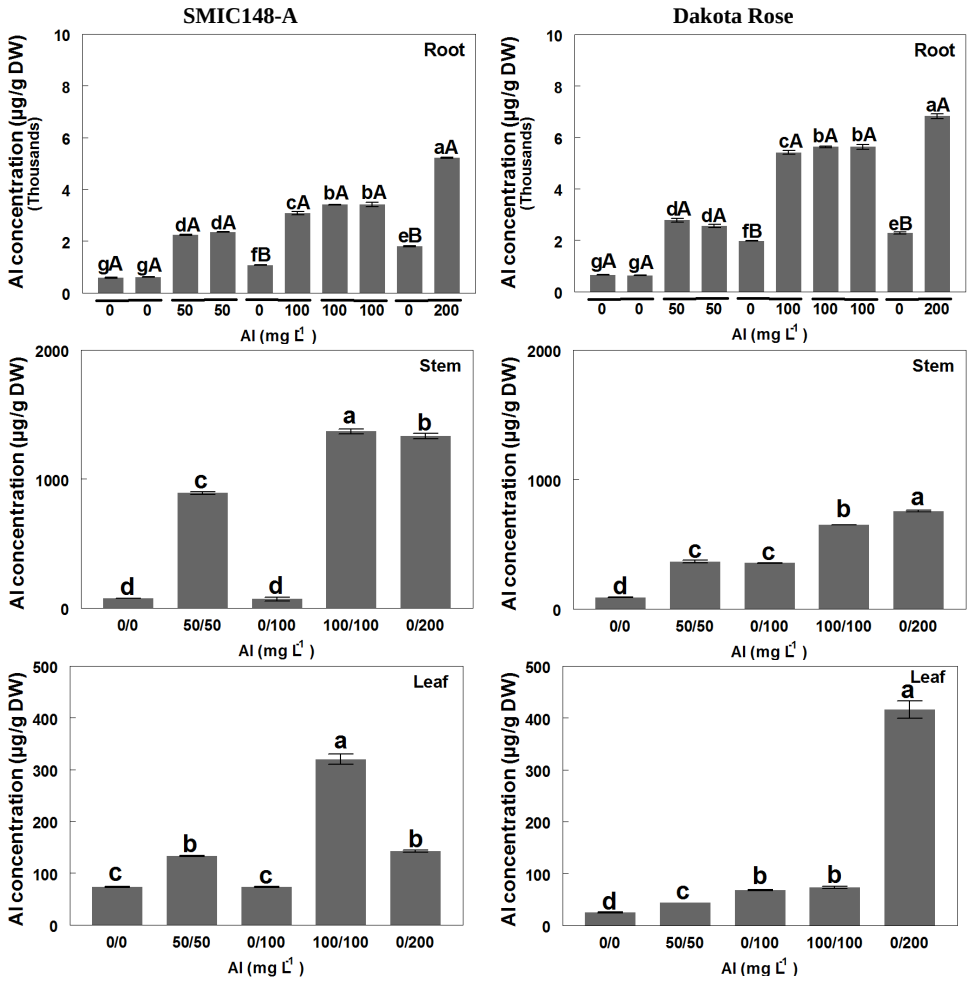

Figure 4.2 – Effect of Al concentrations on Al concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

due to Al translocation via phloem. Several researchers showed that Al might be transfered via phloem (BRITEZ et al., 2002; WATANABE; JANSEN; OSAKI, 2005; ZENG et al., 2013). ZENG et al. (2013) showed that Al was present in the phloem of oil tea petioles and that Al in oil tea could also be redistributed. These authors observed that higher concentrations of Al were found in leaves when Al was supplied to a different leaf of the same plant. In addition, Al was present in newly emerging roots of oil tea seedlings in which all original roots were excised prior to treatment, and a positive correlation existed between Al content in the newly formed roots and that in the leaves.

In the Al-tolerant potato genotype, the stem and leaf Al concentration was significantly increased in the treatments with both sides of the root system exposed to Al (treatments: 50/50

and 100/100) (Fig. 4.2), as well as when plants were supplied with 200 mg Al L^{-1} to only half of the root system (treatment: 0/200). However, in the plants where Al was supplied to only half of the root system (treatment: 0/100), it was not observed a significant difference when compared to the control plants (treatment: 0/0) (Fig. 4.2). In the Al-sensitive genotype, stem and leaf Al concentration was significantly increased when plants were supplied either with 100 mg Al L^{-1} or 200 mg Al L^{-1} to only half of the root system (treatments: 0/100 and 0/200), as well as when both halves of the root system were treated with 50 mg Al L^{-1} or 100 mg Al L^{-1} (treatments:50/50 and 100/100) (Fig. 4.2). These data show that these genotypes differ in both Al uptake and translocation/allocation, and that the Al-tolerant genotype has a more efficient ability to prevent Al uptake and/or accumulation in the roots, but also to prevent its translocation to upper parts, mainly for the leaves, that are the most metabolically active tissues in plants. The exact mechanisms by which the Al-tolerant potato genotype prevents Al uptake and translocation/allocation are still unknown. A possible mechanism that can contribute to lower the Al uptake can be the increased release of Al^{3+}-chelating compounds (e.g., organic acids) (MA; RYAN; DELHAIZE, 2001; HAYES; MA, 2003; SHEN; IWASHITA; MA, 2004; TOLRA et al., 2005). Furthermore, the lower Al translocation of root to upper parts in the Al-tolerant genotype can be due to Al^{3+} binding to negative sites in the cell walls and/or chelate the Al entering the root cells, with subsequent transport and sequestration into subcellular compartments (e.g., vacuoles) (INOSTROZA-BLANCHETEAU et al., 2012).

Aluminium localization by hematoxylin

The hematoxylin method has been used to measure the Al sensitivity of roots of many plant species (POLLE; KONZAK; KATTRICK, 1978). In the present study, with increasing of the exposure time to Al it was observed an increase in the dark purple stain in the root, evidencing an increase in Al concentration in the root tissues (Fig. 4.3). Independetly of the exposure time (6, 12, 24 and 168 h) in both Al-tolerant and Al-sensitive genotypes was observed the presence of the Al in the root tips of plants where both root halves were exposed to 50 mg Al L^{-1} or 100 mg Al L^{-1} (Fig. 4.3).

In this experiment, in both Al-sensitive and Al-tolerant genotypes, after short exposure time (6, 12 and 24 h) to Al was not observed dark purple stain in the Al-untreated root half in plants where only one root half was exposed to Al (treatment: 0/100). However, after long exposure time (168 h) the roots of Al-sensitive genotype that ware not treated with Al showed dark

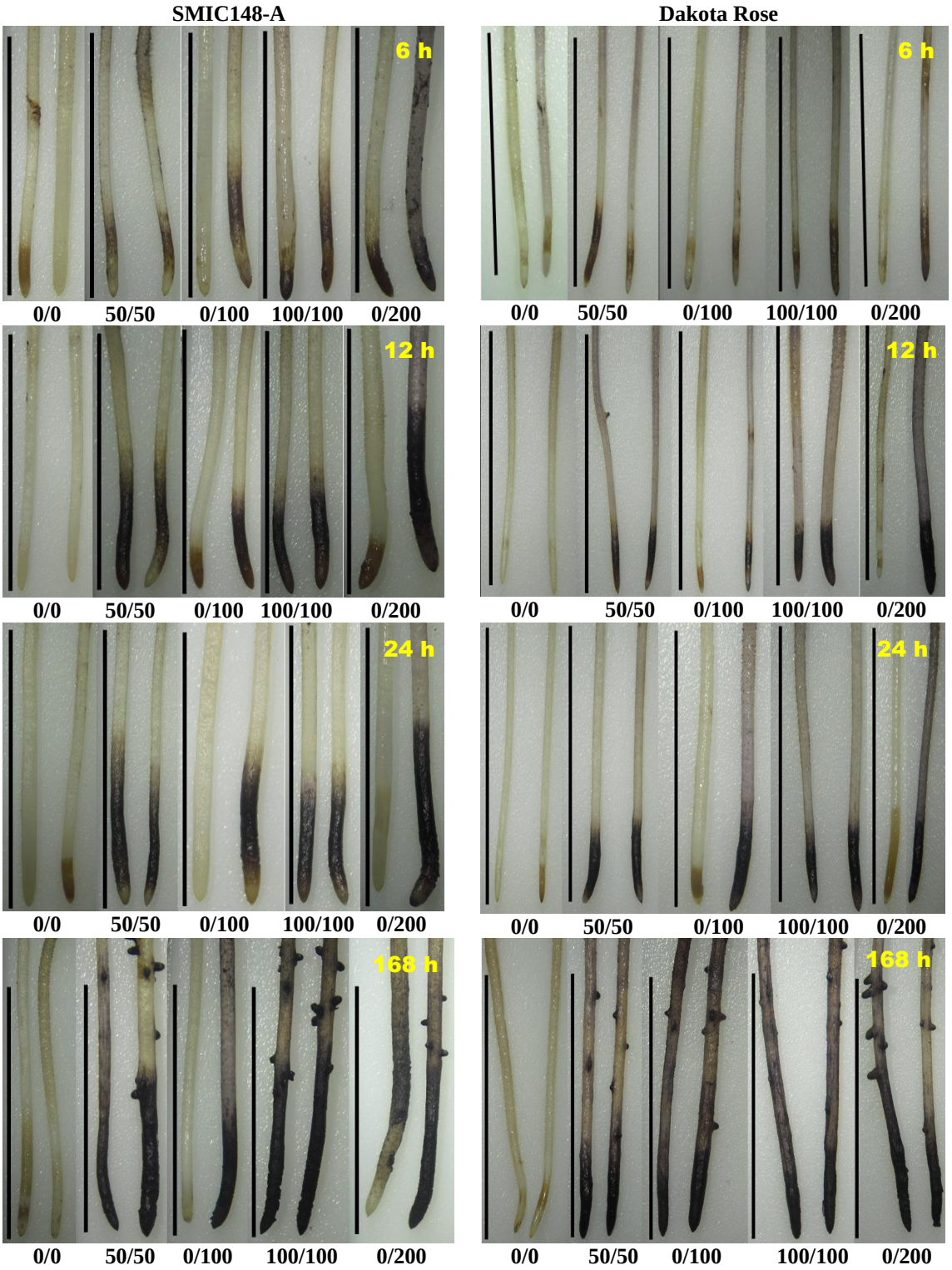

Figure 4.3 – Effect of Al concentrations on Al concentration in root tips of SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes exposed to Al for 6, 12, 24 and 168 h in a split-root system. Roots stained with hematoxylin to visualize root Al content. Scale bars: 1 cm.

purple stain suggesting that occurred Al translocation from the Al-treated root to Al-untreated root (Fig. 4.3). Interestingly, in the Al-tolerant genotype this response was not observed.

On the other hand, in the Al-tolerant genotype, in the plants where one root half was exposed to 200 mg Al L^{-1} (treatment: 0/200) during short exposure time (6 h) both root halves presented dark purple stain. However, with increase of the exposure time (12, 24 and 168 h) it was observed a decrease in the dark purple stain in the Al-untreated root tips (Fig. 2). RINCÓN; GONZALES (1992) observed in a Al-tolerant wheat genotype a decrease of the hematoxylin staining intensity after 6 to 24 h of Al exposure. VÁZQUEZ et al. (1999) observed that Al-tolerant maize is highly responsive to low Al concentrations (20 μM) after 4 h of exposure, but is poorly affected after 24 h. Cell wall thickening and disturbance of apoplastic and symplastic stainable cations occurred only during the initial period of the Al treatment (4 h), but not after longer (24 h) exposure times. In this experiment Al-induced alteration of both cell wall ultrastructure and cation homeostasis was chronologically related to the change in root-elongation rate.

Plants may have the capability to recover from the Al-induced injury when the concentration of Al is not deadly, although the mechanism of recovery is not simple. One mechanism may repair or reduce the injury caused by Al toxicity, and the other mechanism may be the exclusion of toxic Al. Both mechanisms may function simultaneously in some cases (MATSUMOTO, 2000). The increased exudation of organic acids from the roots tips may play an important role in Al detoxification in two species of *Citrus* (YANG et al., 2011; IKKA et al., 2013). Root tips of an Al-sensitive wheat that stain intensely with hematoxylin exhibited no coloration when rinsed with citrate before the staning procedure (OWNBY, 1993). Furthermore, VÁZQUEZ et al. (1999) observed that differences in intracellular tolerance can be due to the presence of metal deposits in vacuoles. In Al-tolerant maize was observed an increase of the vacuolar Al from the 4 h to 24 h Al exposure, mainly through electron-dense deposits containing Al and P or Si (VÁZQUEZ et al., 1999).

However, in the Al-sensitive genotype only after long exposure time (168 h) the Al-untreated root half presented dark color, which suggestes that occurred Al translocation from the Al-treated roots to Al-untreated roots (Fig. 4.3).

In both Al-sensitive and Al-tolerant genotypes was observed the formation of secondary roots in the root half exposed to Al after long exposure time (168 h). Interestingly, only in the Al-sensitive genotype was observed the formation of secondary roots in the Al-untreated

root half, for those plants where only one root half was exposed to 100 mg Al L^{-1} or 200 mg Al L^{-1} (Fig. 4.3). This effect on the morfogenesis of secondary root suggests that the Al is translocated throughout the whole plant. The formation of secondary root is dependent on auxin and ethylene signaling. SUN et al. (2010) showed that the increased ethylene production by Al may act as a trigger to evoke changes in auxin distribution by affecting auxin polar transport systems such as AUX1(auxin influx carrier) and PIN2 (proteins function to mediate auxin efflux). DONCHEVA et al. (2005) and AMENÓS et al. (2009) observed that alterations in auxin transport by Al causes chances in pericycle cell patterning inducing the formation of lateral roots in Al-sensitive maize.

Tissue nutrients concentration

Aluminum interferes with the uptake, transport, and utilization of essential mineral elements including P, K, Ca, Mg, Mn, Cu, Zn and Fe (GUO et al., 2004; GUO; ZHANG; ZHANG, 2007; SCHöLL et al., 2005). The decrease in the uptake of nutrients by Al can be due to poor root growth, which induces less uptake of water and nutrients which disrupts metabolic processes (MATSUMOTO, 2000). Aluminum may bind to the phospholipids heads of the plasma membrane, reduce the negative charge associated with the plasma membrane phospholipids and proteins by binding to these charged groups or shielding the surface potential (KINRAIDE, 2001), and modify the activity of nutrients transporters (KOCHIAN, 1995).

Furthermore, the Al interaction with essential mineral elements such as P is well known. In the present study, to avoid the interaction between P and Al in the nutrient solution, an experimental setup determined that plants should grown for about three days in the presence of 250 μM of P, and, subsequently, during the Al exposure (for 7 days), P was omitted from the nutrient solution. In a previous experiment, (personal data), was observed that potato plants were very well nourished with P and could withstand 12 days in the absence of P in the nutrient solution without showing visible symptoms of P deficiency.

In both Al-sensitive and Al-tolerant genotypes was observed the decrease in P concentration in the both root halves treated to Al (treatments: 50/50 and 100/100), when compared to the control treatment, showed a clear P translocation from the root to shoot (Fig. 4.4). The P translocation can be evidenced by the increase in P concentration in the stem of both genotypes, being more pronounced in the Al-sensitive genotype (Fig. 4.4). However, in the Al-sensitive genotype, P translocation to the leaves seemed not to occur. Conversely, in this genotype it was

observed a decrease in leaf P concentration in all Al treatments (Fig. 4.4).

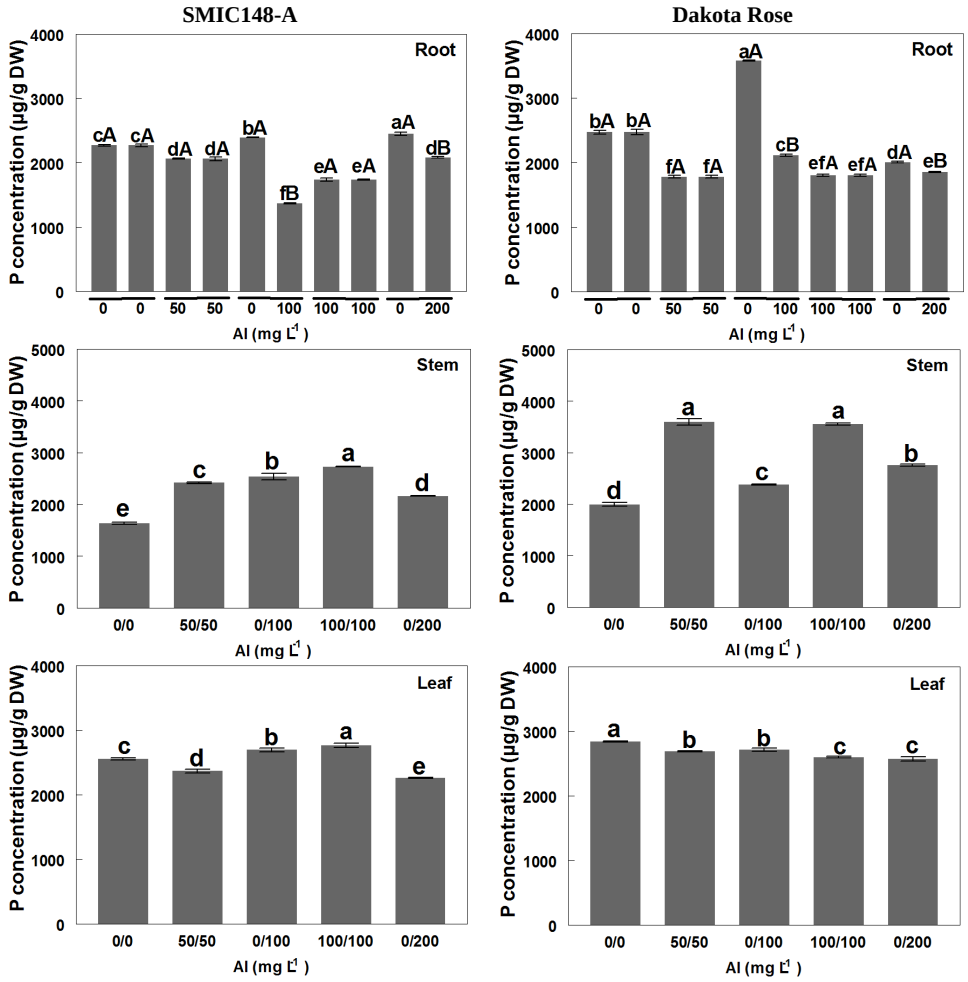

Figure 4.4 – Effect of Al concentrations on P concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

The inhibition of P accumulation by Al was already observed by PIETRASZEWSKA (2001) and SILVA et al. (2010). CHEN et al. (2009) showed that Al decreased the root and leaf P concentration in of *Citrus grandis*. Once within the cell, Al may react with P compounds, and upset the plant P metabolism (ZHENG et al., 2005; VÁZQUEZ et al., 1999). QUARTIN; AZINHEIRA; NUNES (2001) observed that P deficiency is considered to be the key cause of growth reduction in Al-stressed plants.

