UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM AGROBIOLOGIA

CARACTERIZAÇÃO BIOQUÍMICO-FISIOLÓGICA DE GENÓTIPOS DE BATATA (*Solanum tuberosum*) CULTIVADOS EM SOLOS COM ACÚMULO DE COBRE

DISSERTAÇÃO DE MESTRADO

Júlia Gomes Farias

Santa Maria, RS, Brasil

2012

CARACTERIZAÇÃO BIOQUÍMICO-FISIOLÓGICA DE GENÓTIPOS DE BATATA (*Solanum tuberosum*) CULTIVADOS EM SOLOS COM ACÚMULO DE COBRE

Júlia Gomes Farias

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Agrobiologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
Mestre em Agrobiologia

Orientador: Prof. Dr. Fernando Teixeira Nicoloso

Santa Maria, RS, Brasil
2012

Universidade Federal de Santa Maria
Centro de Ciências Naturais e Exatas
Programa de Pós-Graduação em Agrobiologia

A Comissão Examinadora, abaixo assinada, aprova a Dissertação de Mestrado.

CARACTERIZAÇÃO BIOQUÍMICO-FISIOLÓGICA DE GENÓTIPOS DE BATATA (*Solanum tuberosum*) CULTIVADOS EM SOLOS COM ACÚMULO DE COBRE

elaborada por
Júlia Gomes Farias

como requisito parcial para obtenção do grau de
Mestre em Agrobiologia

COMISSÃO EXAMINADORA:

Fernando Teixeira Nicoloso, Dr.
(Presidente/Orientador)

Janette Palma Fett, PhD (UFRGS)

Eduardo Girotto, Dr.(IFRS)

Santa Maria, 09 de março de 2012.

DEDICATÓRIA

Aos meus cientistas preferidos.

Aos mais autênticos, felizes e inspiradores que conheci:

Meus queridos vô Antônio e minha mãe Iria.

Que alegria conhecer e poder conviver com pessoas maravilhosas!

Descobrir na ciência uma paixão!

Que bom!

AGRADECIMENTOS

Ao professor Fernando Nicoloso, pela orientação, ensinamentos e incentivos constantes. É realmente gratificante poder trabalhar ao lado de um profissional com tamanha competência, audácia e dedicação, em um meio onde ainda hoje encontramos tantas dificuldades, desde infraestrutura à mão de obra e equipamentos.

À professora Luciane, pela disponibilidade, atenção e ajuda na correção dos trabalhos.

Ao Programa de Pós-Graduação em Agrobiologia, pela oportunidade de realização do curso de mestrado.

À CAPES pela concessão da bolsa de estudo, além de juntamente com CNPq e FAPERGS, tornar possíveis a realização de projetos como este aqui apresentado.

Aos meus colegas de laboratório Sibila, Pedro, Leonardo, Franciele, Marcos, Darlene, Liana, Jader, Marlo, Suzi, Fernando, Gabriel, Denise, Luciane, Jamile e Bibiana, pelo apoio e empenho, sem os quais não seria possível a realização deste trabalho.

Ao departamento de Fitotecnia da UFSM, pela utilização da infraestrutura, em especial ao professor Dilson pelos ensinamentos e fornecimento dos genótipos utilizados.

Ao laboratório de bioquímica toxicológia da UFSM, pela ajuda em reagentes e utilização de equipamentos, em especial a professora Maria Rosa.

Ao laboratório de fertilidade do solo da UFSM, em especial ao Eduardo, Alcione e ao Paulo, pela atenção, ensinamentos, paciência e compreensão de minhas dificuldades; e ao Tadeu pela ajuda nas análises de solo.

Ao laboratório de química da UFSM, em especial à Fabiane e ao professor Valderi, pela ajuda nas análises de tecido.

Aos meus colegas do programa de Pós-Graduação em Agrobiologia e Graduação em Agronomia, pela amizade, carinho e apoio; em especial à Núbia, Jussianne e ao Eduardo.

À minha família e amigos, pelo apoio e amor incondicionais, em especial a minha mãe, por nunca deixar de acreditar em mim e me inspirar sempre a buscar o melhor, a ser o melhor e nunca desistir. E é claro à Rose, quem me apresentou ao laboratório de Biotecnologia vegetal tornando a realização deste sonho possível.

Em um mundo dito cruel e competitivo, tive a alegria de conviver e trabalhar com pessoas dispostas ao bem, a ajudar, fiz amigos que levarei comigo a vida toda. Dentre tanto carinho e amizade, todas as tristezas e dificuldades ficam tão pequenas que só me resta o desejo de continuar. Meus queridos, meu muito obrigado!

EPÍGRAFE

“É melhor tentar e falhar, que preocupar-se e ver a vida passar.
É melhor tentar, ainda que em vão que sentar-se, fazendo nada até o final.
Eu prefiro na chuva caminhar, que em dias frios em casa me esconder.
Prefiro ser feliz embora louco, que em conformidade viver.”

Martin Luther King

RESUMO

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

CARACTERIZAÇÃO BIOQUÍMICO-FISIOLÓGICA DE GENÓTIPOS DE BATATA (*Solanum tuberosum*) CULTIVADOS EM SOLOS COM ACÚMULO DE COBRE

AUTORA: JÚLIA GOMES FARIAS
ORIENTADOR: FERNANDO TEIXEIRA NICOLOSO
Data e Local da Defesa: Santa Maria, 09 de março de 2012.

Solos cultivados com videiras e com longo histórico de aplicação de fungicidas cúpricos tendem a acumular cobre (Cu), podendo este atingir níveis tóxicos às plantas. Os efeitos ecotoxicológicos do Cu são uma preocupação global, entretanto existe uma carência de informações relacionando fitointoxicação por metais pesados com eficiências nutricionais em plantas. Este trabalho teve como objetivo estudar o processo de estresse induzido por excesso de Cu, efeito na distribuição de nutrientes minerais, bem como definir preditores para toxicidade de Cu em diferentes tecidos vegetais de genótipos de batata, diferindo quanto a eficiência de uso e resposta ao fósforo (P). As plantas foram cultivadas em solos de vinhedos com diferentes níveis de Cu (2,2, 5, 36,3, 67, 95,7, 270,5 e 320,70 mg kg^{-1}) durante os períodos de safra e safrinha em casa de vegetação semi-climatizada. O aumento da concentração de Cu em tecidos de plantas foi dependente da concentração externa de Cu, sendo as concentrações mais elevadas observadas em tecidos da raiz e estolão e maior parte de Cu absorvidofoi acumulado em tubérculos. Durante a safrinha, as plantas pré-classificadas como não eficientes mas responsivas ao P apresentaram a maior sensibilidade ao excesso de Cu em termos de concentração de nutrients e de crescimento. Estas respostas incluíram plantas sem folhas expandidas e sem produção de tubérculos, enquanto genótipos pré-classificados como eficientes no uso de P foram capazes de expandir folhas e produzir tubérculos em todos os solos testados. Durante a safrinha, houve um aumento na concentração de malondialdeído (MDA) nas folhas durante o ciclo da planta em todos os solos testados. Já no período de safra, as concentrações de MDA e H_2O_2 foram ligeiramente diferentes entre as coletas. Além disso, os genótipos pré-classificados como eficientes e não responsivos ao P apresentaram maior incremento na concentração de H_2O_2 em solos com alto Cu, enquanto o genótipo pré-classificado como não eficiente e responsivo a P apresentou maior incremento de H_2O_2 no tratamento com déficit de P. Em geral, as enzimas testadas, incluindo ascorbato peroxidase, superóxido dismutase e catalase, tiveram aumento na atividade com o aumento do Cu externo. Entretanto, nossos resultados fornecem evidências de que o sistema antioxidante não é suficiente para evitar danos biológicos mediados por ROS em altas concentrações de Cu, que resulta em efeitos deletérios. Concentrações de P e Cu apresentaram alta correlação com toxidez de Cu em Cambisolos, enquanto Fe e K foram mais correlacionados em Argisolos. Além disso, os nossos dados sugerem a utilização de folhas apicais e medianas para investigação da toxicidade de Cu em plantas de batata. Este estudo apresenta evidências de absorção não-competitiva de Cu e Fe por plantas de batata; e que a eficiência do uso de P confere maior tolerância ao excesso de Cu.

Palavras chave: fitointoxicação, metais pesados, nutrição mineral, plantas de batata, sistema antioxidante.

ABSTRACT

Master Dissertation
Agrobiology Graduate Program
Universidade Federal de Santa Maria

BIOCHEMICAL-PHYSIOLOGICAL CHARACTERIZATION OF POTATO GENOTYPES (*Solanum tuberosum*) CULTIVATED IN SOILS WITH COPPER ACCUMULATION

AUTHOR: JÚLIA GOMES FARIAS
ADVISER: FERNANDO TEIXEIRA NICOLOSO
Date and Place of Presentation: Santa Maria, March 09th 2012

Soils cultivated with grapevines and with a history of cupric fungicide application may accumulate copper, which may be toxic to plants. The ecotoxicological effects of copper (Cu) are a global concern; comparatively there is a lack of information relating phytotoxicity of heavy metals in plants with nutritional efficiency. This work aimed to study the process of stress induced by Cu excess, effect on the mineral nutrients distribution, and to define predictors of Cu toxicity in different tissues of potato genotypes, differing in the efficiency of use and response to phosphorus (P). Plants grown in vineyards soils with different levels of Cu (2.2, 5, 36.3, 67, 95.7, 270.5 and 320.70 mg kg^{-1}) during periods of fall and spring growing season, in a greenhouse. Tissue Cu concentration was dependent on the external Cu level and the higher concentrations were observed in root and stolon tissues and most of absorbed Cu was accumulated in tubers. During the fall growing season, plants pre-classified as not efficient but responsible to P had the highest sensitivity to Cu excess in growth and nutritional terms. These responses included plants without expanded leaves and without tubers production, while genotypes pre-classified as efficient in the use of P were able to expand leaves and to produce tubers in all tested soils. During the fall growing season, there was an increase of malondialdehyde (MDA) concentration in leaves during the plant cycle in all tested soils. In addition, during the spring growing season, the concentrations of MDA and H_2O_2 were slightly different between the samples. The genotypes pre-classified as efficient and responsive to P showed a greater increase in H_2O_2 concentration in soils with high Cu, while genotype pre-classified as non-efficient and responsive to P showed a higher increase of H_2O_2 treatment with deficit P. In general, the enzymes tested, including ascorbate peroxidase, superoxide dismutase and catalase activities were increased with increasing external Cu. However, our results provide evidence that the antioxidant system was not sufficient to prevent biological damage by ROS in high concentrations of Cu, resulting in deleterious effects. Concentrations of P and Cu were highly correlated with Cu toxicity in Cambi soils, while Fe and K were more correlated in Ultisols. In addition, our data suggest the use of medians and apex leaves to investigate toxicity of Cu in potato plants. This study presents evidence of non-competitive uptake of Cu and Fe for the potato plants, and that the P efficiency of use confers greater tolerance to Cu excess.

Keywords: antioxidant system, copper toxicity, heavy metals, mineral nutrition, potato plants.

LISTA DE FIGURAS

ARTIGO 1

ARTIGO 2

LISTA DE TABELAS

ARTIGO 1

ARTIGO 2

SUMÁRIO

INTRODUÇÃO

Nutrientes são necessários para o crescimento ótimo das plantas, o qual só é alcançado após controlar o nível de minerais essenciais (MARSCHNER, 2003). O cobre (Cu) é um metal de transição que atua em plantas como co-fator de diversas proteínas (LINDER e GOODE, 1991) e transportadores de elétrons, devido à capacidade de alternar-se entre o estado oxidado Cu (II) e o estado reduzido Cu (I) (LIPPARD e BERG, 1994). Além disso, atua na dismutação do superóxido, receptor hormonal, modelagem da parede celular, metabolismo de compostos fenólicos, tolerância a compostos salinos, diferenciação da parede celular, cicatrização, resposta a patógenos, conversão de fenóis dihidroxi para ortoquinonas e, possivelmente, desempenhe funções na reprodução e expansão celular (ABDEL-GHANY, 2009; BOWLER et al., 1992; CARR e WINGE, 2003; DONG et al., 2005; KIM et al., 2003; KLIEBENSTEIN et al., 1998; MARINA et al., 2008; PESARESI et al., 2009; PIGNOCCHI et al., 2003; RODRIGUEZ et al., 1999; WEIGEL et al., 2003; WELCHEN et al., 2004). Tendo como proteínas relacionadas a plastocianina, citocromo c oxidase, Cu/Zn superóxido dismutase (SOD), lacase, ascorbato oxidase, amino oxidase e polifenol oxidase (ARNON, 1949; MAYER, 2006; SCHUBERT et al., 2002; THIPYAPONG et al., 1997).

O suprimento de Cu às plantas depende basicamente da ocorrência e da disponibilidade do Cu presente no solo, que é dependente principalmente da composição do material de origem e dos processos de formação do solo (ABREU et al., 2012). Os solos originários de rochas básicas apresentam maiores teores de Cu comparados àqueles desenvolvidos a partir de granitos, gnaisses, arenitos e siltitos (OLIVEIRA, 1996; TILLER, 1989). Entretanto, em função do uso excessivo de produtos fitossanitários que contêm Cu em suas formulações ou de outras atividades antrópicas, como a mineração e a fundição, tem-se observado aumento nos teores de Cu nos solos que podem alcançar níveis deletérios ao meio ambiente. (PIETRZAK e McPHAIL, 2004).

Neste contexto a viticultura se destaca pelo combate às doenças fúngicas com produtos à base de Cu (calda bordalesa), utilizados na cultura desde o final do século XIX (LARGE, 1940). No Rio Grande do Sul (RS), são cultivados cerca de 28.000 hectares de videiras destinadas à fabricação de vinhos. Nele, a Serra Gaúcha, região Nordeste do estado corresponde a maior e mais antiga região vitivinícola. Sendo áreas de campo natural da região da Campanha do RS, região Sudeste, incorporadas ao sistema de produção de uva nos últimos anos. Em ambas as regiões, as características climáticas da região (verão úmido e inverno chuvoso) promovem uma pressão fúngica constante, o que leva à utilização de tratamentos

preventivos em geral à base de Cu.

A calda bordalesa é uma suspensão coloidal obtida pela mistura de sulfato de cobre, hidróxido de cálcio e água, utilizada para prevenir a incidência de fungos, principalmente, *Plasmopara viticola* (LARGE, 1940). Porém, o uso contínuo pode adicionar quantidades expressivas de Cu ao sistema de cultivo e, devido a sua reatividade, o Cu concentra-se na superfície do solo (ARIAS et al., 2004; PARAT et al., 2002), ultrapassando a capacidade máxima de adsorção (CASALI et. al., 2008). Adicionalmente, estudos realizados no sul do Brasil relataram alta concentração de Cu em solos de áreas vitivinícolas e, consequente, toxidez aos vegetais (GIROTTO, 2010; MIRLEAN et al., 2007).

A contaminação do solo por metais pesados apresenta graves problemas ambientais e requer soluções para mitigá-los. Além da natureza do material de origem, outros fatores como o conteúdo e composição da fração argila, o teor de matéria orgânica e condições físico-químicas podem influenciar a disponibilidade de Cu (OLIVEIRA, 1996). Neste sentido, a escolha da espécie vegetal a ser implantada bem como a caracterização físico-química do solo é muito importante, pois a toxidez de Cu às plantas varia em relação às espécies vegetais e às propriedades do solo, sobretudo em solos ácidos (BRUN et al., 1998), nos quais há maior solubilização do Cu e, consequente, aumento da biodisponibilidade do mesmo (MENCH, 1990). Algumas espécies vegetais apresentam maior capacidade de liberar compostos orgânicos capazes de complexar o Cu, o que diminui a absorção e a toxidez causada pelo elemento (MENCH et al., 1987). Em plantas que não apresentam estes mecanismos de adaptação, processos como a fotossíntese são afetados, principalmente devido às alterações nos cloroplastos e transporte de elétrons (PANOU-FILOTHEOU et al., 2001; YRUELA, 2005), além de alterações na nutrição mineral (GUO, 2011).

Fatores não relacionados diretamente às plantas também podem contribuir para minimizar os efeitos causados pelo excesso de Cu, como alterações no teor de fosfato do solo, que, além de aumentar a disponibilidade desse nutriente também pode precipitar metais pesados, tornando-os menos disponíveis às plantas e a outros organismos do solo, diminuindo consideravelmente a fitointoxicação (CAO et al., 2003). Estudos anteriores demonstraram que a adição de fosfato diminuiu o transporte de metais pesados para parte aérea de plantas cultivadas em solos multicontaminados (LEE e GEORGE, 2005) devido à formação de compostos menos solúveis (BROWN et al., 1995). Outros efeitos de proteção podem incluir a diluição do metal no tecido vegetal como resultado do aumento da biomassa radicular e aérea ou quelação via compostos exsudados pela planta na rizosfera (KALDORF et al., 1999).