The increase in P concentration in the root half untreated with Al in the plants when only one root half was treated at 100 and 200 mg Al L^{-1} (treatments: 0/100 and 0/200) showed a clear P translocation from the Al-treated root half to Al-untreated root half via the shoot

(Fig. 4.4). However, in Al-sensitive genotype this response was observed only when one root half was exposed at lower Al level (treatment: 0/100) (Fig. 4.4).

Phosphorus deficiency is the predominant factor of induction of formation and growth of lateral roots. However, the main effect of Al is the inhibition of root growth (MATSUMOTO; MOTODA, 2012). Therefore, the increase of P concentration in the Al-untreated root half could have minimized the toxic effects of Al. In contrast with our results, IQBAL (2013) observed that P translocation was not able to alleviate Al toxicity within plant tissue of both Al-tolerant and Al-sensitive wheat genotypes.

However, in the stem of both Al-tolerant and Al-sensitive genotypes the P concentration increased in all Al treatments, when compared to the control (Fig. 4.4). This increase was more pronounced when both root halves were exposed to 100 mg Al L^{-1} in Al-tolerant genotype and at 50 mg Al L^{-1} and 100 mg Al L^{-1} in Al-sensitive genotype (Fig. 4.4). In addition, in the leaf of Al-tolerant genotype was observed an increase in the P concentration either in the leaves of plants with both root halves exposed to 100 mg Al L^{-1} (treatment: 100/100) or with one root half exposed to 100 mg Al L^{-1} (treatment: 0/100). On the other hand, in the Al-sensitive genotype the leaf P concentration decreased in all Al treatments (Fig. 4.4). It suggests that the immobilization of Al in leaves by precipitation with P might contribute to the fenotypic differences in potato. Furthermore, VÁZQUEZ et al. (1999) reported that the resistance in maize relied on the active transport of Al-P complex from the cell wall to vacuoles.

In both Al-sensitive and Al-tolerant genotypes, root K concentration decreased in plants when both root halves were supplied at 50 mg Al L^{-1} or 100 mg Al L^{-1} (treatments: 50/50 and 100/100), when compared to the control (treatment: 0/0) (Fig. 4.5). In addition, in plants supplied at 100 mg Al L^{-1} or 200 mg Al L^{-1} to only half of the root system (treatments: 0/100 and 0/200), root K concentration decreased in root half exposed to Al, when compared to the control plants (treatments: 0/0).

Membrane transport of K can be mediated either by K channels, utilizing the membrane potential to facilitate transport of K down its electrochemical gradient, or by secondary transporters (GIERTH; MÄSER, 2007). The reduction of K uptake can be correlated with the inhibition of root elongation because K and other cations accumulation contributes to the expansion of cell volume, initiating turgor-driven cell elongation (FRENSCH, 1997). Al can inhibit the K transport blocking the channels at the cytoplasmic side of the plasma membrane (LIU; LUAN, 2001) or the Al may inhibit K in by a direct external block (GASSMANN; SCHROEDER,

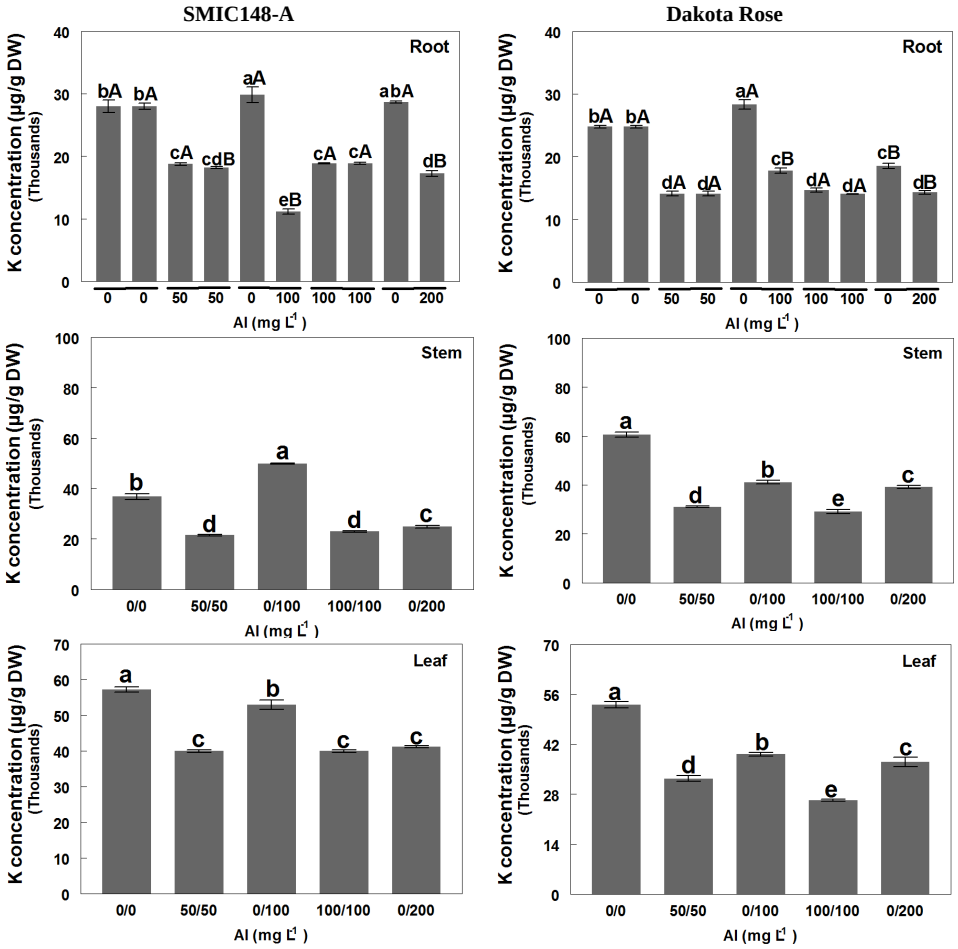

Figure 4.5 – Effect of Al concentrations on K concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

1994), which is consistent with the observation of Al inhibition root elongation. In another work, we observed reduction in root fresh weight in the roots exposed to Al (data shown in manuscript III).

In Al-tolerant genotype the root K concentration was not altered in the Al-untreated root half when only half of the root system was treated with 100 mg Al L^{-1} or 200 mg Al L^{-1}, when compared to the control plants (treatment: 0/0) (Fig. 4.5). However, in Al-sensitive genotype was observed a reduction in root K concentration in the Al-untreated root half when only half of the root system was treated with 200 mg Al L^{-1} (Fig. 4.5). This difference between Al-tolerant and Al-sensitive genotype can be related to minor Al translocation in the Al-tolerant than Al-sensitive genotype (Fig. 4.2)

In the Al-tolerant genotype, the stem K concentration increased when plants were supplied with 100 mg Al L^{-1} to only half of the root system (treatment: 0/100), when compared to the control (treatment: 0/0) (Fig. 4.5). As demonstrated in figure 1, in the Al-tolerant genotype was not observed Al translocation from root to shoot. However, when the plants were supplied with 200 mg Al L^{-1} to only half of the root system (treatment: 0/200), as well as when both halves of the root system were treated with 50 mg Al L^{-1} or 100 mg Al L^{-1} (treatments: 50/50 and 100/100) the stem K concentration decreased, when compared to the control (treatment: 0/0) (Fig. 4.5). In the Al-sensitive genotype, the stem K concentration was significantly decreased in all Al treatments, when compared to the control (treatment: 0/0) (Fig. 4.5). In the leaf, in both Al-tolerant and Al-sensitive genotypes the K concentration decrease in all Al treatments (Fig. 4.5). The reduction of the K concentration in the stem and leaf of the both genotypes can be due to reduction of the K uptake and translocation. Al inhibit of stomatal opening by inward K channels block in guard cell (LIU; LUAN, 2001) causing a reduction in transpiration rate and consequent decrease in uptake and translocation of nutrients.

Root Ca concentration in both Al-sensitive and Al-tolerant genotypes was not altered in root half supplied with 50 mg Al L^{-1} or 100 mg Al L^{-1} to both root halves (treatments: 50/50 and 100/100), when compared to the control (treatment: 0/0) (Fig. 4.6). In Al-tolerant genotype, in plants supplied with 100 mg Al L^{-1} only one half of the root system (treatment: 0/100), root Ca concentration decreased in root half exposed to Al, when compared to the control plants (treatment: 0/0). However, when only one half of the root system was exposed to 200 mg Al L^{-1} (treatment: 0/200) was observed an increased in Ca concentration in the both root halves, when compared to the control (treatment: 0/0). Furthermore, in the Al-sensitive genotype was observed an increased in the Ca concentration in the root half exposed to Al in plants where root half was exposed to 100 mg Al L^{-1} or 200 mg Al L^{-1} (treatments: 0/100 and 0/200), when compared to the control (treatment: 0/0) (Fig. 4.6).

In the Al-tolerant genotype, Ca concentration either increased in stem or did not alter in leaf of plants with only one root half exposed to 100 mg Al L^{-1} (treatment: 0/100) and decreased in the other treatments, when compared to the control (treatment: 0/0). In the Al-tolerant genotype was not observed alteration in the stem and leaf Al concentration in this treatment (Fig. 4.2). However, in the Al-sensitive genotype was observed a decrease in the stem and leaf Ca concentration in all Al treatments (Fig. 4.6). HUANG et al. (1992) suggested that Al inhibits Ca^{2+} influx across the root plasmalemma possibly via blockage of Ca channels.

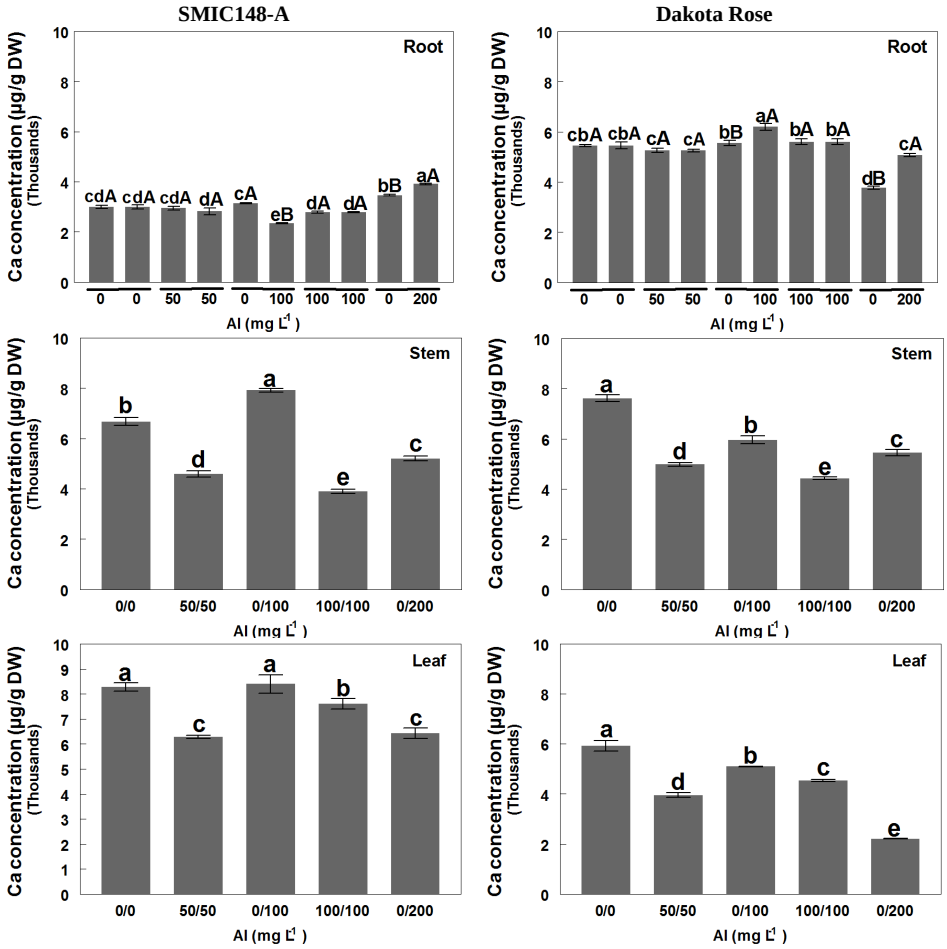

Figure 4.6 – Effect of Al concentration on Ca concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

BASSET; MATSUMOTO (2008) observed that Ca depletion and Al toxicity lead to death of tobacco cells via damage the membranes and decreasing the osmotic potential that inhibited water influx. Furthermore, works have demonstrated that inadequate levels of foliar Ca can reduce photosynthetic carbon fixation (MCLAUGHLIN et al., 1991). Free Ca^{2+} ions in the cell act as secondary messengers, in the transduction of various hormonal and environmental signals to the responsive elements of cellular metabolism (RENGEL; ZHANG, 2003).

In both Al-sensitive and Al-tolerant genotypes, root Mg concentration decreased in one root half of plants supplied with Al at 50 mg Al L^{-1} or 100 mg Al L^{-1} to both root halves (treatments: 50/50 and 100/100), when compared to the control (treatment: 0/0) (Fig. 4.7). In addition, in plants supplied with Al at 200 mg Al L^{-1} to only one half of the root system (treat-

ment: 0/200), root Mg concentration decreased in both root halves of the root system (treatment: 0/200), when compared to the control plants (treatment: 0/0). However, in both Al-sensitive and Al-tolerant genotypes the root Mg concentration was not altered in the Al-untreated root half when only half of the root system was treated with 100 mg Al L^{-1} (treatment: 0/100), when compared to the control plants (treatment: 0/0) (Fig. 4.7).

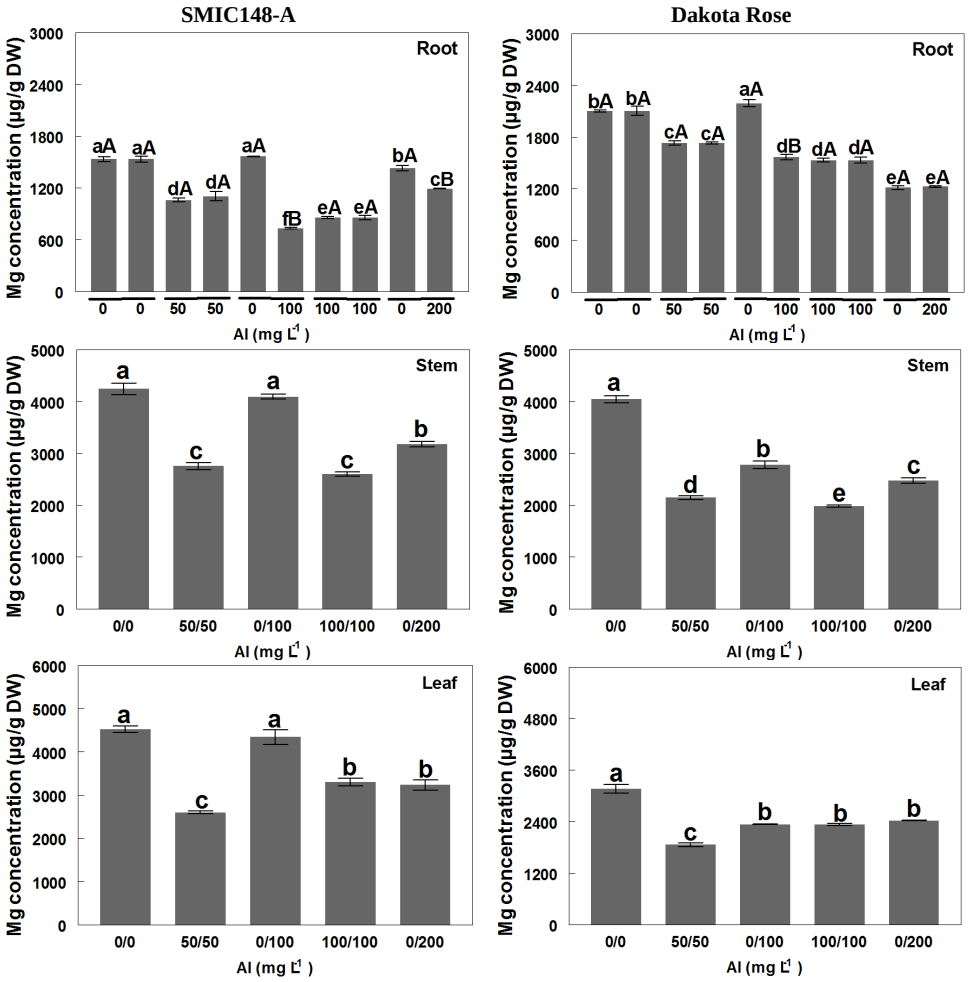

Figure 4.7 – Effect of Al concentration on Mg concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three different replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

The uptake Mg ion can be through the apoplasmic and symplasmic pathway. Further, entry of Mg into endodermis is more effective through the apoplasmic pathaway. However, the Mg apoplasmic pathway can be impaired due Mg-Al interation (BOSE et al., 2013). Large amounts of Al (85%-99% of the total cellular Al), accumulate in the cell wall and intercellular root spaces (MA, 2007). The binding of Al to the negative charges in the cell wall and pre-

cipitation of Al in the apoplast can decrease the uptake of Mg ions into apoplast (BOSE et al., 2013).