O processo de aquisição de P pela planta é dificultado em função da sua concentração na solução do solo ser geralmente baixa. Além disso, a absorção do P pode ser reduzida em espécies que possuem sistema radicular pequeno, como é o caso da batata, limitando significativamente o seu crescimento. A forma de P mais rapidamente absorvida pela planta é o fosfato inorgânico (Pi), desta forma, o P orgânico existente no solo deve ser hidrolisado para que possa ocorrer a absorção (RAGHOTHAMA, 1999). Nesse sentido, vários tipos de enzimas do tipo fosfatases são capazes de aumentar a taxa de desfosforilação (hidrólise) de P orgânico no solo, liberando Pi às plantas (YADAV e TARAFDAR, 2003). As atividades enzimáticas bem como a capacidade de absorção e requerimento nutricional podem variar amplamente entre espécies ou mesmo dentro de uma mesma espécie entre genótipos. Nesse contexto, trabalhos desenvolvidos por nosso grupo demonstraram diferenças genotípicas marcantes em plantas de batata quanto à produção de fosfatases ácidas (TABALDI et al., 2009; TABALDI et al., 2011).

O crescimento e desenvolvimento vegetal dependem de dois componentes principais: a eficiência de aquisição e a eficiência da utilização de P (BAILIAN et al., 1991). O primeiro componente depende da eficiência de absorção e de enraizamento. Já o segundo componente, depende da eficiência de translocação e de conversão em biomassa. Para a caracterização da eficiência de utilização de P, um índice bastante aceito foi proposto por Siddiqi e Glass (1981), através da equação: matéria seca produzida dividida pela unidade do nutriente absorvido. Deste modo, reúne-se num mesmo indicador eficiência de utilização e o crescimento. Fageria e Baligar (1993) consideraram a utilização do nutriente como sendo a produtividade sob baixo nível e a resposta à aplicação de P de acordo com Fox (1978). Esta metodologia foi aplicada recentemente em nosso grupo de pesquisa. A análise gerou quatro grupos distintos para a batata: eficientes e responsivos (ER), não-eficientes e responsivos (NER); não-eficientes e não-responsivos (NENR); eficientes e não-responsivos (ENR) (comunicação pessoal).

Este comportamento diferenciado pode ser a chave em estudos para a caracterização de mecanismos que conferem eficiência ou não-eficiência de utilização e também de resposta ao P, e ainda na caracterização de possíveis interações existentes entre metais pesados e estas eficiências. Neste sentido, justifica-se a escolha de genótipos de batata para estudo de toxidez por Cu, uma vez que existe um banco de dados considerável com respostas distintas entre os genótipos em relação ao P.

O presente trabalho teve por objetivos: a) Caracterizar o metabolismo do estresse oxidativo e a atividade do sistema antioxidante enzimático e não-enzimático de genótipos de batata submetidos à toxidez de Cu.

b) Avaliar se os genótipos de batata eficientes no uso de P também são tolerantes à toxidez de Cu.

c) Determinar o efeito do acúmulo excessivo de Cu sobre a nutrição mineral de plantas de batata.

d) Avaliar influência do tipo de solo na toxidez por Cu.

ARTIGOS

Nosso estudo da toxicidade do cobre, acumulado em solos de vinhedos pelas sucessivas aplicações de soluções cúpricas, foi dividido em dois tópicos, que em síntese resultaram em dois manuscritos. Na sequência são apresentados os dois tópicos que contém os resultados, discussão e conclusões dos estudos realizados. São eles:

EFFECT OF ENVIRONMENTAL CONTAMINATION BY COPPER ON BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS OF POTATO GENOTYPES

COPPER EXCESS: THE EFFECTS OF PARTITIONING AND ACCUMULATION ON THE MINERAL NUTRITION OF POTATO GENOTYPES

EFFECT OF ENVIRONMENTAL CONTAMINATION BY COPPER ON BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS OF POTATO GENOTYPES

ABSTRACT

Soils cultivated with grapevines and with a history of cupric fungicide application accumulate copper (Cu), which may be toxic to plants, resulting in growth decrease and oxidative stress. This work aimed to study Cu toxicity in potato genotypes, which differ in efficiency of use and response to phosphorus (P), grown under vineyard soils with a long history of cupric fungicide application presenting different Cu levels (2.2, 5, 36.3, 67, 95.7, 270.5 and 320.70 mg kg^{-1}) during the fall and spring growing seasons. The increase of Cu concentration in leaves was dependent on external Cu concentrations and development stage of the leaves. Plants pre-classified as inefficient but responsive to P showed the highest sensitivity to Cu excess in growth terms, with plants unable to expand leaves and produce tubers. Moreover, genotypes pre-classified as efficient and not responsive to P showed the higher increment of H_2O_2 in soils with high Cu level while the genotype pre-classified as inefficient but responsive to P had higher MDA and H_2O_2 concentrations under P deficiency. Overall, the tested enzymes, including ascorbate peroxidase, superoxide dismutase and catalase, showed increases of activity with Cu increment. The concentrations of non-protein thiol groups and ascorbic acid varied greatly among tissue samples, treatments and genotypes. Therefore, Cu stress triggered a defense mechanism against oxidative stress in potato plants, the magnitude of Cu stress was depended of the genotype and physiological status. In addition, these results provide evidence that the potato antioxidant system are not sufficient to prevent biological damage caused by Cu toxicity.

Keywords: antioxidant system, copper toxicity, heavy metals, phosphorus, *Solanum tuberosum*.

1. Introduction

On the basis of nutritional requirement and potential toxicity to plants, different categories of metals have been distinguished. Some metals are essential to cell metabolism with specific functions and have unknown toxic effects, except at extremely high concentrations [1,2]. However, some elements may either have unknown metabolic function or are highly toxic even at low levels. Interestingly, iron (Fe), zinc (Zn) and copper (Cu) are essential for higher plants and animal life, and these metals may be toxic at higher concentrations [3,4]. This phenomenon is one of the most interesting to consider from the perspective of cellular regulation due to the need to maintain intracellular metals in a relatively constant concentration despite the variation in the supply of metal nutrients and the potential for toxicity [5].

Similar for humans, the deficiency of Cu in plants may result in the reduction of biological function. In addition, Cu is an important metal ion present in chromatin and closely associated with DNA bases in humans [6], this element at high levels enhances the apoptosis-inducing activity of polyphenols (such as the green tea polyphenol, EGCG), which can cause internucleosomal DNA fragmentation in cancer cell lines [7,8]. However, Cu can also cause toxicity to humans by excessive intake [9].

The amplitude of functions and effects on different organisms related to Cu are dependent on Cu concentration and of its oxidation status. Soils naturally contain Cu, but the total Cu content may be insufficient for healthy crop growth in some cases. In contrast, the total Cu content may appear adequate, but the amount of available metal is deficient. Moreover, there are many soils with high contents of Cu, thus rendering them potentially toxic to plants [10,11].

Recently, the impact of heavy metal pollution, such as Cu contamination resulting from anthropogenic inputs, has caused concerns due to Cu persistency in soil, economic loss from a reduction in crop production [12] and its impact on the security of the food chain. It is well recognized that elevated Cu concentrations are toxic to organisms and can have the following effects: growth inhibition; generation of reactive oxygen species (ROS); disturbance of the biochemical and physiological processes, such as photosynthesis, enzyme activity, pigment synthesis, protein synthesis and cell division. These disturbances can result in damages, such as peroxidation of membrane lipids, thus leading to ion leakage [13].

The vineyard soils from the southern region of Brazil represent an example of antropic pollution caused by agricultural practices. These soils have received successive applications

of Bordeaux mixture ($CuSO_4.5H_2O$ $Ca(OH)_2$) to protect the vineyards from fungal diseases. The continued practice of applying Bordeaux mixture adds Cu to the grape production system, which results in increased Cu concentrations on the topsoil due to the low mobility of Cu [14,15,16] and the possibility that Cu may generate toxic effects.

Susceptibility to Cu toxicity varies greatly with plant species. For instance, alfalfa and barley crops are highly tolerant to Cu stress, but rice and potato crops are less tolerant [17]. In soil, Cu is retained by physicochemical processes, and the availability in soil is dependent on the ligand, especially for organic matter and oxides, and the geochemistry of the condition, especially pH, which both define the energy of connection [18]. Additionally, some elements present in soil, such as phosphate, may affect the susceptibility of plants to Cu toxicity. In addition, increased availability of P may also precipitate heavy metals, thereby making them less available to plants and other soil organisms [19]. Previous studies shown a reduction of Cu transport to shoots in plants grown in contaminated soils by phosphate addition [20].

In a comparative transcriptome characterization of *Arabidopsis thaliana* submitted to various rhizotoxic ions (Cu, Al, Cd, and Na), Zhao et al. [21] used microarray techniques to show that the group of genes responsive to all ions contain a large number of genes encoding ROS-scavenging enzymes, which stabilize cellular structures against ROS damage. They also showed a common way to stimulate the gene expression among co-ions, such as Al and Cu.

Our group has previously demonstrated that some potato genotypes are tolerant to Al with lower oxidative stress than Al-sensitive genotypes as demonstrated not only by a lower production of ROS (H_2O_2) and lipid peroxidation but also by the presence of more efficient enzymatic and non-enzymatic antioxidant systems [22,23]. Moreover, we found that these genotypes have significant differences in P response (personal data).

The genotypic differences in response to P observed in potato plants and the shared gene expression profiles between Al and Cu led us to hypothesize that P efficient genotypes are less sensitive to Cu toxicity. Thus, the aim of this work was to characterize the general biochemical and physiological aspects of Cu toxicity in potato genotypes (*Solanum tuberosum*) differing in efficiency of use and response to phosphorus.

2. Materials and Methods

2.1 Plant materials and growth conditions

This study consisted of two experiments conducted with vineyard soils from the southern region of Brazil, which received successive applications of Bordeaux mixture ($CuSO_4.5H_2O$ $Ca(OH)_2$). The tested soils showed different Cu concentrations, as determinated by extraction with 0.01 mol L^{-1} EDTA-Na_2/1.0 mol L^{-1} ammonium acetate. The effects of Cu toxicity were evaluated in potato plants cultivated in contaminated soils in two different periods (the fall growing season and the spring growing season) with potato genotypes pre-classified according to efficiency and responsiveness to P.

During the fall growing season, the SMIE040-6RY (not efficient but responsive) and SMINIA793101-3 (efficient and not responsive) genotypes were evaluated. Due to the contrasting response to Cu excess between the genotypes, a second experiment was proposed. The second experiment consisted of the two genotypes (SMIF212-3 and SMINIA793101; both efficient and not responsive to P) to aprrise the differences in Cu toxicity between two genotypes with the same P response.

For both experiments, four soils were collected from vineyards located in Serra Gaúcha, and three soils were collected from vineyards in Campanha Gaúcha. The concentrations of P, Cu, K, Zn and Fe of these soils are presented at table 1.

Serra Gaúcha soils were collected from vineyards at Embrapa Uva and Vinho experimental areas in Bento Gonçalves (RS), and these soils were classified as Humic Cambisols. Cu levels in the soils from this region were found to be 5.5, 95.7, 270.5 and 320.7 mg kg^{-1}, and these soils were named Cambi C, Cambi VN1, Cambi VN2 and Cambi VN3, respectively (VN indicates that the soils were collected from vineyards). For purposes of comparison, Cambi C was used as a control because it was collected under a native forest. Cambi C+PK was created by correcting the levels of P and K. In this treatment, P (55 mg kg^{-1}) and K (50 mg kg^{-1}) were added based on the results of the soil analysis and according to CQFS-RS/SC (Comissão de Química e Fertilidade do Solo) [16]. The coordenates of the tested soils were 29°9’41,61” S, 51°32’16,70” W; 29°9’41,61” S, 51°32’16,70” W; 29°9’43,56” S, 51°31’40,54” W; 29°9’42,27” S, 51°31’44,35” W; 29°9’41,69” S, 51°31’46,45” W from Cambi C, Cambi C+PK, Cambi VN1, Cambi VN2 and Cambi VN3, respectively. And 30°47’13,87” S, 55°22’9,79” W; 30°47’11,47” S, 55°22’11,02” W; 30°48’28,62” S, 55°23’10,31” W from Ulti, Ulti VN1 and Ulti VN2 respectively.

Soils collected from Campanha Gaúcha were from commercial vineyards located on a property in the municipality of Santana do Livramento (RS) and were classified as Ultisols. Cu levels in the soils from this region were found to be 2.2, 36.3 and 67.2 mg kg^{-1}, and these soils were named Ulti, Ulti VN1 and Ulti VN2, respectively. Ulti was used as a control

because it was collected from a seedling production area with a history of no application of cupric fungicides.

For the assays, 3 kg of each soil was air-dried and placed in pots with a capacity of 5 kg. In each pot, one tuber with a diameter of 2 to 3 cm and an average weight of 8.4 g was sown. Throughout cultivation, the soil humidity was maintained at 80% of field capacity, which was determined with samples deformed in a tension table (1 MPa). Irrigation was performed daily with distilled water to replenish evapotranspired water, which was calculated by weighing the pots daily. Throughout the cultivation of potatoes, two applications of N totaling 70 mg kg^{-1} were applied to the soil. The experimental design was completely randomized with six replicates per treatment.

The experiments were conducted in a greenhouse from March to May (fall growing season) and from September to November (spring growing season) where potato cultivation was conducted in soils with increasing levels of Cu and similar pH values in water (raise to pH 6.0) and exchangeable K levels.

2.2 Cu tissue concentration

Cu tissue concentration was determined in dried plant tissue (between 0.01 and 0.25 g) digested with 5 ml of concentrated HNO_3. Sample digestion was performed in an open digestion system using a heating block Velp Scientific (Milano Italy). Plastic caps were fitted to the vessels to prevent losses by volatilization. The Cu 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.

2.3 Soil analysis

The soils were analyzed for particle size distribution of the soil constituents according to the pipette method [24]. The determination of pH was performed with water in a 1:1 ratio according to the methodology proposed by Tedesco et al. [25].

Table 1. Chemical and physical properties of vineyard soils with application of Cu-based fungicides.

Parameters	Cambi C+PK	Cambi C	Cambi Vn1	Cambi Vn2	Cambi Vn3	Ulti	Ulti Vn1	Ulti Vn2
Sand, g kg^{-1}	34.6	346.0	298.0	345.0	320.0	675.0	661.0	705.0
Silt, g kg^{-1}	391.0	391.0	373.0	353.0	370.0	260.0	264.0	205.0
Clay, g kg^{-1}	263.0	263.0	329.0	302.0	310.0	65.0	75.0	90.0
pH H_2O	5.8	5.8	5.2	5.3	5.3	5.5	5.3	5.2
OM, g kg^{-1}	34.3	33.9	27.3	37.9	35.9	11.2	12.1	9.2
Al, cmol c kg-1	0.0	0.0	0.05	0.02	0.03	0.0	0.06	0.03
H+Al, cmol c kg-1	2.6	2.8	4.2	4.5	3.8	2.3	3.2	2.9
CECef, cmol c kg-1	8.8	8.6	6.0	8.0	8.0	2.4	2.2	1.6
CEC7, cmol c kg-1	11.5	11.4	10.2	12.5	11.8	4.6	5.4	4.4
V. %	76.0	75.4	58.9	64.4	67.4	50.6	40.2	34.9
CuEDTA. mg kg-1	5.0	5,0	95.7	270.5	320.7	2.2	36.3	67.0
Cutot. mg kg-1	29.0	29.8	183.0	408.3	490.3	11.3	51.6	73.1
ZnEDTA. mg kg-1	2.0	2.0	14.0	18.0	21.0	2.0	7.0	10.0
Zntot. mg kg-1	60.6	59.2	81.0	84.6	81.8	8.2	10.8	16.4
Fe oxalate (mg kg^{-1})	101.0	102.0	101.0	110.0	114.0	15.0	16.0	12.0
Mn mg kg^{-1}	280.0	270.0	210.0	210.0	180.0	90.0	85.0	87.0
P. mg kg-1	18.2	4.8	37.0	19.0	27.0	47.1	75.0	60.0
K. mg kg-1	130.0	110.9	260.0	100.0	110.0	129.0	100.0	110.0
Ca. cmolc kg-1	4.7	4.6	3.7	5.9	5.6	1.4	1.3	1.0
Mg. cmolc kg-1	3.7	3.7	1.6	1.9	2.1	0.6	0.6	0.4

The concentration of soil organic matter (OM) was analyzed by wet oxidation using potassium dichromate in a sulfuric acid medium (0.4 N), and the determination of OM was made by titration with 0.1 N ammonium ferrous sulfate according to Embrapa [24]. The total contents of Cu and Zn in the soil samples were extracted with the use of hydrogen peroxide (H_2O_2), nitric acid (HNO_3) and hydrochloric acid (HCl) according to method N^o. 3050B [26]. The extraction of available Cu (CuEDTA) and Zn (ZnEDTA) was performed using 0.01 mol L^{-1} Na_2-EDTA/1.0 mol L^{-1} ammonium acetate with the pH level adjusted to 7.0 according to Chaignon et al. [27]. Both levels of Cu and Zn were measured using an atomic absorption spectrophotometer (GBC brand, model 932 AA).

The extraction of available P and exchangeable K was performed with the Mehlich 1 solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4). The concentration of P extracted by the Mehlich 1 solution was determined according to Murphy & Riley [28]. The concentration of exchangeable K was determined by flame emission spectroscopy. The exchangeable cations (Ca, Mg and Al^{3+}) were extracted with a 1.0 mol L^{-1} KCl solution [24]. The concentration of Al^{3+} was determined by an acid-base titration with a 0.0125 mol L^{-1} NaOH solution, and the concentrations of Ca and Mg were determined by atomic absorption spectroscopy (AAS) [25].

2.4 Growth parameters

At the end of the cycle, the plants were harvested and divided into shoots, tubers, roots and stolons. All tissues were washed three times with distilled water and dried with tissue paper. The effects of Cu toxicity on potato plant growth were evaluated using the following parameters: shoot, root, stolon and tuber fresh weight; average fresh weight of tubers; length of shoots; and number of leaves and tubers per plant.

2.5 Biochemical parameters

For all biochemical assays, the fourth expanded leaves of each plant was collected during the fall growing season, and the third and fourth expanded leaves were collected during the spring growing season. The samples were collected at tuber initiation (approximately 30 days after emergence) and near the end of the cycle (15 or 18 days before harvesting). Once collected, samples were immediately placed in liquid nitrogen and pulverized to a fine powder using a porcelain mortar.

For the first experiment, concentrations of the following components were measured: TBARS and H_2O_2. These analyses were not completed for the SMIE040-6RY genotype grown in Cambi VN2, Cambi VN3 and Ulti VN2 because there were no expanded leaves at tuber initiation. At the second collection (18 days before harvesting), plants grown in these soils still did not have expanded leaves, so all the leaves present in each plant grown in Cambi VN2, VN3 and Ulti VN2 were collected for the biochemical assays. After the biochemical analyses were completed from the fall experiment, a second experiment was proposed with additional analyses, including enzymatic and non-enzymatic analyses, to better characterize the potato response to Cu exposure.

2.6 Estimation of lipid peroxidation

The level of lipid peroxidation products was estimated following the method of El-Moshaty et al. [29] by measuring the concentration of malondialdehyde (MDA) as an end product of lipid peroxidation by reaction with thiobarbituric acid (TBA). Frozen leaves samples were homogenized in 0.2 M citrate phosphate (pH 6.5) containing 0.5% Triton X-100 at a ratio of 1:10 (w/v). The homogenate was 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) TBA. The mixture was heated at 95ºC for 40 min and then quickly cooled in an ice bath for 15 min. After centrifugation at 3,600 rpm for 15 min, the absorbance of the supernatant was measured at 532 nm. A correction of non-specific turbidity was made by subtracting the absorbance value taken at 600 nm.

2.7 Determination of hydrogen peroxide (H_2O_2)

The H_2O_2 concentration of potatoes was determined according to Loreto and Velikova [30]. Approximately 0.05 g of the frozen sample was homogenized in 2 ml of 0.1% (w/v) TCA. The homogenate was mixed with 0.5 ml of a 10 mM potassium phosphate buffer (pH 7.0) and 1.0 mL of 1 M KI, and the mixture was centrifuged at 12,000 g for 15 min at 4ºC. The H_2O_2 concentration of the supernatant was evaluated by comparing its absorbance at 390 nm with a standard calibration curve.

2.8 Enzyme activities of antioxidant system

Frozen samples of leaves were used for the enzyme analysis. The samples were composed of 0.6 g of tissue homogenized in 2.0 mL of a 0.05 M sodium phosphate buffer (pH 7.8) containing 1 mM EDTA and 2% (w/v) PVP. The homogenate was centrifuged at 13,000 g for 20 min at 4ºC. The supernatant was used for enzyme activity and protein content assays [31].

Catalase (CAT) activity was assayed following the method of Aebi [32] with slight modifications. The activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm of a reaction mixture containing 15 mM H_2O_2 in a potassium phosphate buffer (pH 7.0) and 30 μL of extract with a final volume of 2.0 mL.

Ascorbate peroxidase (APX) was measured according to Zhu et al. [31]. The reaction mixture consisted of a total volume of 2 mL of a 25 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA, 0.25 mM ascorbate, 1.0 mM H_2O_2 and 100 μL of enzyme extract. H_2O_2-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm (e = 2.8 mmol L^{-1} cm^{-1}).

Superoxide dismutase (SOD) activity was assayed according to Misra and Fridovich [33]. The assay mixture consisted of a total volume of 1 ml of a glycine buffer (pH 10.5) containing 1 mM epinephrine and enzyme material. Epinephrine was the last component to be

added. Adrenochrome formation in the 4 min following the addition of epinephrine was recorded at 480 nm using an UV-Vis spectrophotometer. One unit of SOD activity is expressed as the amount of enzyme required to cause 50% inhibition of epinephrine oxidation in the experimental conditions. This method is based on the ability of SOD to inhibit the autoxidation of epinephrine at an alkaline pH. Because the oxidation of epinephrine leads to the production of a pink adrenochrome, the rate of increase of absorbance at 480 nm, which represents the rate of autoxidation of epinephrine, can be conveniently followed. SOD can inhibit this radical-mediated process.

2.9 Ascorbic acid (AsA) and non-protein thiol groups (NPSH) concentrations

Frozen samples were homogenized in a solution containing 50 mM Tris–HCl and 10% Triton X-100 (pH 7.5), and the samples were then centrifuged at 2,600 rpm for 10 min. The supernatant was removed, and 10% TCA was then added at a ratio of 1:1 (v/v) to the supernatant followed by centrifugation (2,600 rpm for 10 min) to remove protein. The supernatant was used to determine AsA and NPSH concentration. AsA determination was performed as described by Jacques-Silva et al. [34]. An aliquot of the sample (300 μl) was incubated at 37ºC in a medium containing 100 μL of 13.3% TCA, 100 μl of deionized water and 75 μL of DNPH. After 3 h, 500 μl of 65% H_2SO_4 was added, and samples were then read at 520 nm. A standard curve was constructed using L(+) AsA.

NPSH concentration in the potatoes plants was measured spectrophotometrically with Ellman's reagent [35]. An aliquot of the extract sample (400 μL) was added to a medium containing 550 μL of 1 M Tris–HCl (pH 7.4). The reaction was read at 412 nm after the addition of 5.0 μL of 10.0 mM 5-5-dithio-bis (2-nitrobenzoic acid) (DTNB). A standard curve using cysteine was used to calculate the concentration of thiol groups in the samples.

2.10 Protein determination

For all the enzyme assays, protein was measured by the Coomassie Blue method according to Bradford [36] using bovine serum albumin as a standard.

2.11 Statistical analysis

The experiments were performed using a randomized design. The analyses of variance were computed on statistically significant differences determined based on appropriate F-tests. Results were presented as means ± SD of at least three independent replicates. The mean differences were compared using Tukey's test ($P<0.05$).

2.12 Multivariate analysis

Principal component analysis (PCA) was used to evaluate the relationship among variables and possible patterns in the data distribution obtained from different seasons during the potato cycle.

Initially, data from experiments cultivated in different seasons were transformed by ranking on a scale ranging from 1 to 10. The average value of the evaluated parameters corresponded to 5 on the scale with 1 being the lowest assessed value and 10 being the highest assessed value. The average data were analyzed using CANOCO® statistical software (version 4.5, Fa. Biometris). The data matrix was submitted to PCA analysis to compound variables, thus providing information about the factors responsible for these patterns.

3. Results

3.1 Tissue Cu concentration

In both growing seasons (fall and spring), the Cu concentration in leaves increased with increasing Cu levels in Ulti soils (Table 2). Remarkably, regardless of the developmental stage of leaves, genotypes or growing season, there was a significant difference between the Ulti control and the other treatments. Additionally, higher Cu tissue concentrations were observed in the second set of samples (15 or 18 days before harvesting) as compared to the first set of samples (tuber initiation).

Even with low values of Cu content in soil, Cambi C (Table 1) treatment lead to Cu concentrations in leaves closely to values from contaminate soil leaves. In addition, Cu concentration in leaves tissues was affected by PK fertilization. At second harvest, overall PK addition reduced about 60% of Cu concentration in leaves (Table 2).

During the fall growing season, the SMINIA793101-3 genotype had the maximum Cu concentration in leaves in Cambi VN2, Cambi VN3 and Ulti VN1 treatments at both harvests.

In contrast Cu tissue concentration of the SMIE040-6RY genotype did not differ among the Cambi VN2, Cambi VN3 and Cambi C samples at the second harvest (Table 2).

Interestingly, during the spring growing season, in Cambi soils, all genotypes showed the same pattern in relation to Cu increment, which resulted in a significant and continuous increase of Cu concentration from Cambi C to Cambi VN2. At the second harvest, SMIF 212-3 plants had the highest Cu concent ration in Ulti VN1 not differing from Cambi VN2 and Cambi VN3 values (Table 2).

Table 2. Effect of increasing Cu level on leaf Cu concentration in potato genotypes grown in vineyard soils.

	Cu concentration (µg/g dry weight)			
	Fall growing season			
soil treatment	SMINIA 793101-3	SMIE 040-6RY	SMINIA 793101-3	SMIE 040-6RY
	tuber initiation		*18 days before haversting*	
Cambi C+PK	3.40 ± 0.09 dA	3.80 ± 0.09 dA	3.67 ± 0.05 eB	4.34 ± 0.09 cA
Cambi C	7.23 ± 0.3 cA	6.10 ± 0.09 cA	10.00 ± 0.2 dA	11.23 ± 0.09 bA
Cambi VN1	11.00 ± 0.3 bA	12.00 ± 0.5 bA	19.13 ± 0.4 bA	20.45 ± 0.2 aA
Cambi VN2	20.01 ± 0.1 a	ND*	29.00 ± 0.3 aA	14.45 ± 0.4 bB
Cambi VN3	15.34 ± 0.2 ab	ND*	28.55 ± 0.3 aA	13.00 ± 0.4 bB
Ulti	3.90 ± 0.09 dB	5.20 ± 0.06 cdA	4.69 ± 0.09 eB	9.76 ± 0.3 bA
Ulti VN 1	17.00 ± 0.1 aA	19.35 ± 0.1 aA	25.10 ± 0.2 aA	21.97 ± 0.1 aB
Ulti VN 2	14.23 ± 0.1 b	ND*	16.49 ± 0.3 cA	18.66 ± 0.2 aA
	Spring growing season			
soil treatment	SMINIA 793101-3	SMIF 212-3	SMINIA 793101-3	SMIF 212-3
	tuber initiation		*15 days before haversting*	
Cambi C+PK	3.40 ± 0.09 dA	3.80 ± 0.08 dA	3.00 ± 0.07 eB	4.20 ± 0.09 eA
Cambi C	5.70 ± 0.3 cA	6.80 ± 0.1 cA	10.70 ± 0.2 cdA	10.50 ± 0.3 dA
Cambi VN1	15.70 ± 0.6 abA	16.50 ± 0.3 bA	19.01 ± 0.3 bA	18.00 ± 0.1 bA
Cambi VN2	20.00 ± 0.2 aA	21.00 ± 0.1 aA	25.50 ± 0.1 aA	24.00 ± 0.2 aA
Cambi VN3	18.50 ± 0.2 aA	18.55 ± 0.2 aA	23.00 ± 0.1 aA	24.50 ± 0.2 aA
Ulti	3.30 ± 0.09 dA	2.30 ± 0.09 eB	6.80 ± 0.3 dA	4.70 ± 0.09 eB
Ulti VN 1	9.00 ± 0.5 bcB	15.00 ± 0.4 bA	13.00 ± 0.5 cB	25.50 ± 0.2 aA
Ulti VN 2	13.34 ± 0.4 bA	11.50 ± 0.09 bB	14.32 ± 0.2 cA	14.00 ± 0.4 cA

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and collect ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and collect ($p < 0.05$). ND*(data not available).

3.2 Effects of Cu toxicity on shoot production

Contamination of Cu in Ulti soils resulted in growth inhibition with a reduction of shoot fresh weight, shoot length and number of leaves during the fall growing season for both

genotypes tested (Table 3). In contrast, only SMIF 212-3 plants showed sensitivity to Cu increments during the spring growing season in Ulti soils. Remarkably, the shoot fresh weight of the SMINIA 793101-3 genotype was not affected by increased Cu concentrations, actually shoot length and number of leaves increased with increasing Cu concentrations for this genotype (Table 3).

Table 3. Effect of increasing Cu level on shoot fresh weight, shoot lengh and numbers of leaves in potato genotypes grown in vineyards soils.

	Fall growing season		***Spring growing season***	
	SMINIA 793101-3	SMIE 040-6RY	3	SMIF 212-3
	Shoot Fresh weight per plant (g)			
Cambi C+PK	65.33 ± 2.3aA	33.60 ± 1.2aB	38.51 ±2.1 aA	23.60 ± 2.4abB
Cambi C	19.52 ± 3.1cA	17.07 ±0.7bA	29.29 ± 1.7aA	14.07 ± 4.8cB
Cambi VN1	42.69 ± 1.5bA	39.40 ± 1.2aA	32.64 ±8.9 aA	29.40 ± 3.3aA
Cambi VN2	11.25 ± 0.7dA	3.80 ± 0.3cB	19.82 ± 2.3bA	7.20 ± 1.7dB
Cambi VN3	7.20 ±2.4 dA	3.20 ± 0.27cB	9.88 ± 1.2cA	8.80 ± 1.6dA
Ulti	20.23 ± 6.3cA	22.30 ± 1.8aA	17.90 ±3.4 bB	28.30 ± 5.4aA
Ulti VN 1	14.10 ± 3.1cdA	14.50 ± 3.1bcA	18.87 ± 2.9bB	22.50 ± 3.8bA
Ulti VN 2	4.90 ±1.25 dA	3.10 ±1.4 cB	19.82 ± 4.5bA	14.10 ± 1.0cB
	Shoot Lengh (cm)			
Cambi C+PK	50.67 ±14.8 aA	22.17 ±3.8 bB	40.68 ± 12.3aA	29.17 ± 3.7abB
Cambi C	28.20 ± 6.7cdA	21.12 ± 1.8bA	30.20 ±3.2 bA	19.13 ±2.9 bB
Cambi VN1	41.92 ± 5.4abA	32.22 ± 1.9aB	33.92 ±4.5 abA	33.22 ± 1.9aA
Cambi VN2	17.20 ± 3.4dA	3.45 ± 1.8cB	25.80 ± 3.1bcA	12.52 ± 2.1cB
Cambi VN3	22.60 ±1.6 dA	0.89 ±0.04 cB	19.54 ±3.3 cA	13.77 ±3.3 cA
Ulti	38.77 ± 10.1bcA	26.35 ±7.6 abB	24.87 ± 5.2bcB	37.35 ± 5.4aA
Ulti VN 1	36.85 ± 12.5bcA	31.97 ± 7.8aA	36.05 ± 6.7aA	27.93 ±2.1 abB
Ulti VN 2	23.32 ±6.7 dA	1.80 ± 0.9cB	34.75 ± 4.8aA	14.61± 1.6cB
	Number of leaves per plant			
Cambi C+PK	16.00 ± 0.7aA	13.25 ± 2.1abA	9.25 ± 1.0 aA	8.66 ± 1.25a A
Cambi C	12.00 ±1.5 bcA	8.25 ± 1.0bB	8.25 ± 2.0abA	7.75 ± 1.5ab A
Cambi VN1	14.25 ± 2.0abA	15.00 ±1.5 aA	9.25 ±1.25 aA	7.00 ± 1.25b B
Cambi VN2	10.00 ± 1.0cA	5.00 ±1.0 cB	7.25 ± 1.0bA	7.00 ±2.0 b A
Cambi VN3	10.25 ±1.5 cA	6.50 ±1.0 cB	6.75 ± 2.0bcA	5.25 ±1.25 c B
Ulti	13.50 ± 2.3abA	11.00 ± 2.0abA	6.75 ± 2.0cA	7.33 ± 1.5b A
Ulti VN 1	13.25 ± 1.5abA	12.75 ± 1.0abA	7.66 ±1.0 bA	6.25 ± 2.0bc A
Ulti VN 2	10.25 ±2.8 cA	4.20 ± 1.5cB	7.75 ±1.25 bA	6.33 ± 1.0bcA

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letter indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

In addition, Cambi soils with the high Cu levels (Cambi VN2 and Cambi VN3) led all the tested genotypes to reduce shoot fresh weights as compared to Cambi + PK and Cambi C without fertilization (Table 3). Moreover, a significant difference in the SMINIA 793101-3 and SMIE040-6RY genotypes during the fall growing season was found between Cambi C

and Cambi C+ PK for shoot fresh weight and plant length. In contrast, during the spring growing season, a smaller difference was observed in SMINIA793101-3 plants cultivated in Cambi soils with or without PK fertilization for shoot fresh weight and length, with higher shoot production under PK fertilization (Table 3).