In the Al-sensitive genotype, stem and leaf Mg concentration was significantly decreased in all Al treatments (Fig. 4.7). In the Al-tolerant genotype, the stem and leaf Mg concentration was significantly decreased in the treatments with both sides of the root system exposed to Al (treatments: 50/50 and 100/100) (Fig. 4.7), as well as when plants were supplied with 200 mg Al L^{-1} to only half of the root system (treatment: 0/200). The decrease in the loading of Mg ions may be due to inhibition of Mg-permeable cation channels and Mg transporters by Al (BOSE et al., 2013). However, in the plants where Al was supplied to only half of the root system (treatment: 0/100), it was not observed significant difference when compared to the control plants (treatment: 0/0) (Fig. 4.7). The no reduction of the Mg concentration in the Al-tolerant in the stem and leaf in this treatment can be due to no translocation of Al into shoot parts. The Al concentration in the stem and leaf did not show significant difference when Al was supplied to only half of the root system (treatment: 0/100), when compared to the control plants (treatment:0/0) (Fig. 4.3).

Root Mn concentration in Al-tolerant genotype increased in one root half of plants supplied with 100 mg Al L^{-1} to both root halves (treatment:100/100) and in both root halves of the plant when only half of the root system was treated at 200 mg Al L^{-1} (treatment: 0/200), when compared to the control (treatment: 0/0) (Fig. 4.8). On the other hand, in the Al-sensitive genotype the root Mn concentration increased in all Al treatments (Fig. 4.8). In stem, the Mn concentration decreased in all Al treatments in the Al-sensitive genotype. However, in the Al-tolerant genotype was observed a decrease only in the plants when both root halves was treated with 100 mg Al L^{-1} (treatment: 100/100) and when only one root half was treated with 200 mg Al L^{-1} (treatment: 0/200) (Fig. 4.8).

However, leaf Mn concentration decreased in both Al-tolerant and Al-sensitive genotypes in all Al treatments, when compared to the control (treatment: 0/0) (Fig. 4.8). Furthermore, plants supplied either with 100 mg Al L^{-1} or 200 mg Al L^{-1} to only half of the root system (treatments: 0/100 and 0/200) showed increase in leaf Mn concentration in relation to plants that both root halves of the root system were treated with 50 mg Al L^{-1} or 100 mg Al L^{-1} (treatments: 50/50 and 100/100) (Fig. 4.8).

The Mn transport through the xylem from roots to the shoot (stem and leaf) of plants is performed by the transpiration rate (MARSCHNER, 1995). The Al can reduce transpiration

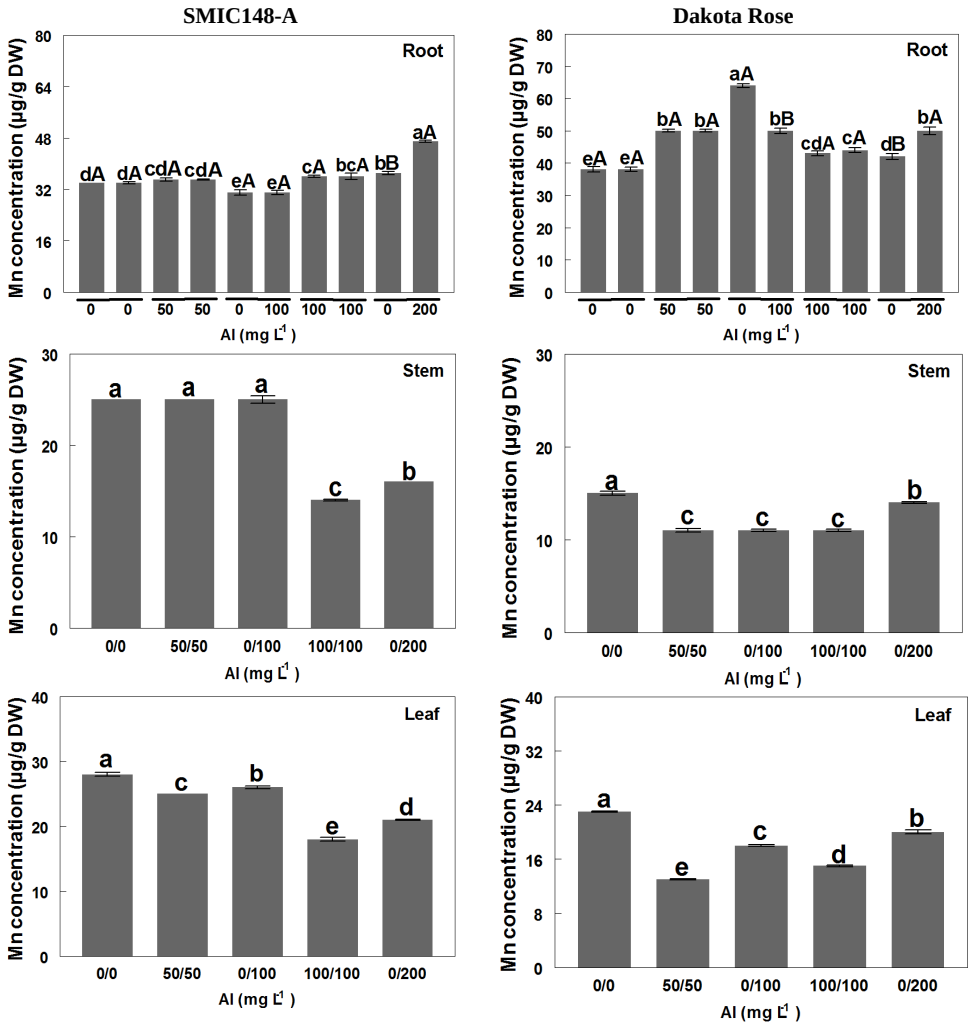

Figure 4.8 – Effect of Al concentration on Mn concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

rate due to blockage of K channels in guard cell, inhibiting the stomatal opening (LIU; LUAN, 2001). As micronutrient, low Mn levels are necessary for normal nutrition and development of plants. Mn is involved in metabolic processes such as respiration, photosynthesis, synthesis of amino acids and hormone activation (MILLALEO et al., 2010).

Root Cu concentration in Al-tolerant and Al-sensitive genotypes decreased when the plants were supplied with Al in all treatments, when compared to the control (treatment: 0/0) (Fig. 4.9). Furthermore, in both root halves of the plant that only half of the root system was treated with 100 mg Al L^{-1} or 200 mg Al L^{-1} (treatments: 0/100 and 0/200) was observed decrease in the root Cu concentration, when compared to control (0/0) (Fig. 4.9).

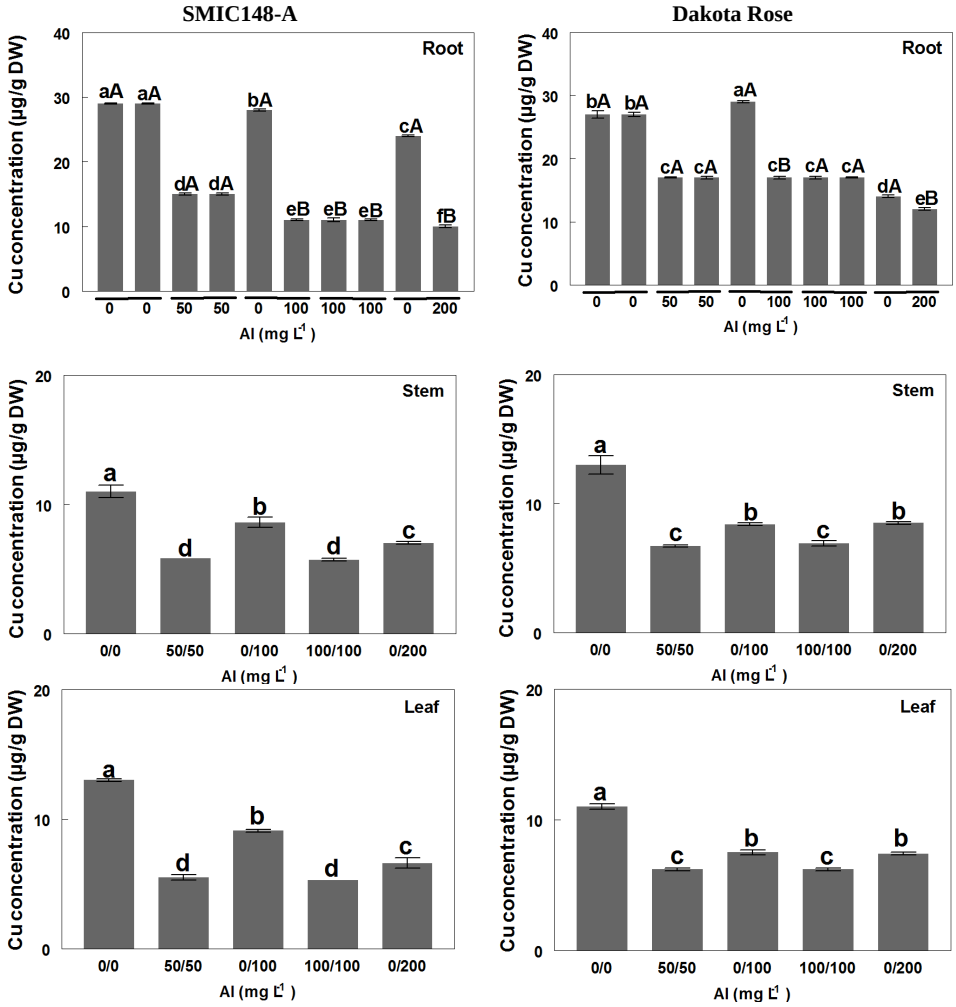

Figure 4.9 – Effect of Al concentration on Cu concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

In both Al-tolerant and Al-sensitive genotypes the stem and leaf Cu concentration decreased in all Al treatments, when compared to the control (treatment: 0/0) (Fig. 4.9). Furthermore, plants supplied either with 100 mg Al L^{-1} or 200 mg Al L^{-1} to only one half of the root system (treatments: 0/100 and 0/200) showed increase in the stem and leaf Cu concentration in relation to plants that both halves of the root system were treated with 50 mg Al L^{-1} or 100 mg Al L^{-1}, respectively (treatments: 50/50 and 100/100) (Fig. 4.9).

In both Al-sensitive and Al-tolerant genotypes, root Fe concentration increased in roots supplied at 50 mg Al L^{-1} or 100 mg Al L^{-1} to both root halves (treatments: 50/50 and 100/100), when compared to the control (treatment: 0/0) (Fig. 4.10). Furthermore, in plants

supplied at 200 mg Al L^{-1} to only one half of the root system (treatment: 0/200), root Fe concentration increased in both root halves of the root (treatment: 0/200), when compared to the control (treatment: 0/0). However, in both Al-sensitive and Al-tolerant genotypes the root Fe concentration either was not altered (Al-tolerant) or decrease (Al-sensitive) in the Al-untreated root half when only half of the root system was treated with 100 mg Al L^{-1} (treatment: 0/100) (Fig. 4.10).

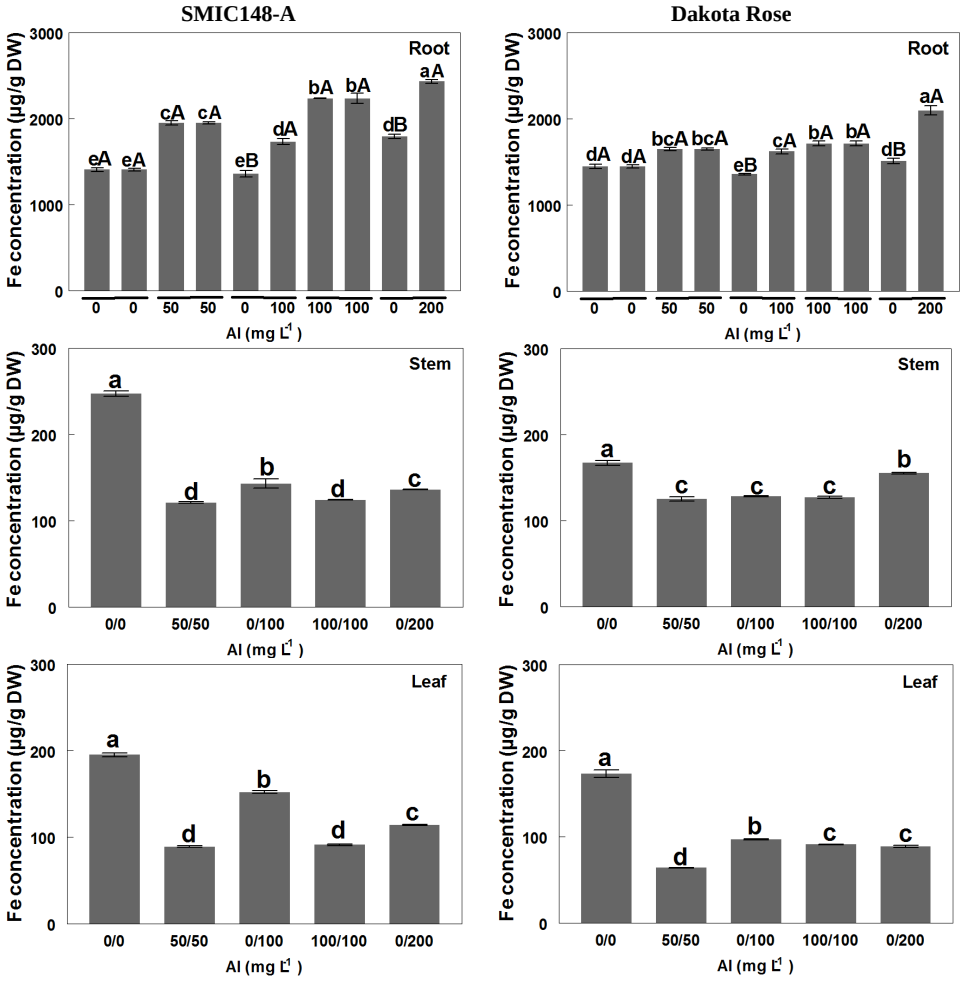

Figure 4.10 – Effect of Al concentration on Fe concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

The increase of Fe concentration in the roots exposed to Al in both genotypes can be due to the fact that the Al precipitates with Fe in root apoplast, reducing translocation to shoots. Furthermore, ionic radius of the trivalent cations Al^{3+} (67.5 pm) and Fe (78.5 pm) are quite close. Due to its ability to mimic Fe, the Al disturbs Fe homeostasis and consequently interferes with

essential biochemical processes dependent on this redox-active metal . This interaction leads to a further increased in the intracellular pool of Fe (WARD; ZHANG; CRICHTON, 2001). However, WATANABE; JANSEN; OSAKI (2006) observed in *Melastoma malabathricum*, an Al accumulator specie, that Al application significantly decreased the Fe concentration in roots.

In the Al-sensitive and Al-tolerant genotype, the stem and leaf Fe concentration was significantly decreased in all Al treatments, when compared to the control (treatment: 0/0) (Fig. 4.10). HAJIBOLAND et al. (2013) observed the same response in *Camellia sinensis* exposed at 200 μM Al. The lower accumulation of Fe in the stem and leaves of the both genotypes exposed to Al in comparison to control can be due to the competition between Fe and Al for citrate as a ligand in xylem loading and long-distance transport. The main form of Al transported from roots to shoot in the xylem sap is Al-citrate (MORITA et al., 2004). Citrate is also a major ligand for Fe in plants (HAJIBOLAND et al., 2013).

In the Al-tolerant genotype, the stem and leaf Fe concentration was significantly decreased in the treatments with both halves of the root system exposed to Al (treatments: 50/50 and 100/100), when compared to stem and leaf of the plants where only half of the root system were treatment with 100 mg Al L^{-1} or 200 mg Al L^{-1} (Fig. 4.10). In addition, in the stem of the Al-sensitive genotype Fe concentration decreased in the treatments with both halves of the root system exposed to Al (treatment: 100/100), when compared to Fe concentration of the plants where only half of the root system were treated with 200 mg Al L^{-1} (Fig. 4.10). In the leaf, Fe concentration decreased in the treatments with both halves of the root system exposed to Al (treatment: 50/50), when compared to Fe concentration of the plants where only half of the roots were treatment with 100 mg Al L^{-1} (Fig. 4.10).

In both Al-sensitive and Al-tolerant genotypes, root Zn concentration decreased in one root half of plants supplied with 50 mg Al L^{-1} or 100 mg Al L^{-1} to both root halves (treatments: 50/50 and 100/100), when compared to the control (treatment: 0/0) (Fig. 4.11). Furthermore, in plants supplied with 100 mg Al L^{-1} or 200 mg Al L^{-1} to only one half of the root system (treatments: 0/100 and 0/200), root Zn concentration decreased in both root halves of the root, when compared to the control plants (treatment: 0/0). However, in both genotypes the root Zn concentration in the Al-untreated root half was higher than in Al-treated root half (Fig. 4.11).

The stem Zn concentration in both Al-tolerant and Al-sensitive genotypes was significantly decreased in all Al treatments, when compared to the control (treatment: 0/0) (Fig. 4.11).

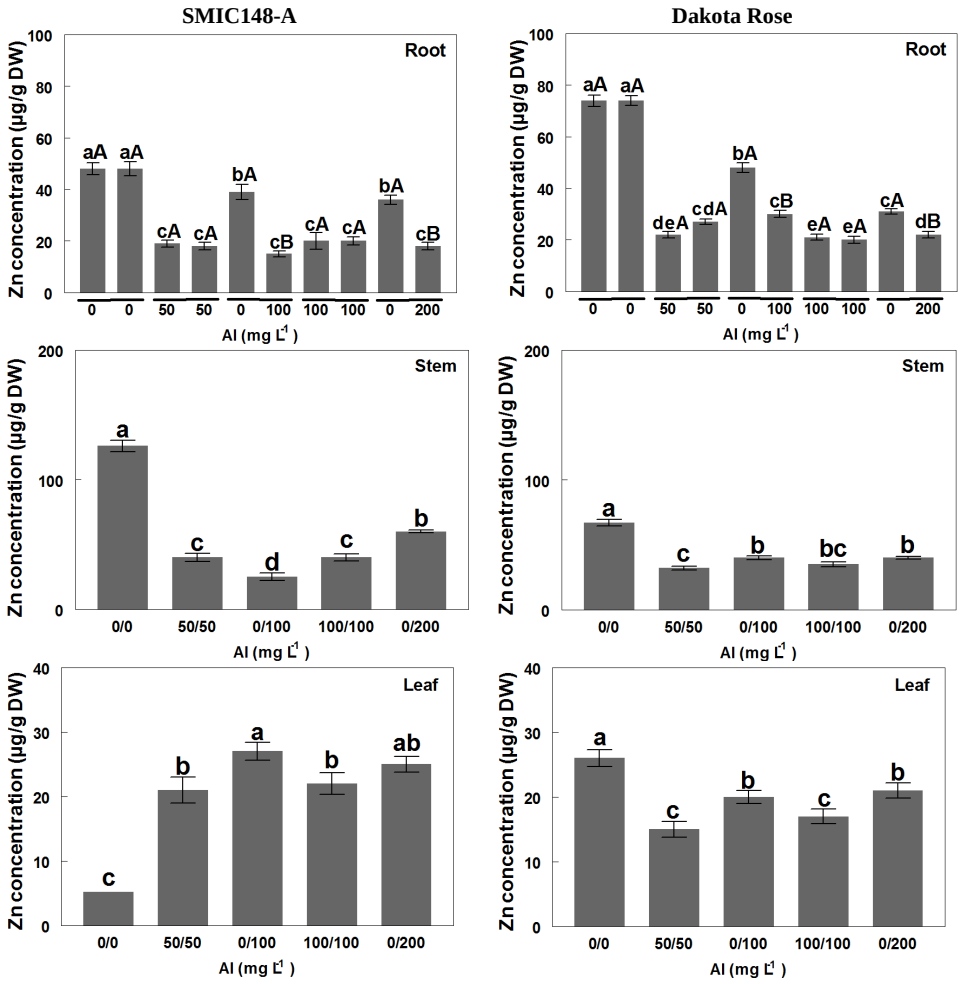

Figure 4.11 – Effect of Al concentration on Zn concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

The same response was observed to Al-sensitive genotype for leaf Zn concentration (Fig. 4.11).

Zinc deficiency in plants affect photosynthesis due to altered chloroplast pigments biosynthesis (KÖSESAKAL; ÜNAL, 2010). In the Al-sensitive genotype was observed reduction in the total chlorophyll and carotenoids pigments (data shown in manuscript III). Interestingly, for the Al-tolerant genotype, the Zn leaf concentration increased in all Al treatments, when compared to the control (treatment: 0/0) (Fig. 4.11).

SAMREEN et al. (2013) observed that Zn application increased plant chlorophyll and protein contents in control. In the Al-tolerant genotype was observed an increased in the total chlorophyll and carotenoids pigments in the plants exposed to both root halves to 50 mg Al L^{-1} and only root half to 200 mg Al L^{-1} (treatments: 50/50 and 0/200), when compared to the

control (treatment: 0/0) (data shown in manuscript IV). Furthermore, Zn supplementation, at low level, restored and enhanced the functional activity of these enzymes (SOD, CAT, APX and GR), decrease lipid peroxidation level and restored the chlorophyll content as compared to plants treated with Cd (CHERIF et al., 2012). Therefore, these results show that Al tolerance in the SMIC148-A genotype can be correlated to lower Al translocation from root to the shoot and a higher nutrient translocation, mainly P and Zn.

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5 BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES IN TWO POTATO GENOTYPES (*Solanum tuberosum*) THAT DIFFER IN AL-AVOIDANCE BY LOCALIZED SUPPLY OF ALUMINUM IN A SPLIT-ROOT SYSTEM

Abstract

This study aimed to evaluate whether Al oxidative stress differs in potato genotypes Dakota Rose (Al-sensitive) and SMIC148-A (Al-tolerant), which present distinct degrees of Al - avoidance. Plants were cultivated in a split-root system for 7 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. In general, Al exposure decreased shoot length, leaf, stem and root fresh weight and leaf area of both genotypes. However, only in Al-sensitive genotype was observed decrease in the stolon fresh weight and stolon number. Furthermore, Al-tolerant genotype was able to increased total chlorophyll and carotenoids concentration whereas in the Al-sensitive genotype a decrease occurred with Al exposure. In the Al-tolerant genotype plants with one root half exposed at 100 mg Al L^{-1} did not differ in Al shoot concentration, when compared to the control plants. Both genotypes showed decrease P concentration in Al-treated root half. However, in Al-untreated root half the P concentration increased, mainly in the Al-tolerant genotype. In the root tissue, the acid phosphatase activities decreased with Al exposure in both genotypes. The Al-sensitive genotype also decreased leaf acid phosphatase activity under Al exposure. Compensatory effects were noticed for soluble protein, APX and CAT activities for both genotypes, with only one root half was exposed to Al. Additionaly, in Al-sensitive genotype, was observed increase in the leaf and root lipid peroxidation in plants exposed to higher Al treatments. On the other hand, in the Al-tolerant genotype was not observed increase in the plants exposed at higher Al treatments. These results show that Al-tolerant genotype, showed higher Al-avoidance when compared to Al-sensitive genotype. This response can be associated to lower Al translocation to the leaves, mainly in the plants with one root half exposed to Al and the higher ability of this genotype to remobilize P to the Al-untreated root half.

Keywords: *Solanum tuberosum*, aluminum, oxidative stress, split-root

5.1 Introduction

Potatoes (*Solanum tuberosum* L.) rate fourth in world production among various agricultural products, following wheat, rice and maize (**?**), with an overall annual production of 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; FENNELL, 1985).

Acid soils, which comprise 30–40% of the world's arable lands (VITORELLO; CAPALDI; STEFANUTO, 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; MUTERT, 1995). Under this condition, Al is solubilized in acidic pH into the toxic cation Al^{3+} which is toxic to plants.

Aluminum is known to inhibit plant growth (ČIAMPOROVÁ, 2002), mainly the root system (BALESTRASSE; GALLEGO; TOMARO, 2006; TABALDI et al., 2007). Symptoms of Al toxicity are also manifested in the shoot and are regarded as a consequence of root system injuries (VITORELLO; CAPALDI; STEFANUTO, 2005). 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., 2007) and, consequently, formation of reactive oxygen species (ROS) in plants, including superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$) and hydroxygen peroxide (H_2O_2).

These ROS can cause oxidative damage to the biomolecules such as lipids, proteins (TABALDI et al., 2007), photosynthetic pigments and nucleic acids, which leads to cell membrane peroxidation, loss of ions, protein hydrolysis, and even DNA strand breakage (GUO; ZHANG; ZHANG, 2007). To mitigate the oxidative damage initiated by ROS, plants have developed a complex defense antioxidant system, which may possibly protect cells from Al toxicity (MITTLER, 2002).

Besides enzyme activity, morphological changes may also play an important role in Al avoidance. In this view, plant roots are characterized by very high adaptability. Their growth and development involve complex interactions with both the soil environment and the shoot (MARSCHNER, 1995). 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). This rel-

ative 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.

Potential alternatives to the direct amelioration of subsoil acidity include the use of Al-tolerant germplasm (FOY, 1988). In our previous study (TABALDI et al., 2009), utilizing a homogeneous supply of Al to the roots of potato genotypes grown in a hydroponic growth system, it was demonstrated that the SMIC148-A genotype was Al-tolerant, whereas the Dakota Rose genotype was Al-sensitive. Moreover, it was observed that Al supply induced oxidative stress, mainly in the Al-sensitive genotype. Therefore, we formulated the hypothesis that potato genotypes 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 genotype, since the response to local supply of Al is reduced under this condition.

5.2 Material and Methods

Plant materials and growth conditions

Microtubers of potato genotypes (*Solanum tuberosum* L.) Dakota Rose (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 (mg L^{-1}): 7.54 P; 85.31 N; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 176.76 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe. The pH solution was adjusted to 4.0 ± 0.1 with a 1 M solution of HCl or NaOH.

Fourteen-day-old plants (shoot length of five centimeters) were 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 three days. After that acclimatization period, these plants with split-roots were cultivated for 7 days in a new nutrient solution (without P and pH 4.0 ± 0.1) with five treatments (ten 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. At harvest, the plants of both genotypes were divided into shoot (leaf and stem), left root and right root to evaluate nutrient and Al concentration, and growth parameters.

Growth parameters

Growth of potato genotypes was determined by measuring the fresh weight of leaves, stem, roots and stolon, shoot length, stolon number, and leaf area. For the leaf area, all leaves of the plant were scanned using a portable measuring instrument (Area Meter AM300).

Al and P determination

Al and P concentration was determined in roots, stem and leaves. Dried plant tissues, between 0.01 and 0.25 g, were ground and digested with 5 mL of concentrated HNO_3. Sample digested was carried out in an open digestion system, using a heating block Velp Scientific (Milano, Italy). Heating was set at 130°C for 2 h. Plastic caps were fitted to the vessels to prevent losses by volatilization. The Al and nutrient concentration was determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-EOS), using a PerkinElmer Optima 4300 DV (Shelton, USA) equipped with a cyclonic spray chamber and a concentric nebulizer.

Chlorophyll and carotenoid determination

Chlorophyll (a+b) and carotenoids were extracted following the method of HISCOX; ISRAELSTAM (1979) and estimated with the help of Lichtenthaler's formulae (LICHTENTHALER, 1987). Fresh leaves (0.1 g) were incubated at 65°C 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. Chlorophyll and carotenoid concentrations were expressed as mg 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 20,000 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 95°C for 40 min and then quickly cooled in an ice bath for 15 min, and centrifuged at 10,000 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}.

Enzyme activities of antioxidant system

Frozen root and leaf samples were used for enzyme analysis. One gram tissue were homogenized in 3 mL of 0.05 M sodium phosphate buffer (pH 7.8) including 1 mM EDTA and 1% Triton X-100. The homogenate was centrifuged at 13,000 g for 20 min at 4 °C. Supernatant was used for enzyme activity and protein content assays (ZHU et al., 2004).

Catalase (CAT) activity was assayed following the modified AEBI (1984) method. The activity was determined by monitoring the disappearance of H_2O_2 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 potassium phosphate buffer (pH 7.0) and 30 μL extract.

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 mmol l^{-1} cm^{-1}).

Guaiacol Peroxidase (POD) was measured according to ZERAIK; DE SOUSA; FATIBELLO-FILHO (2008). The reaction mixture contained 1.0 mL potassium phosphate buffer (100 mM (pH 6.5), 1.0 mL of guaiacol (15 mM) and 1.0 mL de H_2O_2 (3 mM). After the homogenization this solution was added 50 μL of plant extract. The guaicol oxidation to tetraguaiacol was measured through of increase in the absorbance at 470 nm. The results were expressed at μmol

min^{-1} mg^{-1} protein. For the calculation using the molar extinction coefficient of 26.6 mM^{-1} cm^{-1}.

Acid phosphatases assay

Frozen roots and leaves were homogenized and centrifuged at 20,000 g for 30 min and the resulting supernatant was used for enzyme assay. Acid phosphatase activity was determined according to TABALDI et al. (2007) 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 was added to the reaction mixture, except in controls, and pre-incubated 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% TCA to a final concentration of 5%. Inorganic phosphate (Pi) was quantified at 630 nm using malachite green as the colorimetric reagent and KH_2PO_4 as standard for the calibration curve. All assays were performed using PPi as substrate at a final concentration of 3.0 mM.

Soluble phosphorus content (Pi)

The same supernatant utilized in the acid phosphatases assay was utilized to quantify the soluble phosphorus content. An aliquot of the diluted sample (800 μL) was incubated at 45°C for 45 min in a medium containing 2.5 N sulfuric acid, 4.8 mM ammonium molybtate and 35 mM ascorbic acid in a total volume of 1 mL. A standard curve was constructed using KH_2PO_4. After cooling at room temperature the samples were read at 650 nm.

Protein determination

For all the enzyme preparations, protein was determined following the method of BRADFORD (1976) using bovine serum albumin for the calibration curve.

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$\pm$S.D. of three independent replicates.

5.3 Results

Growth parameters

After 7d in a split-root system, a significant decrease in the shoot length was observed in the Al-tolerant and Al-sensitive genotypes when both or only one root half was exposed to Al (Fig. 5.1). Furthermore, both genotypes decreased leaf fresh weight when both root halves were exposed to Al (treatments: 50/50 and 100/100). However, in the Al-sensitive genotype an increase in the leaf fresh weight and leaf area occurred in plants when only one root half was exposed at 100 mg Al L^{-1} (treatment: 0/100) (Fig. 5.1). However, Al-tolerant genotype showed decrease in leaf area in plants where both or only one root half was exposed to Al (Fig. 5.1).

Interestingly, the Al-tolerant genotype did not show alteration in stem fresh weight when both or only one root half was exposed to Al. Additionally, in the plants where both root halves were exposed at 50 mg Al L^{-1} (treatment: 50/50) an increase occurred, when compared to the control (treatment: 0/0) (Fig. 5.1). In the Al-sensitive genotype the Al exposure provoked decrease in stem fresh weight. However, when only one root half was exposed at 100 mg Al L^{-1} (treatment: 0/100) no significant difference in stem fresh weight was observed, when compared to the control (treatment: 0/0) (Fig. 5.1).

In both genotypes, the root fresh weight decreased in the root halves exposed to Al (Fig. 5.2), when compared to the control. Both genotypes showed similar values of root fresh weight in Al-untreated root half of treatment 0/100, when compared to the control (Fig. 5.2).

Furthermore, in Al-sensitive genotype it was observed decrease in stolon growth (stolon fresh weight and stolon number) where one root half was exposed to Al, when compared to the control (Fig. 5.2). However, where the root half was not exposed to Al, the stolon formation was not inhibited (Fig. 5.2). Interestingly, the Al-tolerant genotype did not show inhibition in growth of stolon (stolon fresh weight and stolon number) at any Al treatment (Fig. 5.2).

Al and P determination

In both genotypes root Al concentration increased upon Al exposure (Fig. 5.3). In plants supplied with Al at 100 and 200 mg Al L^{-1} to only one half of the root system (treatments: 0/100 and 0/200), root Al concentration in the Al-untreated root half was much lower when

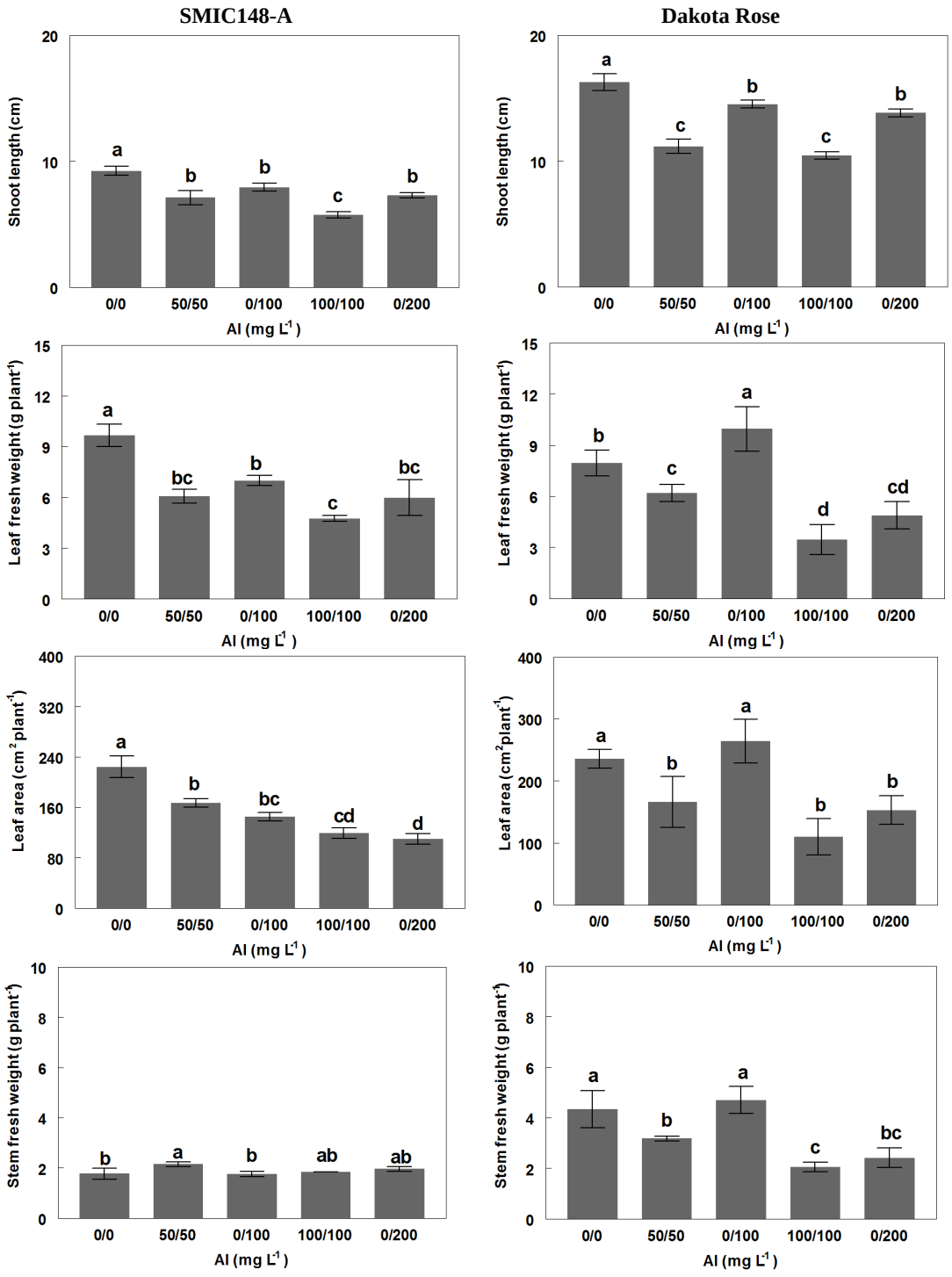

Figure 5.1 – Effect of Al concentration on shoot length, leaf fresh weight, leaf area and stem fresh weight in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