The response to Cu toxicity varied not only among the genotypes, but also between the parameters tested. Interestingly, during the spring growing season, SMINIA793101-3 plants cultivated in Ulti soils were not affected in fresh weight terms by Cu addition, but respond positively in the number of leaves and shoot length. On the other hand, SMIF 212-3 plants were not affected in number of leaves by Cu addition, but significantly reduced shoot fresh weight and shoot length under this same situation. Moreover, the SMINIA 793101-3 genotype maintained higher values in shoot parameters in Cu-contaminated soils in both growing seasons as compared to the other genotypes.

3.3 Effects of Cu toxicity on stolon, root and tuber production

The response of stolon fresh weight for genotypes cultivated in Ulti soils during the fall growing season to Cu toxicity was negative in Ulti VN1 and Ulti VN2. During the spring growing season, however, overall there were no significant differences in the stolon fresh weight in the genotypes among the tested soils (Table 4). In Cambi soils, plants grown during the fall showed no significant difference in stolon fresh weight among the control without PK addition and the other treatments with higher Cu concentrations (Cambi VN2 and Cambi VN3). In contrast, during the spring season, the stolon production for the SMINIA793101-3 and SMIF 212-3 genotypes was significantly reduced in Cambi soils with added Cu.

Unlike the response observed in shoot fresh weight, root fresh weight was not reduced with Cu increment in Ulti soils. In addition, the root fresh weight was increased by approximately 50% in SMINIA793101-3 plants grown in Ulti VN2 as compared to the Ulti control during spring growing season. Conversely, in Cambi soils, the root fresh weight production only significantly decreased at spring in Cambi VN3 as compared to Cambi C (Table 4). Moreover, higher root fresh weights were observed during the spring as compared to the fall growing season. For SMINIA793101-3 plants, the root production was increased by 3.34 and 3.0 fold in Ulti VN2 and Cambi C soils, respectively, during the spring as compared to the fall.

Table 4. Effect of increasing Cu level on stolon and root fresh weight in potato genotypes grown in vineyards soils.

	Fall growing season		***Spring growing season***	
	SMINIA 793101-3	SMIE 040-6RY	SMINIA 793101-3	SMIF 212-3
	Stolon Fresh weight per plant (g)			
Cambi C +PK	3.31 aA	2.30 bB	1.34 bB	3.00 aA
Cambi C	1.44 bcA	1.21 bcA	3.61 aA	2.21 bB
Cambi VN1	3.20 aA	2.15 bcB	2.40 bA	1.15 cB
Cambi VN2	1.83 bA	2.05 bcA	3.83 aA	0.53 dB
Cambi VN3	1.99 bA	0.73 cB	1.90 bcA	0.63 dB
Ulti	2.10 bA	2.80 aA	0.91 cB	3.10 aA
Ulti VN 1	1.30 bcA	1.70 bcA	1.30 cA	1.50 cA
Ulti VN 2	0.93 cB	1.20 bcA	0.98 cB	2.80 aA
	Root Fresh weight per plant (g)			
Cambi C +PK	3.05 aA	2.93 bA	5.81 cB	8.23 aA
Cambi C	2.90 abA	2.66 cA	8.82 abA	3.14 cB
Cambi VN1	2.50 bB	3.60 aA	5.91 bcA	3.60 cB
Cambi VN2	3.20 aA	2.93 bA	4.17 cA	2.82 cdB
Cambi VN3	3.23 aA	2.50 cB	4.30 cA	1.90 dB
Ulti	3.00 aA	2.50 cA	6.05 bA	5.17 bA
Ulti VN 1	2.80 abB	3.40 aA	7.34 bA	2.08 dB
Ulti VN 2	3.02 aA	2.50 cB	10.09 aA	4.17 bB

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

The pattern of response to Cu toxicity in tuber fresh weight was similar between the genotypes and growing seasons (Fig. 1A, B). However, there was higher tuber fresh weight during the spring season as compared to the fall season (Fig. 1B) and the higher numeric production (Fig. 2) at fall, which lead to a higher average of tuber weight at spring (Fig. 3).

Thus, there were visual differences between plants grown in Cambi and Ulti soils with Cu excess (data not shown). At fall, in cambi soils VN2 and VN3 SMIE040-6RY had a critical response to Cu toxicity, resulting in plants without expanded leaves. The genotype showed thickening of the stem, giving a similar form to the bulb with violet staining, with dark green leaves in these same treatments. On the other hand, both genotypes tested at fall showed a slight reticular chlorosis in Ulti VN2 treatment (data not shown).

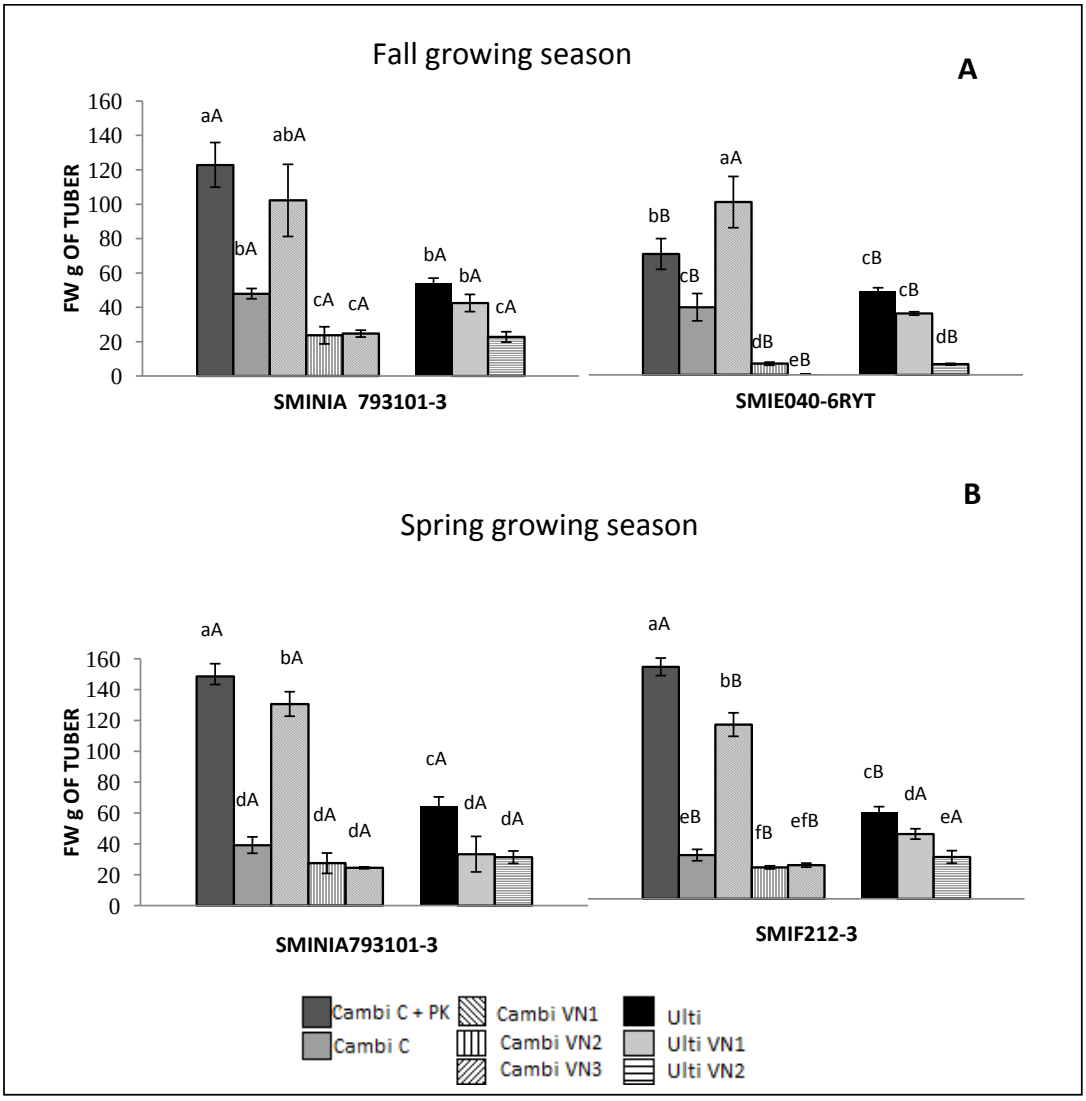

Fig. 1. Effect of increasing Cu levels on tubers fresh weight (FW) per plant at fall (A) and spring (B) growing seasons. Data represent the mean±S.D. of six replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Regardless of the season and genotype, plants grown in Ulti soils had continuous decreases in total tuber fresh weight with the increase of Cu. In Cambi soils, however, only plants grown during the fall growing season in the control without fertilization had significant difference in tuber fresh weights as compared to plants grown in the Cambi VN2 and Cambi VN3, with no difference during the spring time. Nevertheless, Cambi C+PK and Cambi VN1 promoted the highest tuber fresh weight values, and these values were statistically higher than the values in the others Cambi soils regardless genotype and growing season (Fig. 1).

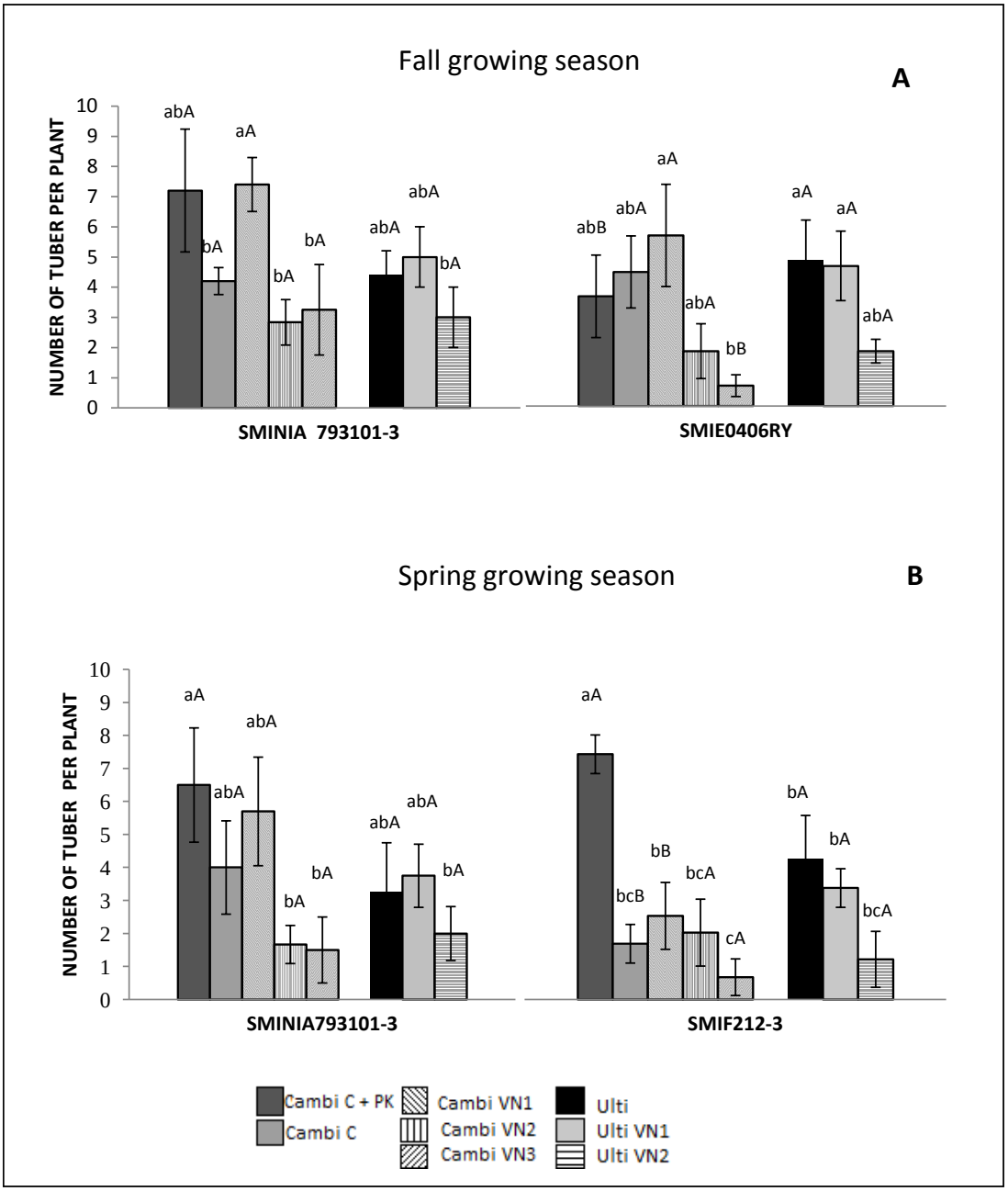

Fig.. 2. Effect of increasing Cu levels on number of tubers per plant at fall (A) and spring (B) growing seasons. Data represent the mean ± S.D. of six replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level ($p < 0.05$).

Interestingly, the difference between Cambi C and Cambi C+PK treatments in number of tubers produced during the spring growing season was numeric higher in SMIF 212-3 in relation to SMINIA793101-3 genotype. In contrast, SMINIA793101-3 plants did not differ as in Ulti VN1 and VN2. Similarly, the number of tubers per plant in Cambi VN2 and Cambi VN3 did not differ from Cambi C for all genotypes tested (Fig. 2).

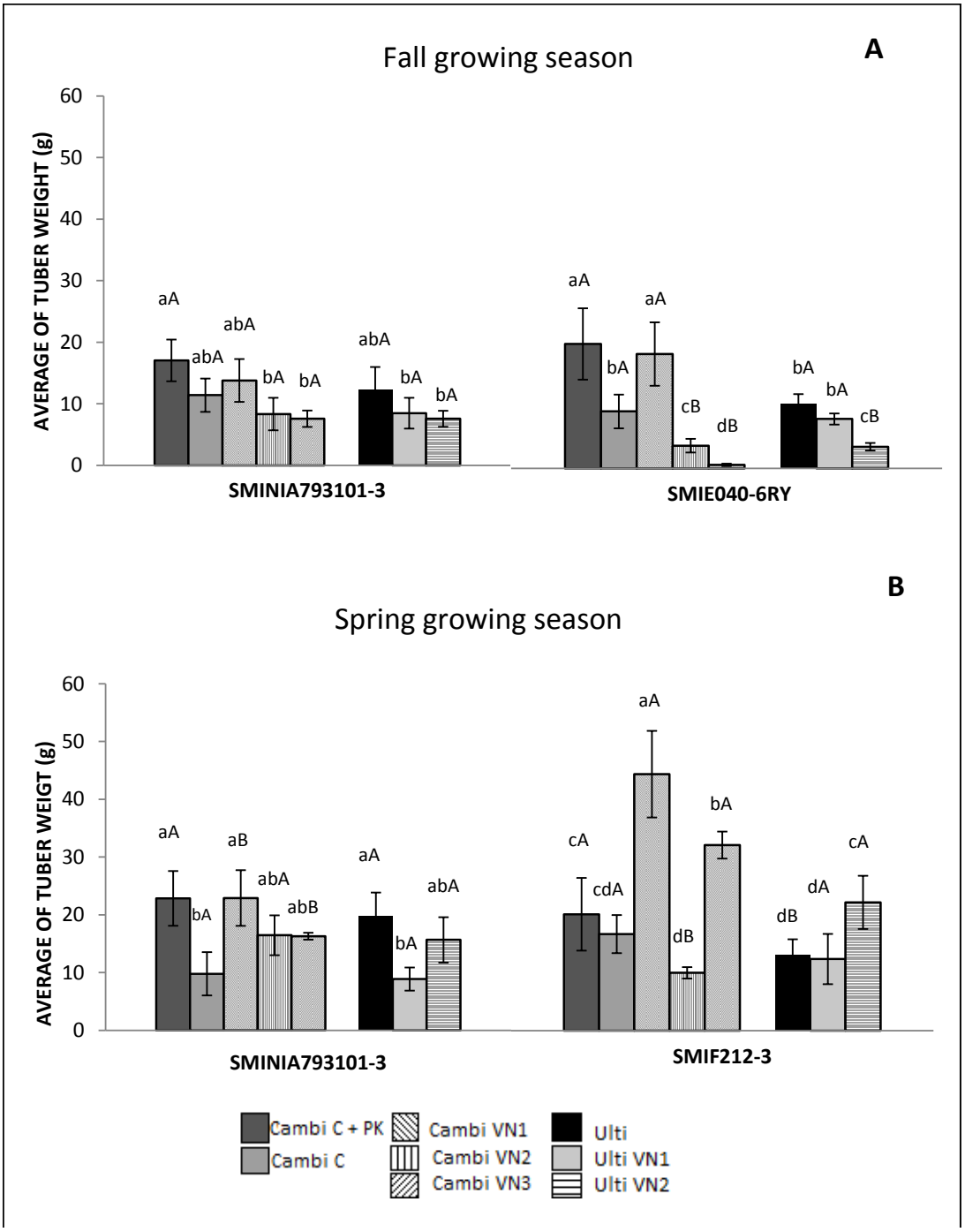

Fig. 3. Effect of increasing Cu levels on average of tuber weight at fall (A) and spring (B) growing seasons. Data represent the mean ± S.D. of six replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Despite the great variation of Cu concentration in the tested soils and growing seasons, the SMINIA793101-3 genotype showed little variation in the average weight of tubers among treatments (Fig. 3). However, the SMIE040-6RY and SMIF212-3 genotypes showed a wide variation in the average weight of tubers in response to Cu levels (Fig. 3). In Ulti soils, the average tuber weights of SMIE040-6RY plants were decreased with increased Cu

concentrations. In contrast, the average tuber weights of SMIF212-3 plants were stimulated for the Cu rise.

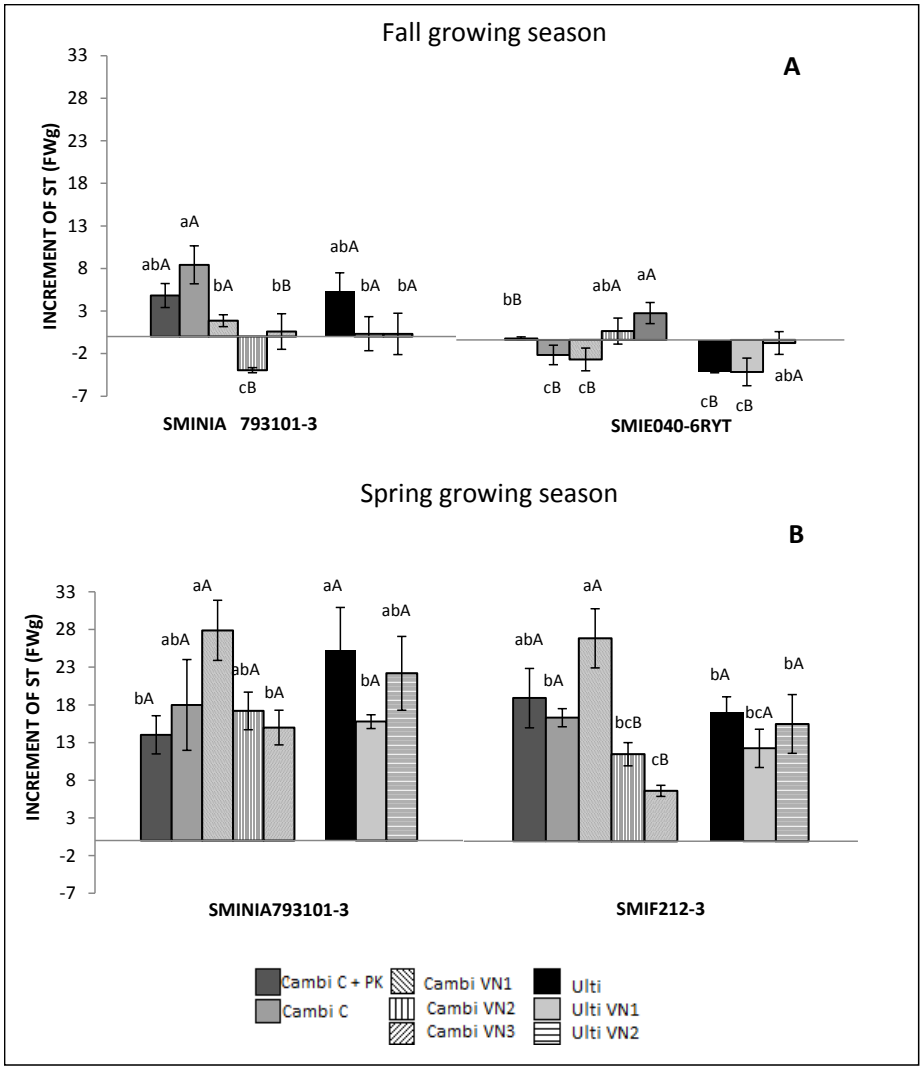

Fig. 4. Effect of increasing Cu levels on seed tuber (ST) increment per tuber at fall (A) and spring (B) growing seasons. Data represent the mean ± S.D. of six replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Interestingly, a large difference in the absorption and/or enhancement of the seed tuber was observed among the genotypes and growing seasons (Fig. 4). During the fall growing season, SMINIA793101-3 plants absorbed much of the seed tuber in soils with high Cu content, and the seed tuber weight of SMIE040-6RY plants was increased in the same soils. Conversely, in soils with lower Cu concentrations, SMIE040-6RY plants had the opposite response, which resulted in absorption of the seed tuber. By the spring period, there was an

increase in the seed tuber weight regardless of soil and genotype compared to seed tubers initial weight (Fig. 4).

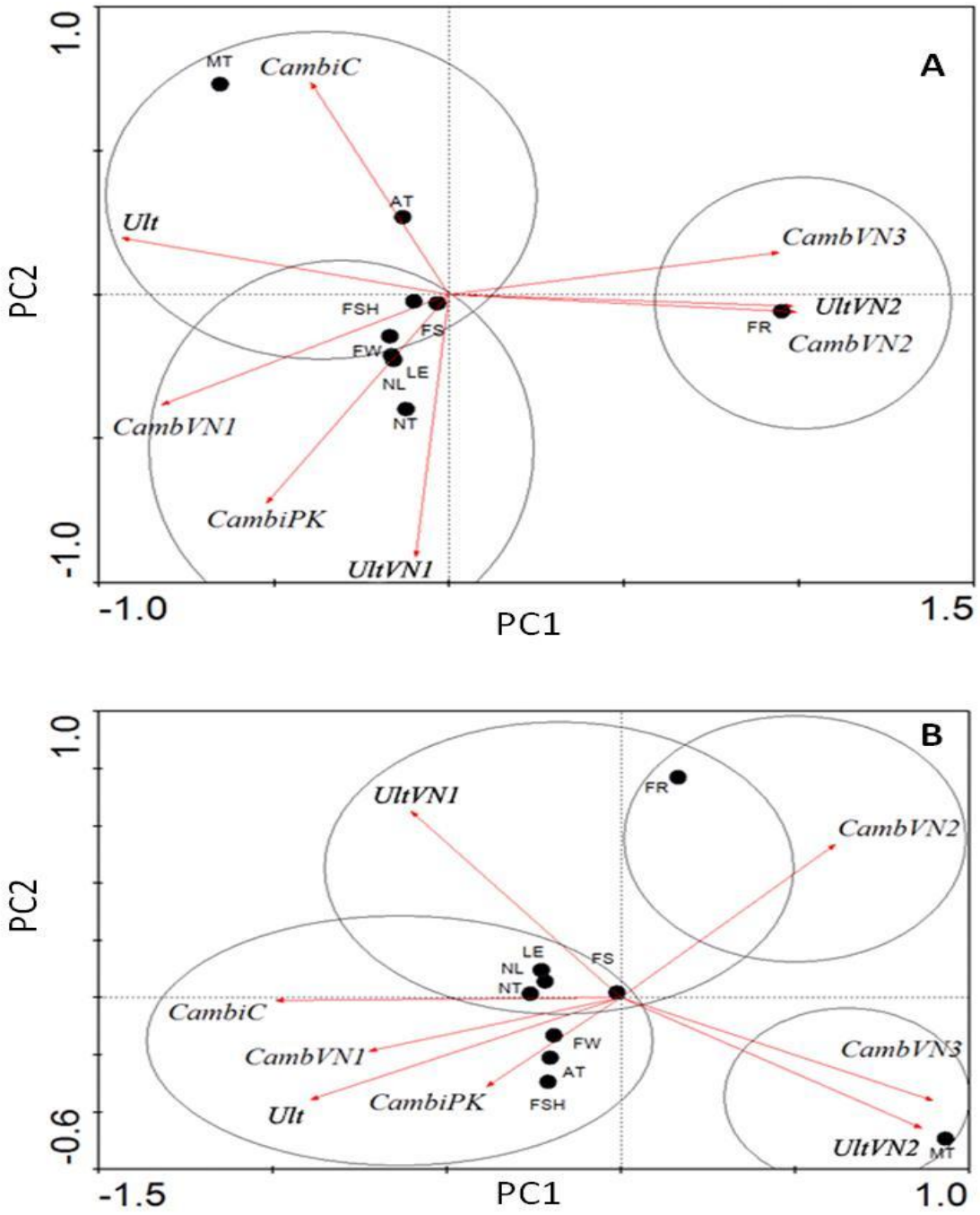

Fig. 5. Biplot graphic of scores and weights (loadings) for the first two principal components (PC1 and PC2) for growth parameters and tested soils at fall growing season for SMINIA793101-3 (A) and SMIE040-6RY (B) genotypes. Average of tuber weight (AT); number of tubers per plant (NT); Tubers fresh weight per plant (FW); seed tubers increment per tuber (MT); number of leaves per plant (NL); length of shoot (LE); shoot fresh weight per plant (FSH), stolon fresh weight per plant (FS) and root fresh weight per plant (FR).

Using multivariate analysis, the formation of three distinct clusters in relation to growth parameters was observed for SMINIA793101-3 plants in both seasons (Fig.s 5A and 6A). During the fall growing season, the axis of the first PC (PC1) explained 65% of the total variance, and Cambi VN2, Cambi VN3 and Ulti VN2 were positively correlated in this component.

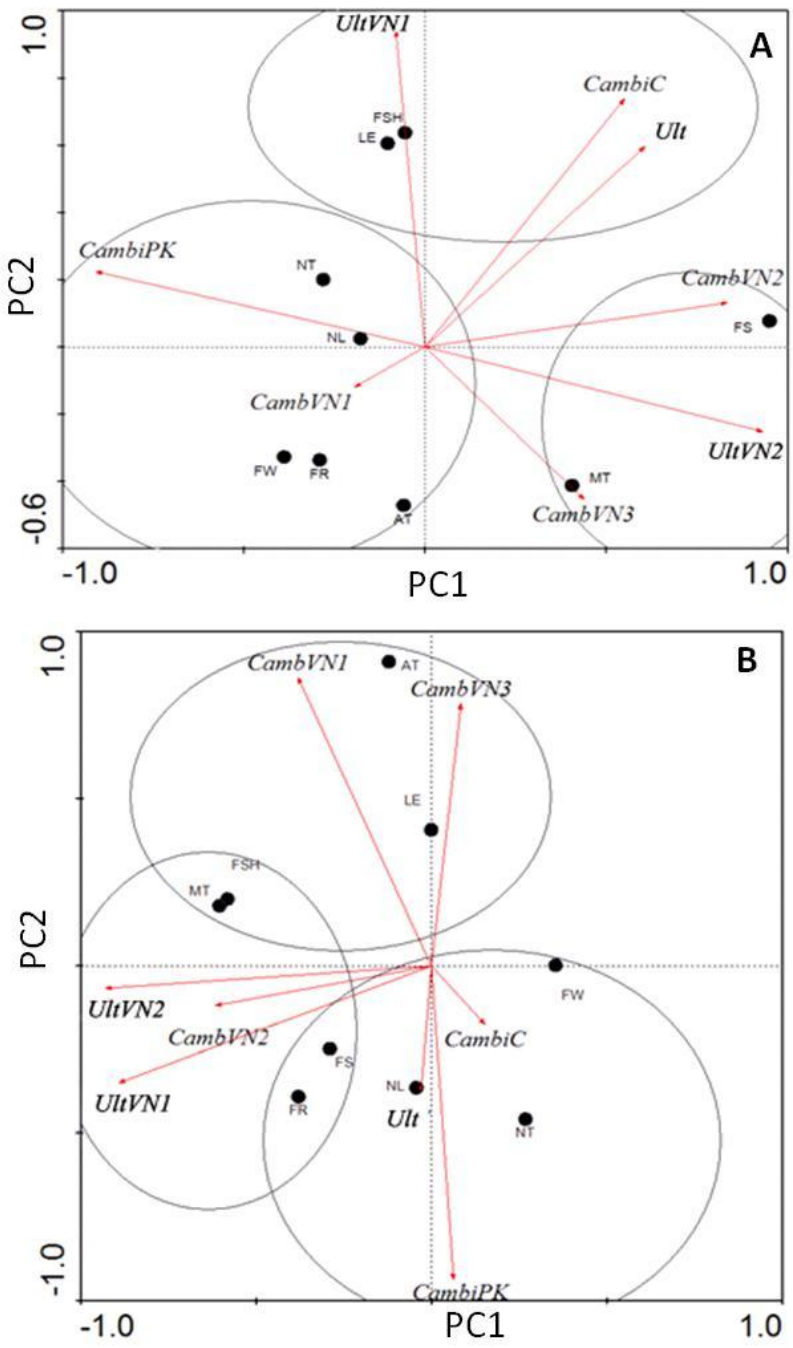

Fig. 6. Biplot graphic of scores and weights (loadings) for the first two principal components (PC1 and PC2) for growth parameters and tested soils at spring growing season for SMINIA793101-3 (A) and SMIF212-3 (B) genotypes. Average of tuber weight (AT); number of tubers per plant (NT); Tubers fresh weight per plant (FW); seed tubers increment per tuber (MT); number of leaves per plant (NL); length of shoot (LE); shoot fresh weight per plant (FSH), stolon fresh weight per plant (FS) and root fresh weight per plant (FR).

The same pattern was observed during the spring season with 43.5% of the total variance explained by PC1. The axis of the second PC (PC2) explained 23% of the total variance with no positive correlation with Ulti and Cambi C controls except for the correlation between Cambi+PK, Cambi VN1 and Ulti VN1. Together, the axes explained 88% of the growth parameter variation in SMINIA793101-3 plants (Fig. 5A). This genotype showed three patterns of response. One pattern was related to Cu toxicity, and the other two patterns involved fertility and absence of Cu contamination. The pattern of response to Cu toxicity clustered Cambi VN2, Cambi VN3 and Ulti VN2 together and related them to root fresh weight.

The other treatments shared the same response for shoot, stolon and tuber fresh weights. The Cambi C and Ulti controls both promoted increased numbers of seed tubers and average tuber weights, and Cambi+PK, Cambi VN1 and Ulti VN1 promoted the same shoot length, leaves number and tuber number. For SMIE040-6RY plants (Fig. 5B), PC1 explained 62% of the total variance, and Cambi VN3 and Ulti VN2 were positively correlated in this component. Cambi C, Cambi+PK, Cambi VN1 and Ulti were the main variables positively correlated in PC2 (17%). Together, PC1 and PC2 explained 79% of the variation of growth parameters (Fig. 5B).

Similar to the SMINIA793101-3 results, it was possible to identify four separate groups by soil type effect for the SMIE040-6RY plants. In PC1, there was a tendency to have root fresh weight and seed tuber number. However, in PC2, the shoot and tuber parameters showed positive scores. Moreover, there was no tendency for stolon fresh weights. During the spring growing season (Fig. 6), the SMINIA793101-3 plants grown in Ulti VN1, Cambi C and Ulti controls had tendencies related to shoot length and fresh weight with a positive correlation in PC2, which explained 26% of the total variance. Together, the axis of PC1 and PC2 explained 69.5% of the growth parameter variation of the SMINIA793101-3 genotype (Fig. 6A). Another group was formed by Cambi+PK and Cambi VN1, and it covered tuber and leaves production (number of tubers and leaves; tuber and root fresh weights; and average weight of tuber) as shown in Fig. 6A.