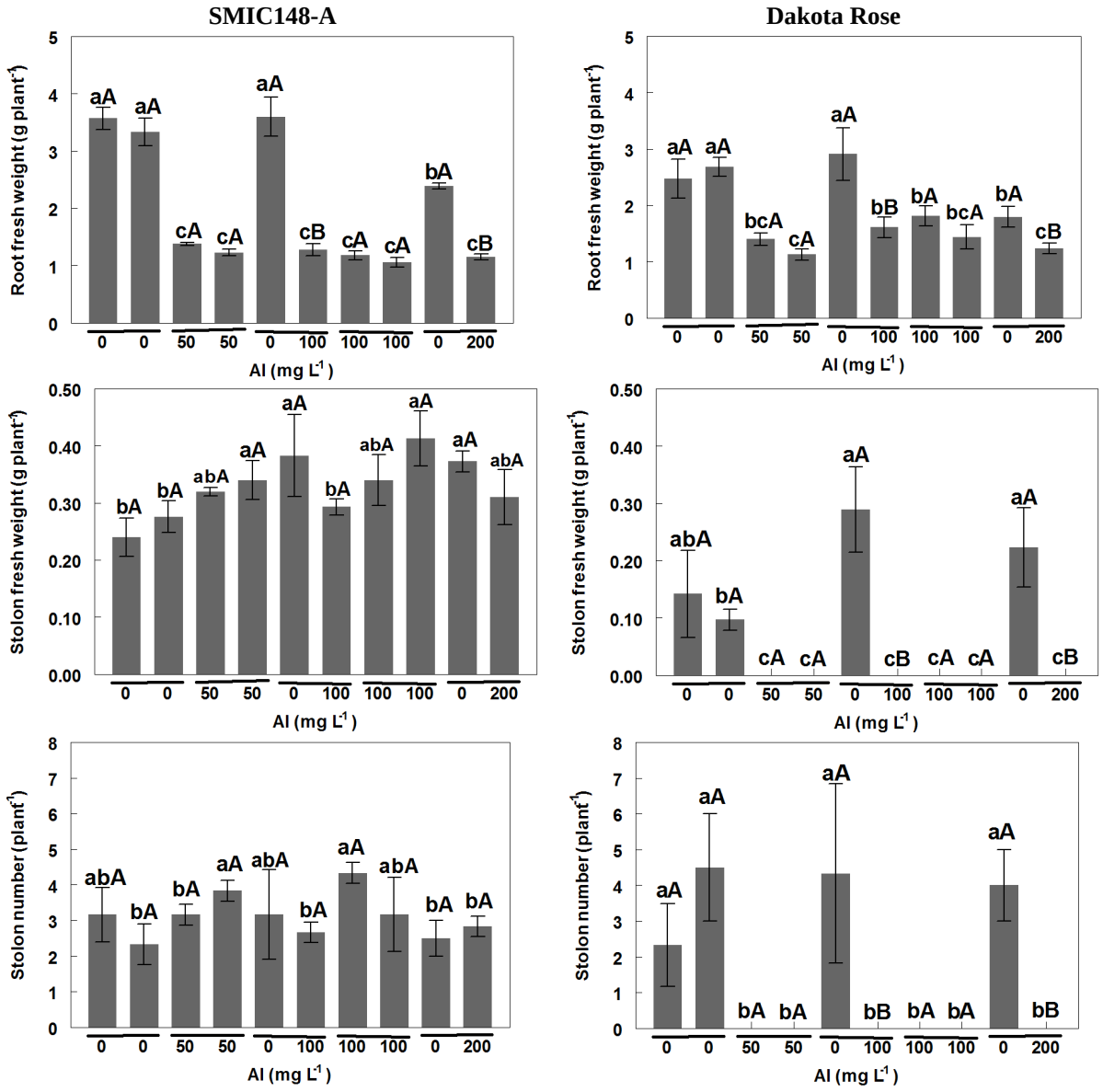

Figure 5.2 – Effect of Al concentration on root fresh weight, stolon fresh weight and stolon number in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

compared to Al-treated root half. However, these root halves showed higher Al concentration, when compared to the control (treatment: 0/0) (Fig. 5.3). Furthermore, in Al-tolerant genotype the percentage of Al translocation was lower than that in the Al-sensitive genotype [Al-tolerant (0/100, increase of 80%; 0/200, increase of 203%) and Al-sensitive (0/100, increase of 209%; 0/200, increase of 258%)] (Fig. 5.3).

In Al-tolerant genotype, the stem and leaf Al concentrations increased in the treatments

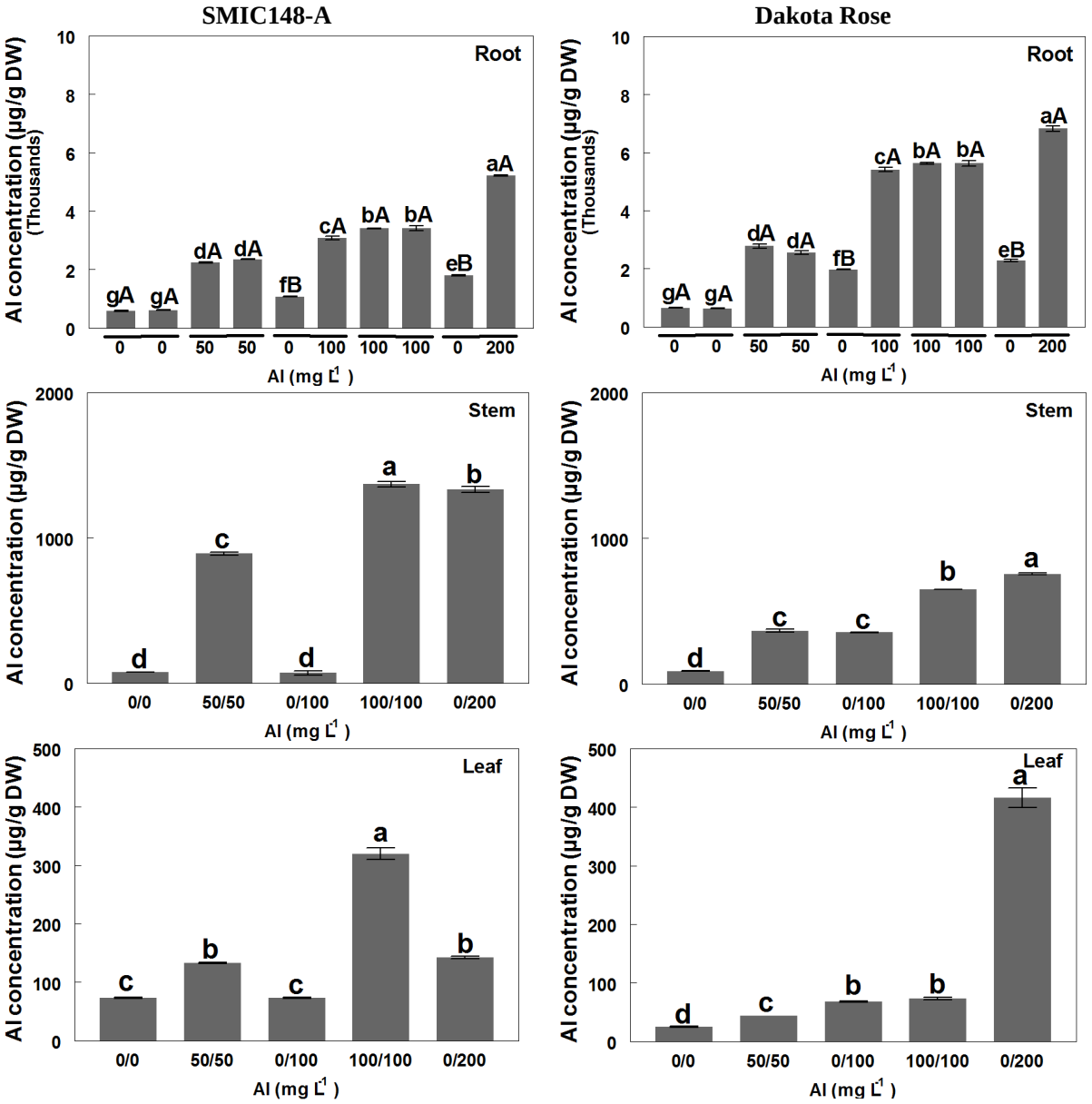

Figure 5.3 – Effect of Al concentration on Al concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

with both root halves exposed to Al (treatments: 50/50 and 100/100) (Fig. 5.3), as well as in plants supplied with different Al levels (treatment: 0/200) (Fig. 5.3). However, in plants where one half of the root system was supplied with 100 mg Al L^{-1} (treatment: 0/100), leaf and stem Al concentrations did not differ from control plants (Fig. 5.3). Conversely, for the Al-sensitive genotype, the stem and leaf Al concentrations increased in the root halves supplied with 100 and 200 mg Al L^{-1} (treatments: 0/100 and 0/200), as well as in the root halves treated with 50

and 100 mg Al L^{-1} (treatments: 50/50 and 100/100) (Fig. 5.3).

In both Al-sensitive and Al-tolerant genotypes, root P concentration decreased in both root halves exposed at 50 and 100 mg Al L^{-1}, when compared to the control (treatment: 0/0) (Fig. 5.4). However, in plants supplied with different Al levels (treatments: 0/100 and 0/200), P root concentration decreased only in the root halves exposed to Al, when compared to the control plants (treatment: 0/0) (Fig. 5.4). In addition, Al-tolerant and Al-sensitive genotypes showed higher P root concentration in the root halves not exposed to Al in the treatments 0/200 and 0/100, respectively (Fig. 5.4). Interestingly, in stem of both Al-tolerant and Al-sensitive genotypes, the P concentration increased when both roots halves were supplied at 50 and 100 mg Al L^{-1} (treatments: 50/50 and 100/100), as well as when only half of the root system were treated with 100 and 200 mg Al L^{-1} (treatments: 0/100 and 0/200) (Fig. 5.4). Furthermore, this increase was more pronounced when both root halves were exposed at 100 mg Al L^{-1} for the Al-tolerant genotype, and 50 and 100 mg Al L^{-1} for the Al-sensitive genotype (Fig. 5.4). The Al-tolerant genotype showed increase in leaf P concentration when both root halves were exposed at 100 mg Al L^{-1} (treatment: 100/100) and when only one root half was exposed at 100 mg Al L^{-1} (treatment: 0/100). On the other hand, in the Al-sensitive genotype, the leaf P concentration decreased in all Al treatments (Fig. 5.4).

Acid phosphatases assay and soluble phosphorus concentration (Pi)

In Al-tolerant genotype was observed decrease in APase activity in the root half treated at 200 mg Al L^{-1} when compared to Al-untreated root half, which did not differ from control (treatment: 0/0) (Fig. 5.5). In Al-sensitive genotype was observed decrease in root APase activity in the Al-treated root half (Fig. 5.5). Furthermore, when only one root half was treated at 100 mg Al L^{-1} (treatment: 0/100), the Al-untreated root half did not show alteration in APase activity, when compared to the control (Fig. 5.5).

Pi concentration, in Al-tolerant genotype, decreased in the root half exposed at 100 and 200 mg Al L^{-1} (treatments: 0/100, decrease of 19%; 100/100, decrease of 16%, and 0/200, decrease of 18%), when compared to the control (Fig. 5.5). However, in the Al-sensitive genotype, the Pi concentration decreased in the root half in all Al treatments (Fig. 5.5).

In Al-tolerant genotype the leaf APase activity was not altered in plants treated with Al (Fig. 5.5). However, in the Al-sensitive genotype was observed decrease in leaf APase activity when both root halves were treated at 50 and 100 mg Al L^{-1} (treatments: 50/50 and 100/100)

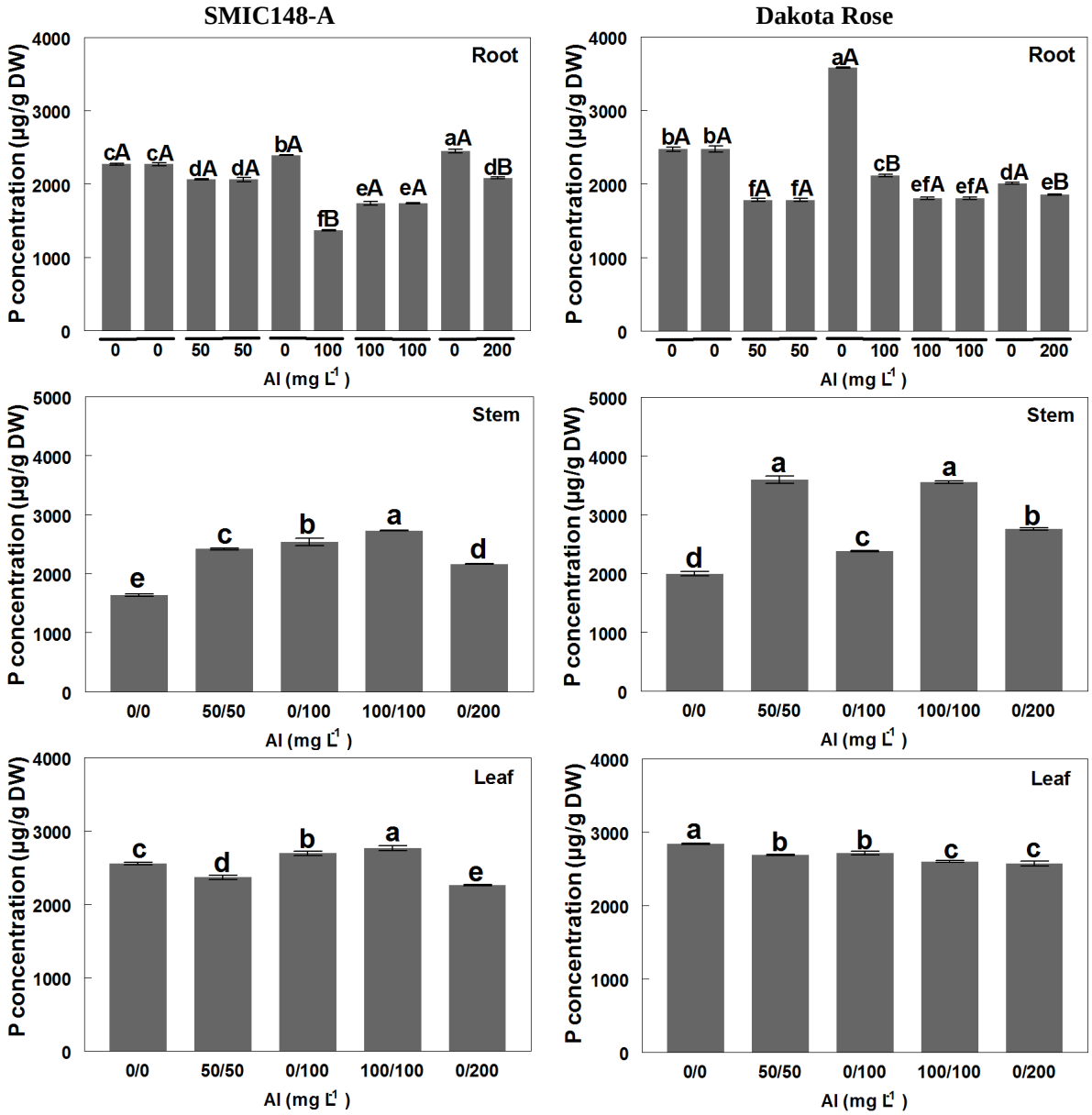

Figure 5.4 – Effect of Al concentration on P concentration in root, stem and leaf in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

and when only one root half was treated at 200 mg Al L^{-1} (Fig. 5.5).

The leaf soluble phosphorus (Pi) concentration in the Al-tolerant genotype increased where both root halves were exposed at 100 mg Al L^{-1} (treatment: 100/100; increase of 55%) and with one root half exposed at 100 and 200 mg Al L^{-1} (treatments: 0/100; increase of 19%, and 0/200; increase of 40%). However, in the Al-sensitive genotype was observed increase of

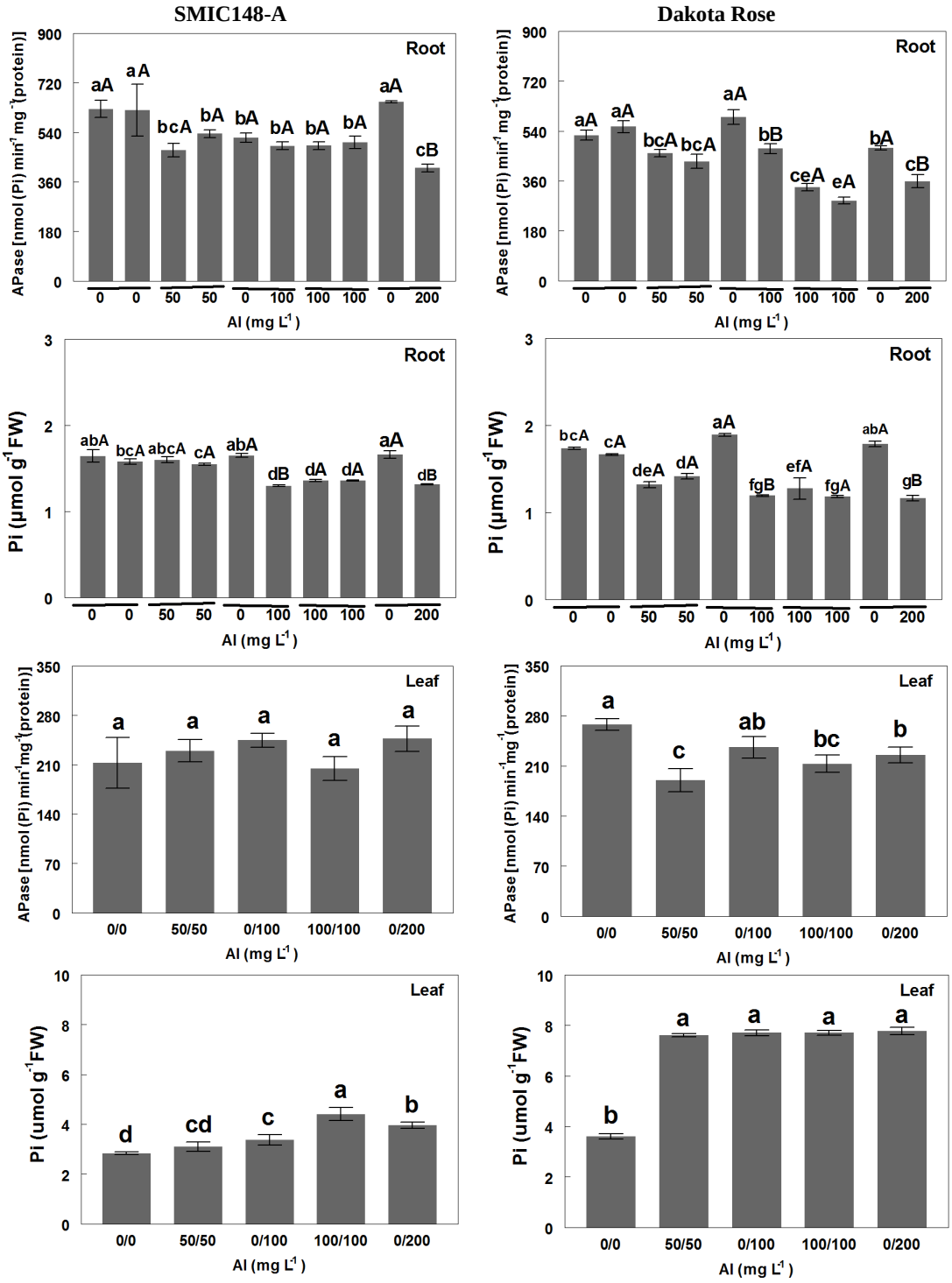

Figure 5.5 – Effect of Al concentration on acid phosphatase activity (APase) and soluble Pi in leaf and root of SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

leaf Pi concentration in all Al treatments (Fig. 5.5).