For SMIF212-3 plants (Fig. 6B), the PC1 axis explained 48% of the total variance, and Cambi VN2, Ulti VN1 and Ulti VN2 were positively correlated in this component. Together, PC1 and PC2 explained 77% of the variation (Fig. 5B). Interestingly, for both SMIE040-6RY and SMIF212-3 genotypes, Cambi VN2 and Cambi VN3 were not clustered in the same group. For either genotype, Cambi VN3 and Ulti VN2 were related to seed tuber increases, and Cambi VN2 corresponded to the group related to root fresh weight.

In both seasons and tested genotypes, Cambi+PK was related to tuber production in numbers and fresh weight, and the opposite pattern was observed in soils with high Cu concentrations (Cambi VN2, Cambi VN3 and Ulti VN2).

3.4 Estimation of lipid peroxidation and hydrogen peroxide concentration

During the fall growing season, there was an increase in tissue MDA concentration regardless of the tested soil for both genotypes during the plant cycle (Fig. 7). SMINIA793101-3 plants showed increased MDA concentration as compared to SMIE040-6RY plants at both sample times. Interestingly, the values of tissue MDA concentration in Cambi C and Ulti controls were markedly higher in SMINIA793101-3 plants during the fall compared to SMIE040-6RY. In Ulti VN2, the MDA concentration of SMINIA793101-3 plants was increased by 43% as compared to the control treatment. In the same soil, the MDA concentration of SMIE040-6RY plants was increased by 103% as compared to the control. These results demonstrated a lower basal level of oxidative stress in the SMIE040-6RY potato plants.

During the spring season, some treatments promoted a reduction in MDA concentration during the plant cycle. Interestingly, in this growing season, SMINIA793101-3 plants showed the highest variation in leaf MDA concentration independent of the soil tested (Fig.7B).

During the fall growing season, SMINIA793101-3 plants showed either a significant decrease in H_2O_2 concentration in Ulti VN1 or an increase in H_2O_2 concentration in Ulti VN2 as compared to the Ulti control (Fig. 8A).

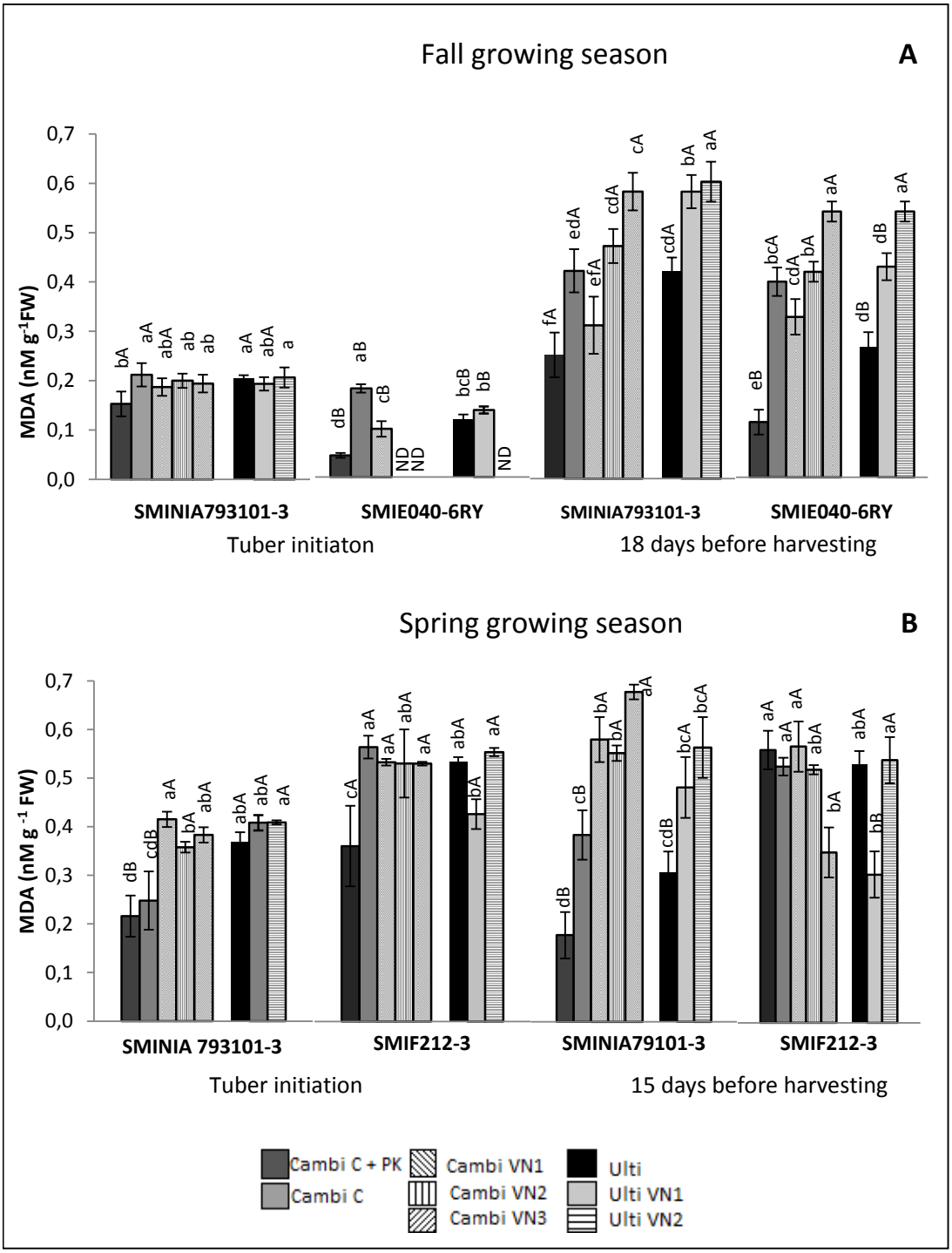

Fig. 7. Effect of increasing Cu levels on leaf lipid peroxidation in different development stages (tuber initiation and 15/18 days before harvesting) of different potato genotypes at fall (A) and spring (B) growing seasons. Data represent the mean ± S.D. of six replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level ($p < 0.05$).

Leaf H_2O_2 concentration was higher 18 days before harvesting than at tuber initiation for both genotypes grown in all Cambi soils with the exception of Cambi VN1. Additionally, both SMINIA793101-3 and SMIE040-6RY plants had the same pattern of variation for leaves H_2O_2 concentration (Fig. 8A).

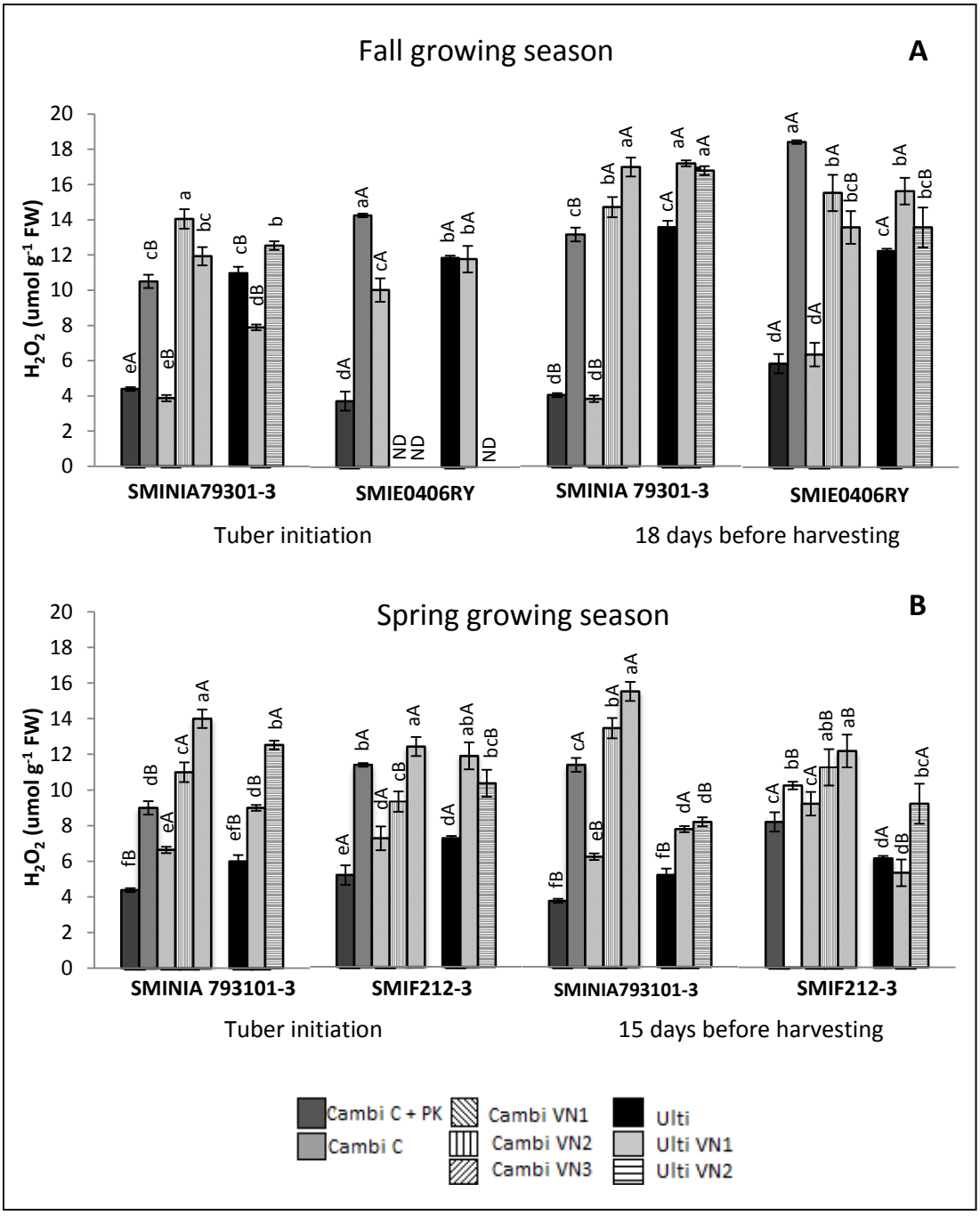

Fig. 8. Effect of increasing Cu levels on leaves H_2O_2 concentration in different development stages (tuber initiation and 15/18 days before harvesting) of different potato genotypes at fall (A) and spring (B) growing seasons. Data represent the mean ± S.D. of six replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level ($p < 0.05$).

At spring, in both SMINIA793101-3 and SMIF212-3genotypes the H_2O_2 concentrations increased with Cu increment, as well as with P deficiency. Unlike the fall growing season, at spring Ulti soils over all promoted lower H_2O_2 concentrations compared to cambi soils (Fig. 8B).

3.5 Enzyme activities of antioxidant systems

As shown in Fig. 9, regardless of the developmental stage of leaves, SOD activity increased with PK fertilization (Cambi C+PK) and with high levels of Cu (Cambi VN2, Cambi VN3 and Ulti VN2). At the tuber initiation period, tissue SOD activity in SMINIA793101-3 was significantly higher in Cambi C+PK, Cambi VN3 and Ulti VN2. However, SOD activity was higher in SMIF212-3 plants grown in Cambi VN3 (Fig. 9A).

CAT activity was more affected by PK addition and Cu toxicity than SOD activity (Fig. 9B). In tissues from both collection times, CAT activity was increased in SMINIA793101-3 plants grown in Cambi C +PK compared to Cambi C and was decreased in SMIF212-3 plants (Fig. 9). For the SMINIA793101-3 and SMIF212-3 genotypes, Cu exposure during the plant cycle (period covering tuber initiation to 15 days before harvesting) increased the CAT activity by 71 and 67%, respectively, in Ulti VN2 and 16 and 146%, respectively, in Cambi VN2. Overall, the CAT tissue activity was higher in SMINIA793101-3 plants cultivated in Ulti soils and in SMIF212-3 plants cultivated in Cambi soils.

In SMINIA793101-3 plants, APX tissue activity was increased when the plants were grown in Cambi VN2 at tuber initiation. At the second tissue collection time, APX tissue activity was increased in these plants grown in Cambi VN3 as compared to Cambi controls (Fig. 9C). Remarkably, in Ulti soils, APX activity was reduced by increased Cu concentrations for the SMINIA793101-3 genotype at the first collection time and for the SMIF212-3 genotype at the second collection time (Fig. 9C).

In general, the SMINIA793101-3 genotype showed a reduction in APX activity between collection times with the exception of Cambi VN3. For the SMIF212-3 genotype, APX activity was increased when the plants were grown in Cambi VN1 and Ulti VN1.

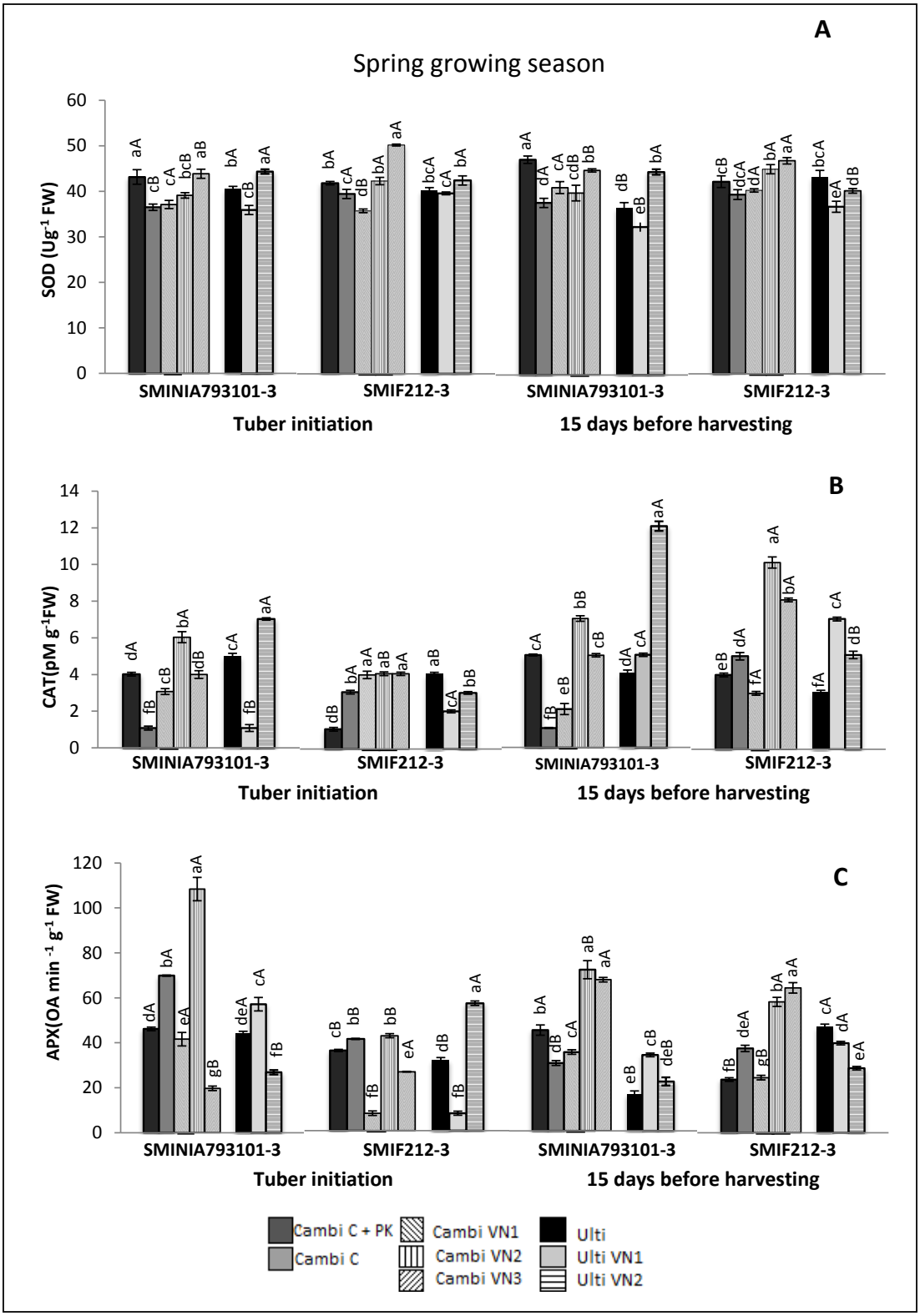

Fig. 9. Effect of increasing Cu levels on SOD (A), CAT (B) and APX (C) leaf activities in different development stages (tuber initiation and 15/18 days before harvesting) of different potato genotypes at spring growing season. Data represent the mean ± S.D. of six replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level ($p < 0.05$).