Chlorophyll and carotenoid concentrations

The total chlorophyll concentration increased in leaves of the Al-tolerant genotype treated at 50 mg Al L^{-1} (treatment: 50/50; increase of 26%) in both root halves and when only one root half was treated at 200 mg Al L^{-1} (treatment: 0/200; increase of 22%), when compared to the control (0/0) (Fig. 5.6). On the other hand, a decrease was observed in total chlorophyll concentration in Al-sensitive genotype exposed to all Al treatments (Fig. 5.6). The exposure

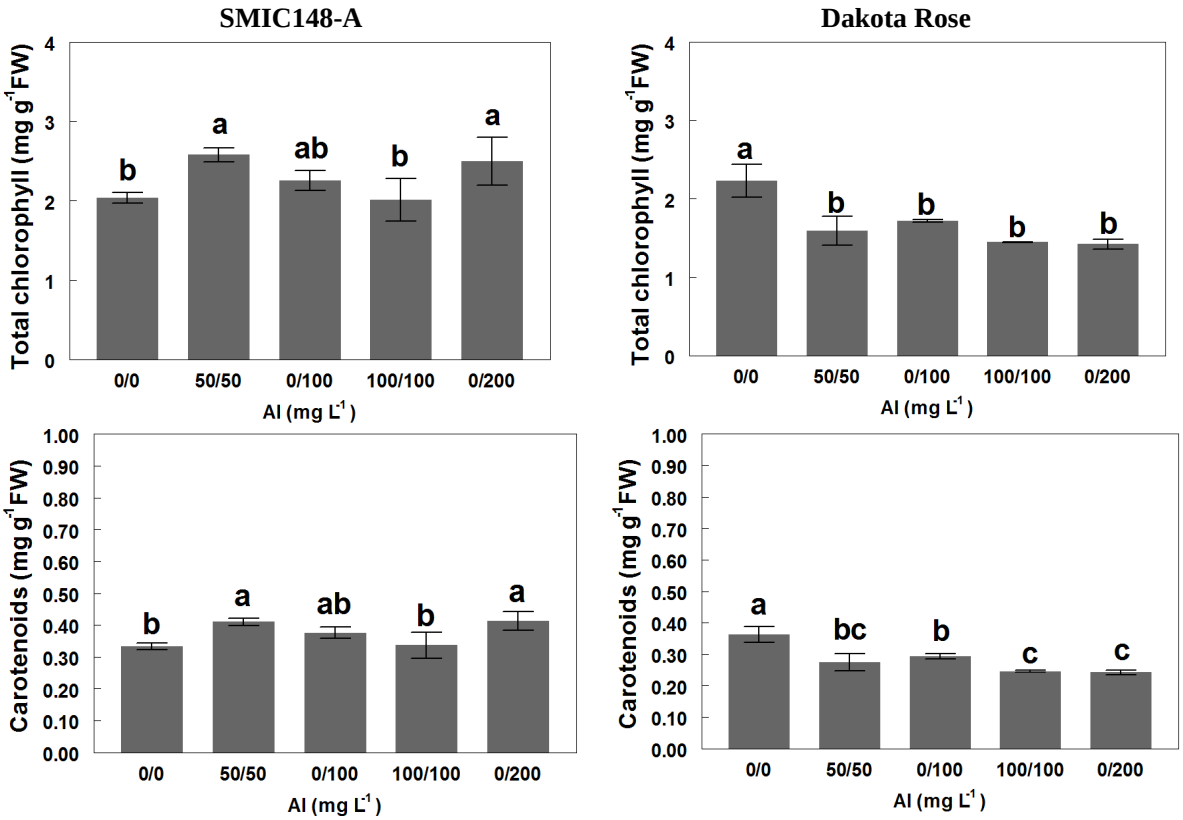

Figure 5.6 – Effect of Al concentration on total chlorophyll and carotenoids in SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

of the Al-sensitive genotype to different Al levels resulted in a decrease of carotenoids concentration (Fig. 5.6). However, in Al-tolerant genotype, the carotenoids concentration increased in plants treated at 50 mg Al L^{-1} to both root halves (treatment: 50/50; increase of 23%) and with Al supplied to only one root half at 200 mg Al L^{-1} (treatment: 0/200; increase of 24%),

when compared to the control (Fig. 5.6).

Soluble protein concentration

The total soluble protein concentration of leaves decreased in Al-tolerant genotype when both root halves were treated at 100 mg Al L^{-1} (treatment: 100/100; decrease of 11%) and with Al supplied to only one root half at 100 and 200 mg Al L^{-1} (treatments: 0/100; decrease of 13% and 0/200; decrease of 20%), when compared to the control (treatment: 0/0) (Fig. 5.7). In Al-sensitive genotype was observed more pronounced decrease in total soluble protein concentration when plants were treated at 100 mg Al L^{-1} to both root halves (treatment:100/100; decrease of 35%) and with Al supplied to only one root half at 200 mg Al L^{-1} (treatment: 0/200; decrease of 19%) (Fig. 5.7). However, in plants where only one root half was exposed at 100 mg Al L^{-1} (treatment: 0/100), the total soluble protein concentration were 12% higher, when compared to the control (Fig. 5.7). Both Al-tolerant and Al-sensitive genotypes showed decrease in total soluble protein concentration in the roots exposed to Al (Fig. 5.7). However, in the plants where only one root half was exposed to Al (treatments: 0/100; 0/200), the Al-untreated root half showed higher total soluble protein concentration, when compared to the control (treatment: 0/0) and with the root half treated with Al (Fig. 5.7).

Estimation of lipid peroxides

In the Al-tolerant genotype leaf lipid peroxidation was not altered upon the addition of Al, except in plants where both root halves were exposed at 50 mg Al L^{-1}, which increased the MDA levels (Fig. 5.7). In Al-sensitive genotype, the leaf lipid peroxidation increased in plants that were treated at 100 mg Al L^{-1} to both root halves (treatment: 100/100; increase of 36%) and with Al supplied to only one root half at 200 mg Al L^{-1} (treatment: 0/200; increase of 24%), when compared to the control (treatment: 0/0) (Fig. 5.7). Furthermore, in plants where only one root half was treated at 100 mg Al L^{-1} (treatment: 0/100; decrease of 23%) was observed decrease in leaf lipid peroxidation, when compared to the control (Fig. 5.7).

Interestingly, in Al-tolerant genotype, the root lipid peroxidation decreased in both root halves where only one root half was treated at 100 mg Al L^{-1} (treatment: 0/100), as well as in the root half untreated with Al in plants where only one root half was treated at 200 mg Al L^{-1} (treatment: 0/200), when compared to the control (Fig. 5.7). Furthermore, in the root half treated at 200 mg Al L^{-1}, lipid peroxidation was not altered, when compared to the

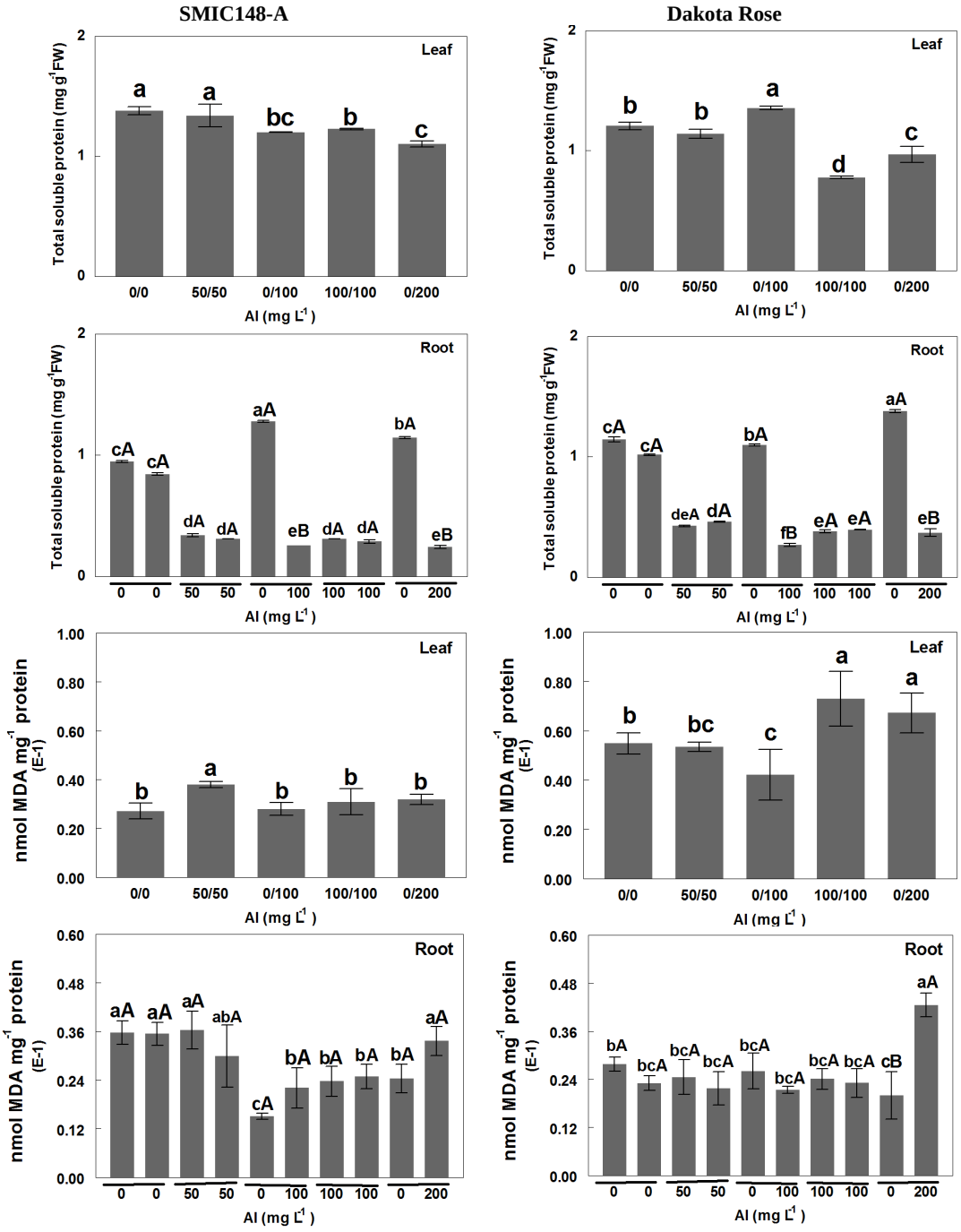

Figure 5.7 – Effect of Al concentration on total soluble protein concentration and lipid peroxidation in leaf and root of SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

control (Fig. 5.7). However, Al-sensitive genotype showed increase of 68% in the root lipid peroxidation for the root half treated at 200 mg Al L^{-1}, when compared to the control (Fig.

5.7).

Enzyme activities of antioxidant system

The activity of leaf APX in Al-tolerant genotype decreased when both root halves were exposed at 50 and 100 mg Al L^{-1} (treatments: 50/50, decrease of 22%; and 100/100, decrease of 26%) and when only one root half was exposed at 200 mg Al L^{-1} (treatments: 0/200, decrease of 65%), compared to the control (Fig. 5.8). Furthermore, in the plants where only one root half was exposed at 100 mg Al L^{-1} was observed increase (11%) in leaf APX activity (treatment: 0/100), when compared to the control (Fig. 5.8). However, in Al-sensitive genotype, leaf APX activity was increased (30 to 60%) in all Al treatments (Fig. 5.8). Both Al-tolerant and Al-sensitive genotypes showed decrease in the root APX activity upon addition of Al levels (Fig. 5.8). However, the root APX activity was not altered in the Al-untreated root half (treatment: 0/100) in both genotypes (Fig. 5.8).

In Al-tolerant genotype leaf CAT activity decreased in plants when both root halves were exposed at 100 mg Al L^{-1} (treatment: 100/100; decrease of 27%) and when only one root half was exposed at 100 mg Al L^{-1} (treatment: 0/100; decrease of 28%), when compared to the control (Fig. 5.8). However, in Al-sensitive genotype, leaf CAT activity decreased in plants when both root halves were exposed at 100 mg Al L^{-1} (treatment: 100/100; decrease of 22%) and when only one root half was treated at 100 and 200 mg Al L^{-1} (treatments: 0/100, decrease of 27%; and 0/200, decrease of 25%) (Fig. 5.8). On the other hand, this genotype showed increase in root CAT activity in the root half treated at 100 and 200 mg Al L^{-1} (treatments: 0/100, increase of 146%; and 0/200, increase of 91%).

In Al-tolerant genotype, root CAT activity increased 303% in Al-untreated root half, when the other root half was treated at 100 mg Al L^{-1}, when compared to the control plants (Fig. 5.8). In addition this genotype also showed increase in leaf POD activity of 23% and 68% with both root halves exposed at 50 and 100 mg Al L^{-1}, respectively; and when only one root half was exposed at 100 mg Al L^{-1} (treatment: 0/100; increased of 23%), when compared to the control (treatment: 0/0) (Fig. 5.9). Similarly, in Al-sensitive genotype, leaf POD activity increased with Al treatment when compared to the control. When both root halves were exposed at 50 and 100 mg Al L^{-1}, POD activity increased 60% and 66% respectively; and 75% with only one root half exposed at 200 mg Al L^{-1} (treatment: 0/200) (Fig. 5.9). Both Al-tolerant and Al-sensitive genotypes showed increase in root POD activity when both or only one root

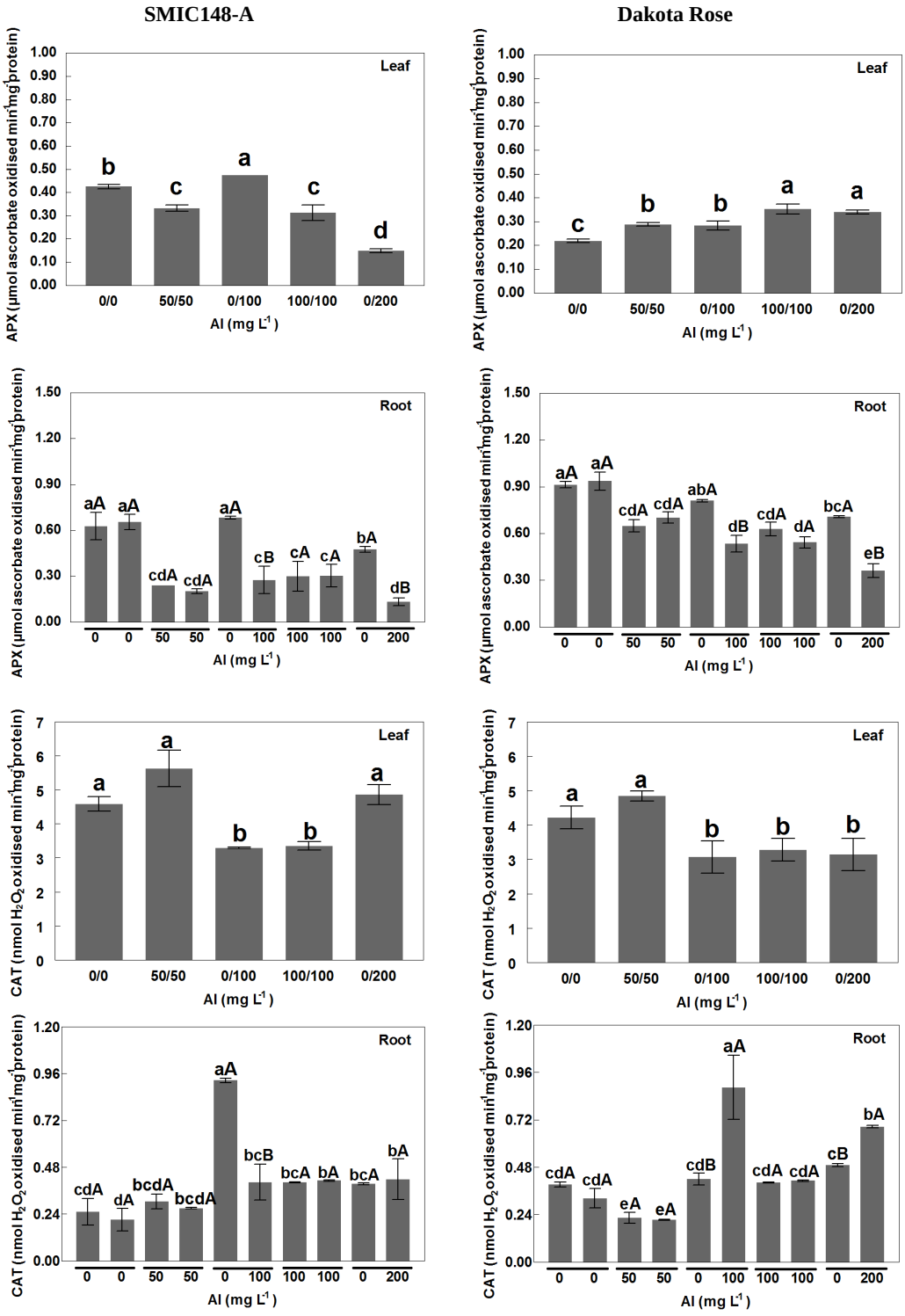

Figure 5.8 – Effect of Al concentration on ascorbate peroxidase (APX) and catalase (CAT) in leaf and root of SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

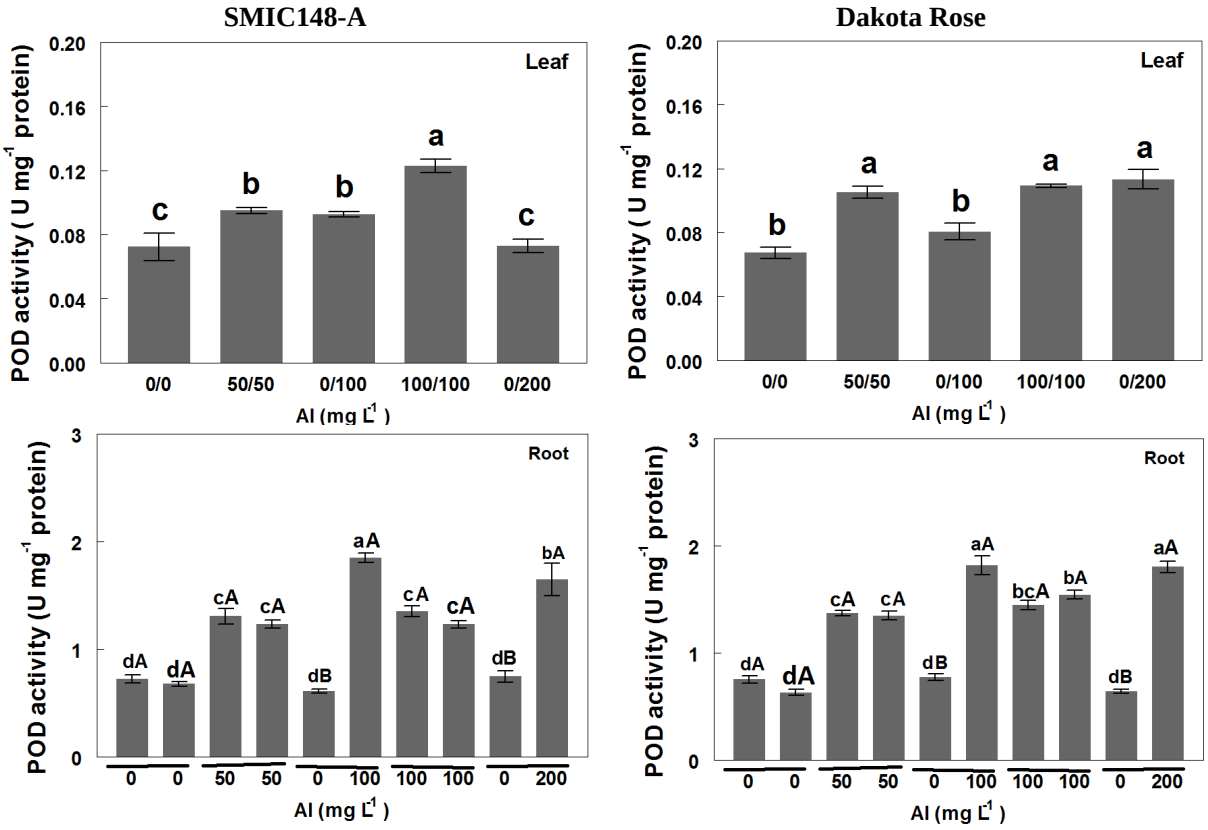

Figure 5.9 – Effect of Al concentration on guaicol peroxidase (POD) in leaf and root of SMIC148-A (Al-tolerant) and Dakota Rose (Al-sensitive) potato genotypes in a split-root system. Data represent the mean±S.D. of three different replicates. Different capital letters indicate significant differences between the root halves of same plant ($p < 0.05$). Different lowercase letters indicate significant differences among Al treatments of same potato genotype ($p < 0.05$).

half was exposed to Al, when compared to the control (Fig. 5.9).

5.4 Discussion

Potato growth parameters, nutrition and Al uptake/translocation

A clear decrease in shoot length was observed upon addition of Al levels in both Al-tolerant and Al-sensitive genotypes (Fig. 5.1). Furthermore, regardless higher Al translocation in the Al-tolerant genotype from the root to the stem, when compared to the Al-sensitive genotype (Fig. 5.3), it was not observed decrease in stem fresh weight (Fig. 5.2). Therefore, in Al-tolerant genotype the stem acted as a barrier, reducing the Al translocation to the leaves. RENGEL (1996) observed that 30-90% of total Al content is localized in the apoplast. The Al in cell walls is mostly bound to OH, amide and CO groups of polysaccharides (WANG et al.,

2013). SMITH; NAIK; CUMMING (2011) observed that apoplastic Al was highest in Al resistant genotypes of *Populus* spp. exposed to higher Al treatment (500 μM) when compared to the sensitive genotypes, and apoplastic Al was positively correlated with root tolerance index. Moreover, WANG et al. (2013) observed thickening of the cell wall of *Rhodotorula* sp. with increasing Al^{3+} levels.

The Al-sensitive genotype showed decrease in stem fresh weight upon addition of all Al treatments, except in the plants with only one root half exposed at 100 mg Al L^{-1} (treatement: 0/100). Moreover, in both Al-tolerant and Al-sensitive genotypes leaf fresh weight and leaf area decreased upon Al addition, with the exception of Al-sensitive genotype under the treatment 0/100 (Fig. 5.1). It is important to point out that for the shoot parameters evaluated, the Al-tolerant genotype seems to be more sensible to Al exposure when compared to the Al-sensitive genotype. In this view, SMIC148-A (Al-tolerant) showed a continuous decrease in shoot length, leaf fresh weight and leaf area upon Al addition. Conversely, Dakota Rose (Al-sensitive) showed higher values of leaf fresh weight, leaf area and stem fresh weight in plants when one root half exposed at 100 mg Al L^{-1} and the other half without Al exposition. This pattern of response suggest a hormetic effect. However, when one root half was exposed at 200 mg Al L^{-1}, plants were not able to increment shoot production; in which a decrease in this parameters was noticed (Fig. 5.1). Growth hormesis represents an over compensation due to a disruption in homeostasis that has been described in relation to different factors (POSCHENRIEDER et al., 2013). Hormetic growth stimulation has frequently been observed in plants exposed to low concentrations of non-essential, toxic metal ions (CALABRESE; BLAIN, 2009). Metal ions can act as elicitors of defense responses that in turn can stimulate the growth of plants, particularly under stress conditions (POSCHENRIEDER et al., 2013). OSAKI; WATANABE; TADANO (1997) observed that the growth of most plants adapted to low pH soils in the tropical and temperate regions was stimulated by Al application. Growth stimulation by Al is considered to alleviate H^+ toxicity at low pH (KINRAIDE, 1993). GHANATI; MORITA; YOKOTA (2005) observed that Al-induced increase in the activities of antioxidant enzymes, resulting in increased membrane integrity and delayed lignification and aging it can be considered as a possible reason for the stimulatory effects of Al on the growth of the tea plants (*Camellia sinensis*).

Aluminum apparently interacts directly and/or indirectly with factors that influence shoot growth, but primarily affecting the root tips (PIETRASZEWSKA, 2001). Effects of Al

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; HORST, 2001). Furthermore, Al can act directly on the shoot development due to cellular and ultrastructural changes in leaves, reduction of stomatal aperture, decreased photosynthetic activity, increase of lipid peroxidation and decrease of enzyme activity (PIETRASZEWSKA, 2001; TABALDI et al., 2007).

The more pronounced symptom observed in plants exposed to Al toxicity has been the reduction in root growth. In both Al-sensitive and Al-tolerant genotypes this reduction was expressed in terms of root fresh weight (Fig. 5.2). This symptom can be caused by increase of the H_2O_2 production in roots exposed to Al stress. HUTTOVÁ; TAMÁS; MISTRIK (2002); ŠIMONOVIČOVÁ et al. (2004) observed that H_2O_2 produced in barley roots during Al stress might play an active role inducing cells' death. Furthermore, Al induces changes in root cytoskeleton (SIVAGURU et al., 1999), callose formation causing an increase in cell wall rigidity (HORST; PÜSCHEL; SCHMOHL, 1997; AHN et al., 2002), and decreased of H^+ - pumping activity (AHN et al., 2002). Al can neutralize the surface charge of the plasma membrane and cause a surface potential change from -120 to +1mV. Such an Al-related change in plasma membrane surface potential causes disturbance in ion transport processes (AHN et al., 2002).

Both potato genotypes demonstrated decrease in root fresh weight of that root half treated at 200 mg Al L^{-1} (treatment: 0/200), when compared to the control plants, as well as to Al-untreated root half (Fig. 5.2). Interestingly, even the Al-untreated root half showed lower root fresh weight when compared to the root halves of control plants. The reduction of root fresh weight in the Al-untreated root half can be due to Al translocation from the Al-treated root half to Al-untreated root half (Fig. 5.3). Both Al-tolerant and Al-sensitive genotypes showed increase in Al concentration in the Al-untreated root half in plants when only one root half was treated at 100 and 200 mg Al L^{-1} (treatments: 0/100 and 0/200) (Fig. 5.3). Additionally, toxic levels of Al could result in root and shoot inhibition through indirect effects such as water uptake and mineral nutrition (COLLET; HORST, 2001)

The Al exposure inhibited the growth of stolons (based on fresh weight and number) in Al-sensitive genotype (Fig. 5.2). Interestingly, in Al-tolerant genotype, it was not observed inhibition of stolon growth in any Al treatment (Fig. 5.2). Although both stolon and roots were in direct contact with the Al in the nutrient solution, only the root fresh weight was decreased at Al exposure. This response indicates that the inhibition of stolon formation was not a local effect of Al, because Al was taken up by the roots and subsequently translocated to shoots

via xilema. In the leaves, Al may have caused reduction in the photoassimilates and hormone balance. Stolon is an etiolated stem, whose development is under hormonal control. Auxins are known to play an important role in plant growth and tissue development. SUN et al. (2010) showed that Al induction of ethylene production may act as a trigger to evoke changes in auxin distribution by affecting auxin polar transport systems such as AUX1 (auxin influx carrier) and PIN2 (proteins function to mediate auxin efflux). In this view, Al exposition could play an important effect on potato hormones balance, thus leading to differences in stolon production.

In the present study, the Al-sensitive and Al-tolerant potato genotypes showed a continuous increase in the root Al concentration with increasing Al levels in nutrient solution and plants accumulated significantly higher Al concentration in roots (Fig. 5.3). The Al accumulation in the root system may indicate that roots serve as a partial barrier to transport Al to the shoots. The root Al-sensitive genotype showed higher Al concentration (Fig. 5.3). In wheat root apices, Al accumulation was considered indicative of Al sensitivity (SAMUELS; KUCUKAKYUZ; RINCÓN-ZACHARY, 1997). Aluminum may bind to the phospholipids heads of the plasma membrane, reduce the negative charge associated with the plasma membrane phospholipids and proteins by binding to these charged groups or shielding the surface potential (KINRAIDE, 1993).

A significant increase in root Al concentration was observed for the Al-untreated root half when the other root half was supplied at 100 and 200 mg Al L^{-1} (treatments: 0/100 and 0/200), when compared to the control plants (treatment: 0/0) (Fig. 5.3). This response can be due to the Al translocation from Al-treated root half via shoot to Al-untreated root half (Fig. 5.3). Furthermore, in the Al-tolerant genotype the percentage of translocation was much lower than in Al-sensitive genotype (Fig. 5.3). The Al remobilization from one root half to the other root half may be due to Al transport via phloem, as suggested by many researchers (BRITEZ et al., 2002; WATANABE; JANSEN; OSAKI, 2005; ZENG et al., 2013). ZENG et al. (2013) showed that Al was present in the phloem of oil tea petioles and that Al could also be redistributed. These authors observed higher concentrations of Al in leaves when Al was supplied to a different leaf of the same plant. In addition, Al was present in newly emerging roots of oil tea seedlings in which all original roots were excised prior to treatment, and a positive correlation existed between Al content in the newly formed roots and that in the leaves.

In the Al-tolerant genotype, the stem and leaf Al concentration was significantly increased in the treatments with both sides of the root system exposed to Al (treatments: 50/50

and 100/100) (Fig. 5.3), as well as when plants were supplied with 200 mg Al L^{-1} to only half of the root system (treatment: 0/200). However, in the plants of Al-tolerant genotype, where Al was supplied to only half of the root system at 100 mg Al L^{-1} (treatment: 0/100), no difference was observed, when compared to the control (treatment: 0/0) (Fig. 5.3). On the other hand, in the Al-sensitive genotype, the stem and leaf Al concentration was increased in all Al treatments (Fig. 5.3). These data showed that Al uptake and translocation/allocation differences existed between the tested genotypes. Thus, it suggests that Al-tolerant genotype has a more efficient ability to prevent Al uptake and/or accumulation in the roots. The exact mechanisms by which the Al-tolerant genotype prevents Al uptake and translocation/allocation are still unknown. However, a possible mechanism that may contribute to the lower Al uptake could be the increased release of Al^{3+}-chelating compounds (e.g., organic acids) (MA; RYAN; DELHAIZE, 2001; HAYES; MA, 2003; SHEN; IWASHITA; MA, 2004; TOLRA et al., 2005). Furthermore, the lower Al translocation from the root to above-ground parts in Al-tolerant genotype can be due to Al^{3+} binding to negative sites in the cell walls and/or chelation of Al, preventing its uptake to the root cells, with subsequent transport and sequestration into subcellular compartments (e.g., vacuoles) (INOSTROZA-BLANCHETEAU et al., 2012).

Besides, all direct damages related to Al, several secondary effects are described in literature. Among these effects, interactions with essential mineral elements such as P are well known. In the present study, to avoid the interaction between P and Al in the nutrient solution, an experimental setup determined that plants could grown for about three days in the presence of 250 μM of P, and, subsequently, during the Al exposure (for 7 days), P was omitted from the nutrient solution. In this setup, was observed that potato plants were very well nourished with P and could withstand 12 days in the absence of P in the nutrient solution, without showing visible symptoms of P deficiency.

In both Al-sensitive and Al-tolerant genotypes, the decrease in P concentration in the both root halves treated with Al (treatments: 50/50 and 100/100), showed a clear P translocation from the root to shoot, when compared to the control treatment, (Fig. 5.4). The P translocation can be evidenced by the increase in P concentration in the stem of both genotypes, being more pronounced in the Al-sensitive genotype (Fig. 5.4). However, in the Al-sensitive genotype, P translocation to the leaves seemed not to occur. Conversely, in this genotype it was observed a decrease in leaf P concentration in all Al treatments (Fig. 5.4).

In soils, Al complexes P in less available forms; in the root such Al-P complexes

also occurs on root surfaces and cell wall. Once within the cell, Al may react with P compounds, and upset the plant P metabolism. QUARTIN; AZINHEIRA; NUNES (2001) observed that P deficiency is considered to be the key cause of growth reduction in Al-stressed plants. PIETRASZEWSKA (2001) and SILVA et al. (2010) noticed a reduction in P accumulation in vegetable tissues by excess of Al. In agreement, CHEN et al. (2009) showed that Al decreased root and leaf P concentration.

Interestingly, in both Al-sensitive and Al-tolerant genotypes, the root P concentration increased in the Al-untreated half root when only half of the root system was treated at 100 mg Al L^{-1} (treatmet: 0/100), when compared to the control plants (treatmet: 0/0). Furthermore, only in the Al-tolerant genotype root P concentration increased in the Al-untreated root half when only half of the root system was treated at 200 mg Al L^{-1} (treatmet: 0/200), when compared to the control plants (treatmet: 0/0) (Fig. 5.4).

Phosphorus deficiency is the predominant factor of induction of formation and growth of lateral roots (RAMAEKERS et al., 2010; WANG; YAN; LIAO, 2010; FANG et al., 2009). However, the main effect of Al is the inhibition the root growth (MATSUMOTO; MOTODA, 2012). Therefore, the increase of P concentration in the Al-untreated root half could have minimized the toxic effects of Al. In contrast with our results, IQBAL (2013) observed that P translocation was not able to alleviate Al toxicity within plant tissue of both Al-tolerant and Al-sensitive wheat genotypes.

In both Al-tolerant and Al-sensitive potato genotypes was observed increase in stem P concentration in plants exposed to all Al treatments, when compared to the control (treatment: 0/0) (Fig. 5.4). However, in Al-sensitive genotype was observed decrease in leaf P concentration in all Al treatments (Fig. 5.4). Additionally, in Al-tolerant genotype, leaf P concentration increased in plants where only one or both root halves were exposed at 100 mg Al L^{-1} (treatments: 0/100 and 100/100) (Fig. 5.4). It suggests that the immobilization of Al in leaves by precipitation with P might contribute to the genotypic differences in potato. Insoluble Al-P precipitates can accumulate on the root surface, in the cell wall, or in the cell vacuole (TAYLOR, 1991) and generally considered nontoxic to plants. The formation of Al-P complexes like $Al_4(PO_4)_3$ may be helpful by retarding the uptake of Al into the citosol (ZHENG et al., 2005). Furthermore, VÁZQUEZ et al. (1999) reported that the resistance in maize relied on the active transport of Al-P complex from the cell wall to vacuoles.

APases activities and soluble phosphorus (Pi) concentration and photosynthetic pigments

In both Al-tolerant and Al-sensitive genotypes the root half exposed to Al showed a decrease in APases activity, when compared to the control (treatment: 0/0). However, in both genotypes when only one root half was exposed to Al, it was not observed decrease in APases activity in the Al-untreated root half (treatments: 0/200, Al-tolerant; 0/100, Al-sensitive) (Fig. 5.5). APases function in the production, transport and recycling of Pi, a crucial element for cellular metabolism and bioenergetics (VELJANOVSKI et al., 2006). Intracellular APases are believed to remobilize and scavenge Pi from intracellular P monoesters and anhydrides in Pi-deficient plants. This is accompanied by marked reductions in cytoplasmic P-metabolic pools during extended P stress (VELJANOVSKI et al., 2006).