3.6 Non-enzymatic antioxidants

For SMINIA793101-3 plants, the increment on ASA production in response to Cu exposure in Cambi soils only appeared near the end of the cycle (15 days before harvesting) in Cambi VN2 (which did not significantly differ from Cambi C and C+PK) as shown in Fig.10.

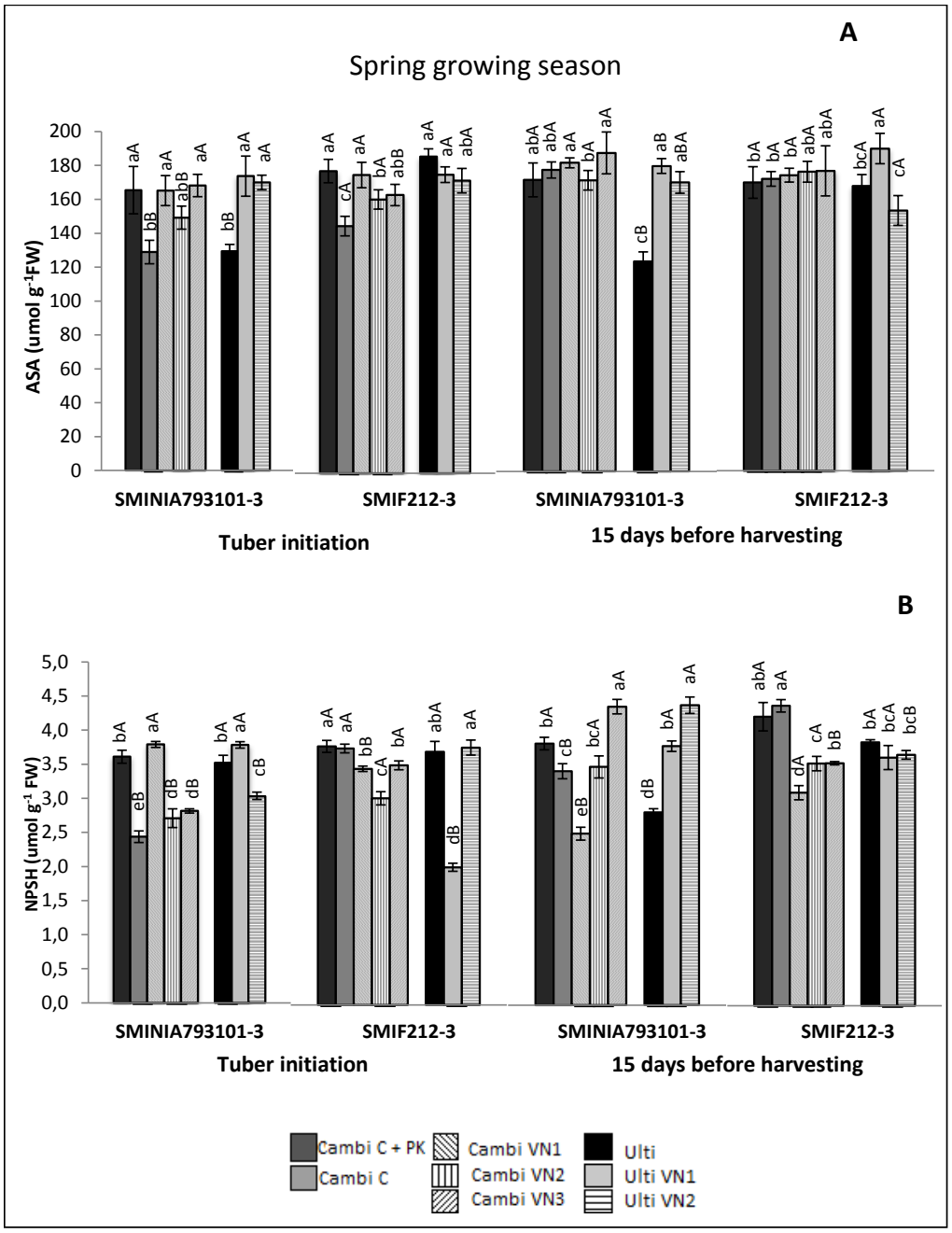

Fig.. 10. Effect of increasing Cu levels on AsA (A) and NPSH (B) leaf concentrations in different development stages (tuber initiation and 15/18 days before harvesting) of different potato genotypes at spring growing season. Data represent the mean ± S.D. of six replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level ($p < 0.05$).

However, in Ulti soils, a pattern of increased ASA concentration was observed in Ulti VN1 and Ulti VN2 as compared to Ulti control at both collection times with a median increase of 33 and 42% at tuber initiation and 15 days before harvesting, respectively, in SMINIA793101-3 plants. SMINIA793101-3 and SMIF212-3 plants had the lowest ASA concentration values in Cambi C at tuber initiation.

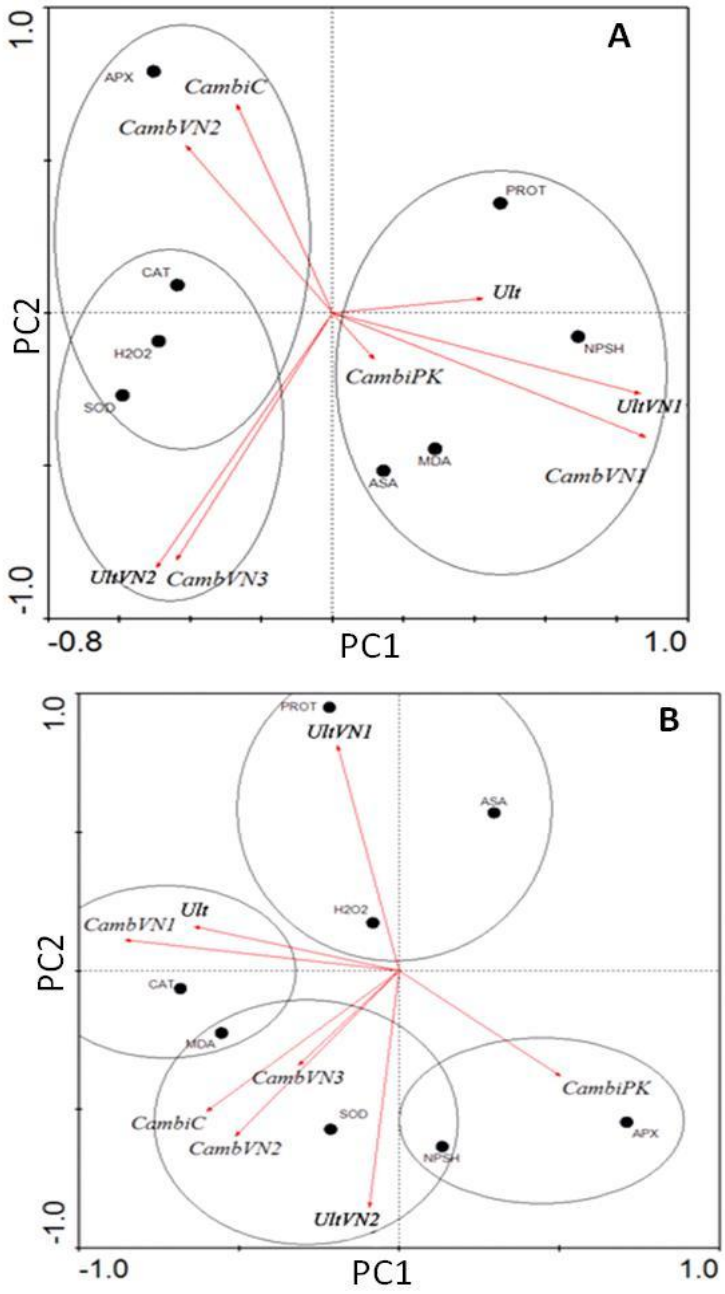

Fig. 11. Biplot graphic of scores and weights (loadings) for the first two principal components (PC1 and PC2) for biochemical parameters and tested soils at tuber initiation for SMINIA793101-3 (A) and SMIF212-3 (B) genotypes. MDA (lipid peroxidation); H_2O_2 (hydrogen peroxide concentration); SOD (Superoxide dismutase activity); CAT (catalase activity); APX (ascorbato peroxidase activity); PROT (protein concentration); AsA (ascorbic acid concentration); NPSH (non-protein thiol groups concentration).

At the second collection time, however, there was no significant difference in ASA concentrations among the Cambi soils. In Ulti soils, the ASA concentration in SMIF212-3 plants was only different 15 days before harvesting with an increase of 13% in Ulti VN1 as compared to Ulti control.

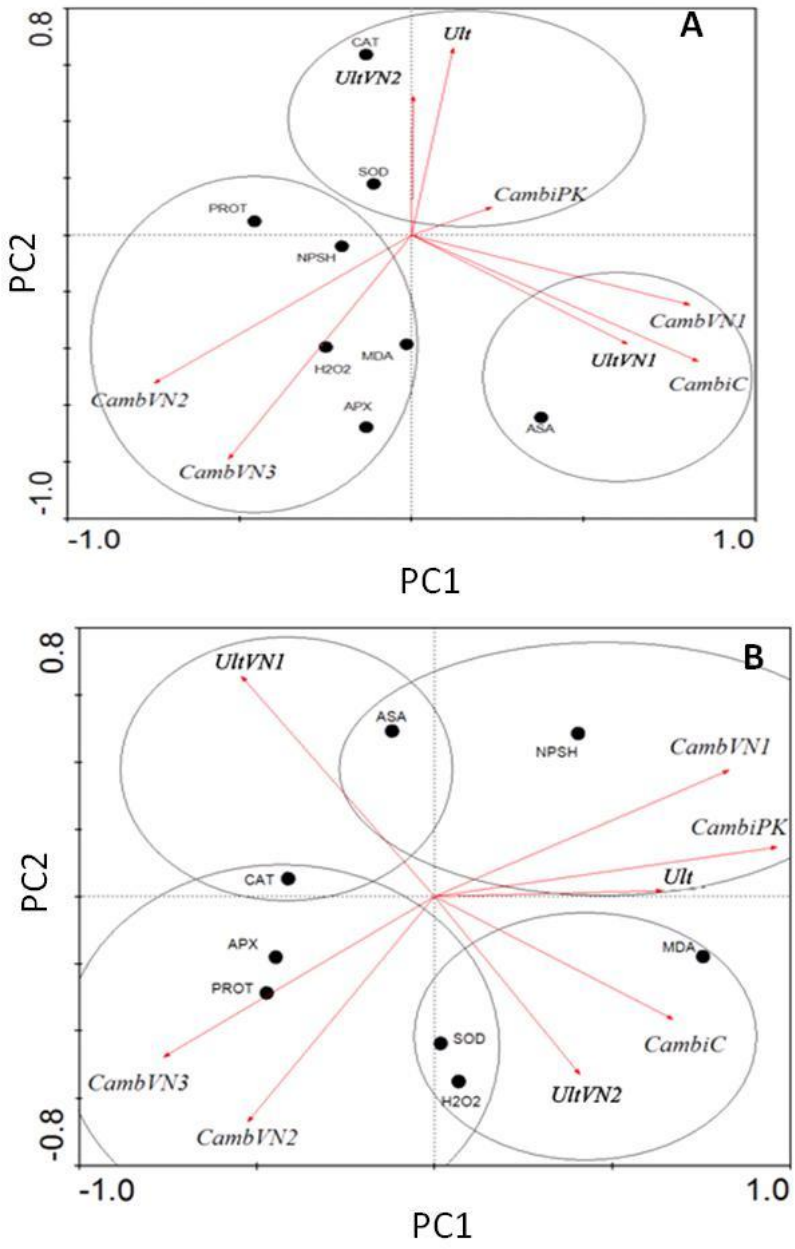

Fig. 12. Biplot graphic of scores and weights (loadings) for the first two principal components (PC1 and PC2) for biochemical parameters and tested soils 15 days before harvesting for SMINIA793101-3 (A) and SMIF212-3 (B) genotypes. MDA (lipid peroxidation); H2O2 (hydrogen peroxide concentration); SOD (Superoxide dismutase activity); CAT (catalase activity); APX (ascorbato peroxidase activity); PROT (protein concentration); AsA (ascorbic acid concentration); NPSH (non-protein thiol groups concentration).

NPSH values decreased with Cu increments in SMINIA793101-3 plants at tuber initiation in both Cambi and Ulti soils, and the opposite response occurred at the second

collection time with higher NPSH values observed in Cambi VN3 and Ulti VN2 (Fig. 10B). SMIF212-3 plants, however, had lower NPSH values in Cambi VN2 and VN3 at both tuber initiation and 15 days before harvesting as compared to Cambi controls. In Ulti soils, this genotype had the lowest NPSH value in Ulti VN1 at tuber initiation, and no significant differences in NPSH values were found among the treatments 15 days before harvesting.

The principal component analysis for biochemical parameters suggested a wider range of response to Cu in the SMIF212-3 genotype, which clustered four groups, and the SMINIA793101-3 data resulted in the formation of three groups at both sampling times (Fig.s 11, 12).

At the first collection time, the PC1 axis of SMINIA793101-3 explained 35% of the total variance, and Cambi+PK, Cambi VN1, Ulti and Ulti VN1 were positively correlated in this component (Fig. 11A). A different pattern was observed at the second harvesting time (Fig. 12A) with 40% of the total variance explained by PC1. The PC2 axis explained 28% of the total variance, and no positive correlation was found for Ulti VN2, Cambi VN3, Cambi C and Cambi VN2. Together, the axes explained 63% of biochemical parameter variation in the SMINIA793101-3 genotype. Interestingly, at tuber initiation, H_2O_2 concentration and CAT tissue activity were clustered together in the SMINIA793101-3 analysis. However, at the second tissue collection time, H_2O_2 concentration was related to MDA concentration. On the other hand, the SMIF212-3 genotype showed MDA concentration grouped with CAT activity at the first collection time, and H_2O_2 concentration was clustered into a different group for the SMIF212-3 genotype (Fig. 12A).

For SMIF212-3 plants (Fig. 11B), the PC1 of the data from the first harvesting time explained 29% of the total variance, and Ulti and Cambi VN1 were positively correlated in this component. Cambi C, Cambi VN2, Cambi VN3 and Ulti VN2 were the main variables with a positive correlation in the PC2 (27%), and both PC1 and PC2 explained 56% of the variation of biochemical parameters (Fig. 11B). Thus, at the second harvesting time, MDA concentration, H_2O_2 concentration and CAT activity were clustered into the same group for the SMIF212-3 genotype. Additionally, at both harvesting times, the CAT activity was clustered with SOD activity for the SMINIA793101-3 genotype, and SMIF212-3 data showed a pathway shared with SOD activity, CAT activity and MDA concentration at the first harvesting time and a pathway shared with SOD activity, CAT activity and H_2O_2 concentration at the second harvesting time (Fig. 12B).

The APX tissue activity and protein concentration was related to Cambi soils with high Cu content (Cambi VN2 and Cambi VN3) at the second harvesting time for both genotypes as well as the protein concentration (Fig. 12A, B).

Overall, ASA and NPSH concentrations were clustered with Cambi+PK and Ulti VN1.

4. Discussion

4.1 Effects of Cu toxicity on tissue Cu concentration and growth parameters

This study demonstrated that higher Cu exposures led to increases in Cu concentration in shoot tissue (Table 2), which has been previously reported for distinct plant species by other authors [10,37]. However, Cu accumulation was also dependent on leaf development stage, with Cu accumulating in expanded leaves, mostly in mature plants (Table 2). Despite the plant development stage, the fitointoxication itself may reduce the transport and consequent accumulation of Cu, once there is a drastic reduction of absorption and translocation of water and solutes from soil as a result to the growth inhibition by Cu stress.

During the fall growing season, SMIE040-6RY plants pre-classified as not efficient but responsive to P showed the highest sensitivity to Cu excess in growth terms (Table 2, 3; Fig. 1, 2, 3). The response to Cu toxicity included plants without expanded leaves and lacking tuber production. Remarkably, the Cu concentration in leaf tissues of plants without expanded leaves grown in Cambi VN2 and Cambi VN3 did not differ from the Cu concentration of plants grown in Cambi C and Ulti controls, but plants with expanded leaves grown in soils with Cu contamination (Cambi VN1, Ulti VN1 and Ulti VN2) had significant increases in Cu tissue concentrations (Table 2). Studies have reported a correlation between Cu excess and ethylene production [38,39], which is mainly associated with growth retardation in plants [40,41], thus agreeing with the data of the present study. Conversely, Lidon et al. [42] reported a decrease of total activity of l-aminocyclopropane-l-carboxylate synthase in both root and shoot tissues at Cu concentrations higher than 0.05 mg L^{-1}, and they reported that the total activity of the ethylene forming enzyme slightly increased when the Cu concentration of 1.25 mg L^{-1} was reached.