Extracellular and intracellular APases activities increase under Pi deficiency in many plant species (CIERESZKO; ŻEBROWSKA; RUMINOWICZ, 2011). However, in this study, in general, APases activity and soluble Pi decreased in the root halves exposed to Al (Fig. 5.5). In Al-tolerant genotype, the decrease in soluble Pi concentration in the root half exposed to Al was only observed at higher Al levels (100 and 200 mg Al L^{-1}), whereas in Al-sensitive genotype this response was observed in all Al levels (50, 100 and 200 mg Al L^{-1}) (Fig. 5.5).

Interestingly, it was observed an increase in leaf Pi concentration in both Al-tolerant and Al-sensitive genotypes when plants were exposed to Al in one or both root half (Fig. 5.5), besides in the Al-sensitive genotype this increase was more pronounced. In Al-sensitive genotype, the increase in leaf Pi concentration was accompanied with a decrease in APases activity (Fig. 5.5). However, in Al-tolerant genotype, it was not observed alteration in APases activity in relation to that increase in leaf Pi concentration (Fig. 5.5). It is important to point out the unit utilized to express Apase activity and Pi concentration. The Apase activity was expressed per mg $^{-1}$ protein, while Pi was expressed per g $^{-1}$ fresh weight. It is universally accept to express the enzyme activity per protein, however this can be a confounding factor. Still in this view, if Apase activity were expressed per mg $^{-1}$ fresh weight, a significant increment in its activity in the root halves exposed to Al, when compared to root halves without exposition, would clearly be noticed. Moreover the amplitude of variation between the root halves exposed or not to Al (treatments: 0/100 and 0/200) would be more pronounced for the Al-tolerant genotype, when compared to the sensitive one (Fig. 5.5).

The level of total chlorophyll and carotenoids is postuled as a simple and reliable indi-

cator of heavy metal toxicity for higher plants (GRATÃO et al., 2005). In general, the results of the present study showed an increase in the total chlorophyll and carotenoids concentration in plants of the Al-tolerant genotype exposed to Al (Fig. 5.6). This result might be related to the reduction in fresh weight (Fig. 5.1) and diminution of leaf area (Fig. 5.1), which would lead to an increase in the concentration of cellular components. Very similar results were reported by CALGAROTO et al. (2010) in *Pfaffia* spp. plants exposed to Hg. In contrast, the Al-sensitive genotype showed decrease in total chlorophyll and carotenoids concentration in plants exposed to Al (Fig. 5.6). It has been suggested that reduction in chlorophyll content in the presence of heavy metals is caused by an inhibition of chlorophyll biosynthesis (PEREIRA et al., 2006).

Protein determination and lipd peroxidation

Aluminium is known to enhance the oxidation of phospholipids and proteins in cell membranes (YAMAMOTO et al., 2002; ACHARY et al., 2008). A significant decrease in the leaf total soluble protein concentration was observed in both genotypes exposed at higher Al levels to one (treatments: 0/100 and 0/200) or both root halves (treatment: 100/100), except in the Al-sensitive genotype for 0/100 treatment. In Al-sensitive genotype was observed decrease more pronounced in total soluble protein concentration when plants were treated at 100 mg Al L^{-1} in both root halves (treatment:100/100; decrease of 35%) and with Al supplied to only one root half at 200 mg Al L^{-1} (treatment: 0/200; decrease of 19%) (Fig. 5.7). Interestingly, in plants exposed to different Al levels (treatments: 0/100 and 0/200), the root halves without Al exposition of both genotypes showed increment in protein concentration, when compared to control plants (Fig. 5.7).

Protein oxidation typically occurs when reduced metal ions like Fe^{2+} or Cu^{+} interact with H_2O_2 in the Fenton reaction and produce the extremely reactive hydroxyl radicals. The hydroxyl radical oxidizes amino acid side chains or causes protein backbone cleavage both resulting in the formation of carbonyl group (MØLLER; ROGOWSKA-WRZESINSKA; RAO, 2011). Reactive oxygen species (ROS) that lead to protein oxidation can be generated via a number of physiological and non-physiological processes, primarily as by-products of normal metabolism (MITTLER, 2002). The Al exposure increased the production of ROS in maize (BOSCOLO; MENOSSI; JORGE, 2003), potato (TABALDI et al., 2009), and oat (PEREIRA et al., 2011). In roots, the decrease in total soluble protein can be due to the increase in protein oxidation (PANDA; SINGHA; KHAN, 2003; BOSCOLO; MENOSSI; JORGE, 2003;

MERIGA et al., 2004; ACHARY et al., 2008) or due to the inhibition of protein synthesis (KUMARI; TAYLOR; DEYHOLOS, 2008). In both genotypes studied was observed increase in the Fe concentration in the roots exposed to Al (date show in the manuscript IV, fig. 4.10).

In both potato genotypes, the root fresh weight in the Al-untreated root half (treatments: 0/100) was not altered, when compared to the control. However, in the Al-untreated root half (treatment: 0/200) the increase in the root total soluble protein concentration was not sufficient to reverse the toxic effects of Al. These toxic effects comprise the interaction of Al ions with lipid components of the plasma membrane (AKESON; MUNNS; BURAU, 1989), depending on the phospholipid charge (JONES; KOCHIAN, 1995, 1997).

In Al-sensitive genotype, it was observed an increase in leaf lipid peroxidation of plants treated at 100 mg Al L^{-1} in both root halves and with Al supplied to only one root half at 200 mg Al L^{-1} (Fig. 5.7). Aluminum cannot by itself catalyze the peroxidation reaction (OTEIZA, 1994), but it enhances the Fe (II or III)-mediated peroxidation by changing the arrangement of membrane phospholipids, causing the packing of fatty acids and thus favoring the propagation of lipid peroxidation (OTEIZA, 1994; XIE; YOKEL, 1996). In wheat, YERMIYAHU; BRAUER; KINRAIDE (1997) observed that the differences in plasma membrane surface negativity and Al sorptive capacity is probably account for some of the difference in genotypic sensitivity to Al^{3+}. However, in both Al-sensitive and Al-tolerant potato genotypes was observed a decrease in the leaf Fe concentration with Al exposure (personal data). Furthermore, CAKMAK; HORST (1991) hypothesized that modification of membrane structures by Al interactions with membrane lipids and proteins may enhance the production of reactive oxygen species and consequent peroxidation of lipids.

However, in Al-tolerant genotype, it was not observed differences in leaf lipid peroxidation of plants exposed to Al, except in plants with both root halves exposed to 50 mg Al L^{-1}. Moreover, in Al-tolerant genotype the lipid peroxidation was not altered in the root half exposed at 50 and 200 mg Al L^{-1}. Interestingly, the root half exposed at 100 mg Al L^{-1} showed lower lipid peroxidation, when compared to the control (treatment: 0/0) (Fig. 5.7). On the other hand, in the Al-sensitive genotype the exposure at 200 mg Al L^{-1} caused an increase in root lipid peroxidation (Fig. 5.7). This indicates a more effective antioxidative system in this genotype, which can protect more efficiently membrane lipids of ROS. TABALDI et al. (2009) observed increase in lipid peroxidation in the Al-sensitive potato, however, in Al-tolerant genotype no alteration in root and shoot lipid peroxidation was observed by Al treatments. In oat,

CASTILHOS et al. (2011) observed that Al-sensitive genotype showed higher H_2O_2 and higher lipid peroxidation in the roots, whereas the Al-tolerant genotype showed similar values among treatments.

Enzyme activities of antioxidant system

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 (MITTLER, 2002). The Al-sensitive potato genotype, in general, showed reduction in leaf CAT activity (Fig. 5.8). This decrease in CAT activity may 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; POLLE, 2002). Conversely, in Al-tolerant genotype, the leaf CAT activity was less affected. In this genotype, it was observed a decrease only in plants where both root halves or only one root half was exposed at 100 mg Al L^{-1}. On the other hand, leaf APX activity had a higher protective effect in the Al-sensitive than in Al-tolerant genotype (Fig. 5.8).

The enzimes APX, CAT, and SOD are major ROS-scavenging mechanisms in plants. The balance between these enzymes is crucial for determining the steady-state level of ROS excess. Different affinities of APX (μM range) and CAT (mM range) to H_2O_2 suggest that they belong to two different classes of H_2O_2-scavenging enzymes: APX might be responsible for the fine modulation of ROS in the sensitive genotype, showing that even small quantities of H_2O_2 caused oxidative stress in this genotype (MITTLER, 2002). This indicates that Al-sensitive genotype is, in fact, more sensitive than the Al-tolerant genotype. This pattern resulted in a more rapid activation of the antioxidant enzimes. The same response was observed by PEREIRA et al. (2011), where the Al-sensitive oat genotype showed lower CAT activity than the tolerant and intermediated genotypes. However, APX had a more protective effect on the sensitive genotype than on the other genotypes.

In the roots, APX activity in both potato genotypes decreased in all Al treatments, with the exception of Al-untreated root halves in plants exposed at 100 mg Al L^{-1} (treatment:0/100). Moreover, in plants with only one root half exposed at 100 mg Al L^{-1} (treatment: 0/100), the Al-untreated roots showed no differences in APX activity, when compared to the control (Fig. 5.8).

On the other hand, the CAT activity had a slight but not significant increase with increasing Al levels, when compared to the control (treatment: 0/0). However, in the plants where only one root half was treated at 100 mg Al L^{-1} (treatment:0/100), the Al-untreated root half showed higher CAT activity (Fig. 5.8).

In Al-tolerant genotype, it was observed a compensatory effect on the CAT and APX activity in plants where only one root half was exposed to Al (treatments: 0/100 and 0/200) (Fig. 5.8). Conversely, Al-sensitive genotype showed an opposite response of CAT activity, where the root half exposed to Al (treatment: 0/100) showing higher activity, when compared to control plants and Al-untreated root half (Fig. 5.8).

In the root tissue, both Al-tolerant and Al-sensitive potato genotypes showed increase in POD activity in the root half exposed to Al, when compared to the control (Fig. 5.9). Furthermore, this increase was more pronounced in Al-treated root half, when only one root half was exposed to Al (Fig. 5.9). In general, both Al-tolerant and Al-sensitive genotypes increased leaf POD activity with Al exposure (Fig. 5.9). However, when one root half was exposed to 200 mg Al L^{-1} (treatment: 0/200; Al-tolerant) and 100 mg Al L^{-1} (treatment: 0/100; Al-sensitive) the POD activity was not altered (Fig. 5.9). These results show that Al-tolerant genotype, showed higher Al-avoidance when compared to Al-sensitive genotype. This response can be associated to lower Al translocation to the leaf mainly in the plants only one root half was exposed to Al, as well as the higher ability of this genotype to remobilize P to the Al-untreated root half. However, the remobilization of P was not associated to an increase in APase activity.

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6 DISCUSSÃO GERAL

Esse estudo contribuiu para uma melhor compreensão dos mecanismos causais da toxicidade do alumínio (Al) em genótipos de batata. Os genótipos de batata foram submetidos a diferentes doses de Al durante 12 dias em um sistema hidropônico utilizando areia como meio de sustentação (manuscrito I e II).

O Al foi absorvido pelas raízes e transportado para a parte aérea em todos os genótipos, mas os efeitos tóxicos desse metal foram diferenciados entre os genótipos. Através de parâmetros de crescimento como comprimento da parte aérea, massa fresca e seca total e consumo de solução nutritiva foi possível separar os genótipos de batata em sensíveis (Dakota Rose, SMIG145-1 e SMIJ319-7), intermediários (SMINIA793101-3 e SMIB106-7) e tolerantes (SMIC148-A, *Solanum microdontum* e SMIF212-3).

Apesar da inibição do crescimento da raiz ser o primeiro sintoma visível de toxicidade do Al, neste estudo, tanto nos genótipos tolerantes quanto nos genótipos sensíveis ao Al não ocorreu diminuição da massa seca da raiz. A inibição do crescimento causada pelo Al ocorreu principalmente na parte aérea. Na presença de Al ocorreu redução da massa seca do caule, da folha e de tubérculo no genótipo Dakota Rose e redução na massa seca da folha no genótipo SMIG145-1, ambos classificados como sensíveis ao Al. As maiores concentrações de Al foram detectadas na raiz, principalmente em genótipos tolerantes. Apesar de vários trabalhos terem observado que a sensibilidade ao Al pode estar relacionada à sua concentração nos tecidos, muitas vezes o metal pode estar ligado a parede celular ou armazenado no vacúolo complexado ao P e a ácidos orgânicos em uma forma não tóxica (ZHENG et al., 2005). O(s) mecanismo(s) de tolerância ao Al existentes nos genótipos tolerantes (SMIC148-A, *S. microdontum* e SMIF212-3) é (são) interno(s), uma vez que o Al foi absorvido e posteriormente transportado para a parte aérea das plantas. Em trabalhos prévios pesquisadores observaram que plantas de batata tolerantes ao Al desenvolveram alguns mecanismos de defesa contra o estresse oxidativo causado pelo Al. No genótipo tolerante (SMIC148-A) foi observado um sistema antioxidante mais eficiente, o qual pode ter resultado na maior tolerância ao Al (TABALDI et al., 2007, 2009).

Além disso, nos genótipos tolerantes SMIC148-A, *S. microdontum* e SMIF212-3 foi observado uma concentração maior de nutrientes nas raízes e nas folhas, sugerindo um mecanismo adicional de tolerância. A tolerância ao Al pode estar relacionada à concentração e ao metabolismo do fósforo. Os genótipos tolerantes ao Al apresentaram um aumento da concentração de

fósforo com o aumento dos níveis de Al, principalmente nas folhas. A tolerância ao Al pode estar associada a maior imobilização do Al na presença do P (ZHENG et al., 2005).

Muitos trabalhos têm verificado o efeito benéfico da aplicação do P sobre a toxicidade do Al. Em batata a adição de P (125 μM) na solução nutritiva juntamente com Al causou aumento na tolerância ao Al nos genótipos Dakota Rose (sensível ao Al) e SMIF212-3 (tolerante ao Al), a qual foi verificada pelo aumento do comprimento da parte aérea, em ambos os genótipos classificados previamente como eficientes ao uso de P. Entretanto, de modo geral, o aumento da concentração de P não alterou a tolerância ao Al nos demais genótipos (SMIC148-A, SMIG145-1) e parâmetros de crescimento avaliados. Portanto, a tolerância ao alumínio nos genótipos tolerantes pode estar vinculada a uma maior eficiência de utilização e translocação do P. Além disso, nos genótipos sensíveis (Dakota Rose e SMIJ319-7) foi observada uma diminuição na eficiência de utilização do P.

Ao contrário do que foi observado no experimento em hidroponia utilizando areia como substrato, quando os genótipos Dakota Rose (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 (manuscrito III e IV), ambos os genótipos apresentaram redução do crescimento do sistema radicular (massa fresca e seca da raiz) quando exposto ao Al. Entretanto, no genótipo tolerante ao Al, quando metade da raiz foi exposta a 100 e 200 mg Al L^{-1}, a metade da raiz não exposta ao Al não sofreu redução na massa seca de raiz e de estolão e no número de estolões. Em ambos os genótipos a concentração de Al aumentou tanto na metade da raiz exposta quanto na metade da raiz não exposta ao metal. Entretanto, no genótipo tolerante foi observada uma menor translocação do Al da porção do sistema radicular tratada para a porção do sistema radicular não tratada com Al.

No experimento de raiz dividida, durante o período de aclimatização as plantas cresceram em solução nutritiva contendo 250μM de P. Entretanto, durante o período de tratamento com Al, o P foi retirado da solução nutritiva para evitar a complexação com o Al. Em ambos os genótipos foi observado um aumento da concentração de P na raiz não exposta ao Al em plantas onde metade da raiz foi exposta a 100 (genótipo sensível e tolerante) e 200 mg Al L^{-1} (genótipo tolerante). O aumento da concentração de P na metade da raiz não exposta ao Al evidencia que ocorreu remobilização e translocação do P da porção do sistema radicular tratada para a porção do sistema radicular não tratada com Al.

A remobilização do P da porção do sistema radicular tratada com Al não foi correlaci-

onada à atividade das fosfatases ácidas nessas raízes. Tanto no genótipo tolerante quanto no genótipo sensível foi verificado redução da atividade das fosfatases ácidas na metade da raiz exposta ao Al. Entretanto, essa resposta pode estar vinculada a atividade de outras enzimas como fosfatases alcalinas e fitases (MA et al., 2009).

Baseado em parâmetros bioquímicos de raízes e da parte aérea, o genótipo tolerante ao Al sofreu danos oxidativos menores, em comparação com o genótipo sensível. Entretanto, não foi observada uma diferença clara entre os genótipos quanto à resposta do sistema antioxidante (APX, CAT e POD) ao suprimento de Al, mesmo tendo uma reação de escape ao Al menor que o genótipo sensível ao Al.

Portanto, vários fatores devem ser considerados no desenvolvimento de protocolos para seleção de genótipos de batata tolerantes ao Al. Além disso, é interessante observar o comportamento desses genótipos expostos a diferentes concentrações de P em um solo caracteristicamente ácido e com alta saturação em Al.

7 CONCLUSÃO

A tolerância ao Al nos genótipos de batata parece estar relacionada com o aumento da concentração nutrientes nos tecidos, principalmente o P. Entretanto, a sensibilidade ao Al pode estar associada ao decréscimo na eficiência de utilização e translocação do P.

A atividade das fosfatases ácidas não foi correlacionada à eficiência no uso do P.

O aumento da concentração de P na solução nutritiva, em geral, não influenciou na tolerância ao Al.

Em genótipos submetidos a suplementação heterogênea de Al, a tolerancia ao Al não foi correlacionada a um aumento da atividade de enzimas antioxidantes. Por outro lado, a tolerância ao Al no genótipo SMIC148-A pode estar associada à menor translocação de Al para as folhas principalmente em plantas onde somente metade da raiz foi exposta ao Al e a maior habilidade de remobilização do P de raízes expostas ao Al para as raízes não expostas.

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