Genotypes pre-classified as P efficient were able to expand leaves and produce tubers in all tested soils (Fig. 1, 2). In view of the internal requirement of the plant nutrient,

efficiency is generally defined as the biomass produced per unit of nutrient applied to the soil, which depends on two main components: efficiency of acquisition and utilization [43]. The first component depends on the efficiencies of absorption and rooting, including changes in root architecture an exudates production [41,44]. The second component depends on the efficiency of translocation and conversion into biomass. These components may mitigate Cu effects throught a good nutrition, which reflects in a superior biomass produced and consequently dilute the tissue metal.

Although with significant differences among genotypes, soils and seasons, overall Cu toxicity inhibited shoot growth in fresh weight terms with stronger inhibition in Cambi VN2, Cambi VN3 and Ulti VN2, which may be due to the inhibition of cell division resulting from the decline of growth [45]. Similar findings have also been reported in *Avena sativa* under the same tested soils by Girotto [37]. Interestingly, for potato plants the numbers of leaves and shoot length were not sensible to Cu stress in comparison to shoot fresh weight. Actually, during the spring growing season SMINIA793101-3 plants were positively affected in these parameters by Cu increment. Other interesting fact was the reduction in number of leaves per plant during the spring season when compared to the fall growing season. This result was similar to that of shoot fresh weight in soils without Cu contamination, but it disagreed with data found in contaminate soils. This data suggests that plants grown during the spring season used the photosynthates to obtain higher leaf area, unlikely of plants in the fall season wich had higher number of leaves.

Contrary to previous reports [46,47], cultivation of potato in soils containing high Cu levels did not negatively affect root fresh weight in SMINIA793101-3 plants, where the root fresh weight increased with increasing Cu levels in Ulti soils during the fall season. Moreover, there was no change in root fresh weight in Cambi VN2 and Cambi VN3 as compared to Cambi C. This response 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 [48]. However, stolon fresh weight decreased in Ulti VN1 and Ulti VN2 as compared to Ulti control during the fall for both cultivars and in Ulti VN1 during spring for SMIF212-3 plants.

Interestingly, upon PK addition, a significant decrease in Cu leaf tissue concentration followed by biomass increase was observed regardless of the season or genotype tested. In agreement, previous studies have shown a reduction of Cu transport to shoots in plants grown in contaminated soils [20] through formation of less soluble compounds with added phosphate [49]. Other protective effects of P may include metal dilution in plant tissue as a result of

larger biomass production and/or chelation by exuded compounds in rhizospheres [50].

In addition, plants grown under high Cu levels but also with suitable P and high K concentrations (Cambi VN1) has the tuber and shoot production not negatively affected, with significant increases observed in these parameters, as compared to Cambi C and Ulti (Table 2; Figs. 1 and 2). K is the most abundant cation in the cytoplasm, and K contributes to the osmotic potential of cells and tissues. Furthermore, many enzymes are either completely dependent on K^+ or stimulated by K^+. Moreover, K is required for protein synthesis and cell extension, which affect many components of photosynthesis [51]. Potato plants are responsive to K fertilization, wich showed high correlation with the number and weight of tuber per plant [52]. In addition, the high soil P levels might contribute to the mitigation of Cu toxicity effects on plant growth and the decrease of Cu in leaf tissue in Cambi C+PK as compared to Cambi C.

Importantly, this study tested two controls for Cambi soils. A positive control (Cambi C+PK), without Cu accumulation and with appropriate nutrient concentrations for the tested culture, as well as a negative control (Cambi C), a soil also with no Cu accumulation but with low availability of P. Therefore, comparison was important for assesseing the genotype variability in relation to P nutrition and for comparison of oxidative stress and growth under Cu contamination and nutritional deficiency.

Overall, the Ulti control, even with agricultural suitable concentrations of P and K and without Cu contamination, had tuber and shoot production values similar to Cambi C, which had a P deficit. Furthermore, the production of tubers and shoots was significantly lower in Ulti control as compared to Cambi C+PK and Cambi VN1. The concentration of Cu found in Ultisols with larger accumulations of Cu (Ulti VN1 and Ulti VN2) was approximately 6.0 times less than that found in Cambisols (Table 1), which can be explained by the natural composition of the soil. The tested Ultisols had lower levels of OM and clay than the Cambisols (data not shown), and this difference may have been the determinant for the lower levels of Cu in the soils. In addition, Rooney et al. [46] used single regressions analysis performed between Cu toxicity threshold values and various soil properties to show that exchangeable calcium, soil cation exchange capacity, iron oxide concentration, soil pH, clay, and organic carbon content are the best predictors for Cu toxicity in barley and tomato plants.

Regardless of the significant difference in Cu content, there was a great reduction in the growth of plants cultivated in Ultisols with Cu accumulation even when Cu was below the established threshold of 200 mg kg^{-1} [53], which was similar to the effects observed in Cambisols with Cu toxicity. The behavior of metals is influenced by attributes of the soil

solid phase, type of adsorbent (organic matter, silicate minerals, iron oxides, manganese, and phosphate groups) and geochemical conditions of the solution, particularly proton concentration and ionic strength [54]. Thus, it is possible to infer that the value established for the Cu threshold is not suitable for sandy soils, such as Ultisols.

The numbers of tubers per plant were similar between the genotypes in the same growing season (Fig. 2). However, the growing season affected tuber production (Fig. 1) with the spring season corresponding to the higher tuber fresh weight and the fall season corresponding to a higher number of tubers, which resulted in a higher tuber average weight in the spring with the exception of Cambi C. These data demonstrated that the type of tuber produced during the fall growing season in this experimental system is less acceptable for commercial production due to the small size and average weight [55].

An important factor to ensure a good yield of tubers in addition to the effect of season and fertilization is the quality of seed tubers, because seed tubers are responsible for the initial plant nutrition and they can also transmit diseases to the plant [56]. In this view, the utilization of homogeneous seed tubers may help to differentiate the genotypes according to initial nutrition requirements. During the fall growing season, plants classified as P efficient (SMINIA793101-3) had a higher absorption of storage nutrients from seed tubers in soils with Cu accumulation, and plants classified as P inefficient (SMIE040-6RY) responded with an increase in the same treatments (Fig. 4). During spring, seed tuber weight increased in all treatments with no statistical differences among Cambi controls, Cambi VN2 and Cambi VN3 for SMINIA793101-3 plants, but a significant decrease in seed tuber weight was found in Cambi VN3 as compared to Cambi controls in SMIF212-3 plants.

In general, the interpretation of growth parameters is performed using univariate analysis or through the correlation among the parameters. However, these variables can interact leading to misinterpretation [57]. PCA analyses detect differences between samples or between different measured variables, thereby reducing the number of variables to explain the same amount of variance [58]. The results obtained by PCA based on the correlation matrix of different soils in relation to growth parameters showed that both genotypes were clustered by response to Cu contamination and soil fertility during the fall season (Fig.5, 6). However, there were distinct patterns of response between the tested genotypes. In general, the SMINIA793101-3 plants were divided into two responses: I) Cu sensitivity, which resulted in the clustering of Cambi VN2, Cambi VN3 and Ulti VN2; and II) fertility response, which was subdivided into a high production response (Cambi+PK and Cambi VN1) and a low production response (Cambi C and Ulti control). The response related to Cu sensitivity was

correlated with high Cu levels. Moreover, the soil fertility response was also related to high Cu levels. For a treatment to be clustered with the fertility response, however, a deficiency or high level of a macronutrient (P or K) must be correlated to Cu toxicity. In addition, fertility was not decisive to define clusters. In SMIE040-6RY plants the most toxic soils were only related to the increment in seed tuber weight and root fresh weight (Fig. 5B). On the other hand, in SMIF212-3 plants these treatments were related to shoot, root and stolon production, with no relation to tubers production in numbers and fresh weight (Fig. 6B).

4. 2 Estimation of lipid peroxidation and hydrogen peroxide concentration

The level of lipid peroxidation products was estimated by reaction with thiobarbituric acid (TBA). The TBA test quantifies malonaldehyde (MDA), which is one of the main decomposition products of polyunsaturated fatty acid hydroperoxides of biomembranes. Increased levels of MDA in plants indicate that the plants are under high levels of oxidative stress [59]. In many plant species, heavy metals have been reported to cause oxidative damage due to production of ROS [60]. The main site of attack by any redox active metal in a plant cell is usually the cell membrane, and oxidation damage can cause a variety of harmful effects, including lipid peroxidation [61]. In the present study, there were differences in MDA levels between seasons, harvesting times and genotypes (Fig. 7). During the fall growing season, there was an increase in MDA concentration in leaves during the plant cycle in all tested soils. This data was partially in agreement with results reported by Girotto [37], who tested *Avena sativa* grown in the same soil types.

The increase in MDA levels may be a result of increased levels of H_2O_2, which occurred in plants grown in Cambi C, Cambi VN2, Cambi VN3, Ulti VN1 and Ulti VN2, thereby indicating the excessive accumulation of Cu in the soil. H_2O_2 is an oxidant that can cause cellular damage, such as carbonylation of proteins, or even cell death (Bienert et al., 2006). In addition, Cu can catalyze the formation of hydroxyl radicals (OH^-) from the non-enzymatic chemical reaction between superoxide (O_2^-) and H_2O_2 (Haber-Weiss reaction) [62]. The main mechanism of Cu toxicity involves the Fenton reaction catalyzed by the presence of Cu, which is characterized by the production of hydroxyl radicals from superoxide and hydrogen peroxide in the presence of free Cu ions in the cell [63]. As reported by Mishra et al. 64], an excessive amount of ROS induces severe lipid peroxidation due to the hydrogen removal from unsaturated fatty acid constituents of cell membranes.

During the spring, the difference between harvesting times for MDA concentrations was slight. In the Ulti soils, there was no increase during the plant cycle for both MDA and H_2O_2 concentrations. In addition, MDA concentration was only statistically different in Ulti VN1 as compared to Ulti control. Environmental stresses, both biotic and abiotic, promote enhanced production of hydrogen peroxide (H_2O_2), superoxide anion (O_2^-) and their accumulation in plants [65]. Because O_2^- and H_2O_2 are the major stable oxidants, the ratio of these redox components is indicative of the redox balance within the tissue [66,67]. Moreover, the effects of higher Cu concentrations on the growth of potato plants observed in the experimental conditions of the present study were more associated with H_2O_2 concentration than MDA concentration, which may be due to enzymatic and non-enzymatic system activation in a way that H_2O_2 was not able to cause lipid peroxidation with the same magnitude.

Tamás et al. [68] suggested that the function of this elevated H_2O_2 formation in tissues may be due to the increase in cell wall loosening. H_2O_2 has a crucial role not only in cell wall modification but also in cell wall synthesis and subsequent cell division. Interestingly, at the second harvesting time, genotypes efficient and not responsive to P showed a higher increase in H_2O_2 levels when grown in Cambi VN2 and Cambi VN3, and the genotype not efficient and responsive to P had the highest H_2O_2 level when grown in Cambi C. Additionally, regardless of the genotype tested, the H_2O_2 concentrations were significantly higher in plants grown in Cambi C as compared to Cambi+PK, which may be attributed to the low availability of P in soil. These results were consistent with those obtained by Tewari et al. [69], who showed that low levels of available P and exchangeable K in soil cause increased MDA levels in maize plants and increased H_2O_2 concentrations in mulberry plants [70].

4.3 Enzyme activities of antioxidant systems

The presence of excess Cu can cause oxidative stress in plants and, subsequently, cause an increase in antioxidant responses due to increased production of highly toxic oxygen free radicals. The oxidative stress caused by excess Cu in plants induces changes in the activity and content of some antioxidant pathway components, such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR), and superoxide dismutase (SOD) [2,71]. Moreover, to control the level of ROS and protect the cells upon exposure to metal stress, plants usually show increased induction of SOD, CAT, and POD isozymes [72,73]. Accordingly, the present study showed a significant increase in

the activity of SMINIA793101-3 SOD tissue activity at both harvesting times with increased Cu levels in Ulti and Cambi soils as compared to Cambi C (Fig. 9). The increase in SOD activity is usually attributed to an increase in superoxide radical concentration, which is due to de novo synthesis of enzyme proteins [74] resulting from the induction of SOD genes by superoxide-mediated signal transduction [75].

In the present study, the CAT activity was increased with Cu addition with the exception of Ulti control because its CAT activity was statistically higher than that of Ulti VN1 at the first harvesting time (Fig. 9). Interestingly, in SMINIA793101-3 plants, both CAT an APX had high activity under Cu contamination. However, CAT was more related to Ulti soils, and APX was more related to Cambi soils. Moreover, CAT activity was increased with the maturation of plants. In contrast, APX had the highest activity at tuber initiation. On the other hand, the APX and CAT activities of SMIF212-3 plants grown in Cambi soils were increased with high Cu levels at the second harvesting time (Fig. 9B, C). These results suggested that the CAT and APX activities (Fig. 9A, B) were more effective than SOD activity to scavenge the excess of ROS (Fig. 9A, B, C). APX is extremely sensitive to ascorbate concentrations. APX loses stability and its activity declines with low AsA contents [79]. However, the lower activity of APX did not correlate with the lower concentration of AsA in this experiment. According to Mittler [76], the different affinities of APX (l M range) and CAT (mM range) for H_2O_2 suggest that they belong to two different classes of H_2O_2-scavenging enzymes where APX may be responsible for the fine modulation of ROS for signaling and CAT may be responsible for the removal of excess ROS during stress. Whereas APX has a high affinity for H_2O_2 and is able to detoxify low concentrations, catalases have a higher V_{max} but a lower affinity for H_2O_2. Catalases also have a key role in maintaining the redox balance in cells exposed to oxidative stresses [77,78].

The response of enzymes involved in the attenuation of ROS (SOD, APX or CAT) to heavy metals greatly depends on the species, plant age and growth conditions [48,60]. Under Cu stress conditions, the analysis of different antioxidant enzymes (SOD, CAT, and APX) by non-denaturating polyacrylamide gel electrophoresis has shown that they have several isoforms in different organs of various plant species [80]. Multiple isoforms of POD, CAT and SOD, which are controlled by different genes, have been reported in higher plants [76]. Interestingly, the patterns of total CAT and SOD activities were significantly different between genotypes in the present study, which may be due to different responses of diverse CAT and SOD isoforms with prevalence for the isoforms with increased activities.

4.4 Non-enzymatic antioxidants

In the present study, the AsA concentrations of SMINIA793101-3 plants in both Cambi and Ulti soils increased with higher Cu concentrations as compared to Cambi C and Ulti controls (Fig. 10), and the AsA concentrations of SMIF212-3 plants were only significantly increased in Cambi VN2 and Cambi VN3 at the first harvesting time and in Ulti VN1 at the second harvesting time. AsA concentrations vary considerably between tissues, and they depend on the physiological status of the plant and on environmental factors [81]. AsA is an essential constituent of higher plants that has key roles in antioxidant defense, cell division and cell elongation [82]. Therefore, AsA contents are generally higher in younger tissues than in older ones, and AsA accumulates in actively growing tissues, such as meristems. In addition, photosynthetic tissues have high ASA concentrations [82]. However, in the present study, the AsA concentration did not considerably vary between the harvesting times and genotypes.

In the present study, the NPSH concentrations in SMINIA793101-3 plants were higher in Cambi VN2 and Cambi VN3 as compared to Cambi C at both harvesting times, but the NPSH concentrations resulting from plants grown in these soils were less than those resulting from plants grown in Cambi+PK and Cambi VN1 at tuber initiation. In Ulti soils, the NPSH concentrations in SMINIA793101-3 plants only gradually increased with Cu increments at the second harvesting time (Fig. 10B). Together, these data suggested an initial suppression of NPSH levels followed by an increase in response to Cu concentration in plant maturation, which has been previously described by Ali et al. [83] in *Panax ginseng* plants. However, SMIF212-3 plants had lower NPSH values with high Cu concentrations even at maturation.

NPSH is affected by the presence of several metals [84]. Among the types of NPSH, glutathione (GSH) is the predominant molecule and has important functions as a redox-buffer, phytochelatin (PC) precursor and substrate for keeping ascorbate in its reduced form in the ascorbate–glutathione pathway [85]. Moreover, GSH is the substrate for PCs synthesis. PCs are involved in the cellular detoxification mechanism due to their ability to form stable metal-PC complexes [86]. Conversely, Murphy and Taiz [87] tested 10 *Arabidopsis* ecotypes for copper tolerance with the expression of 2 metallothionein genes (MT1 and MT2) and NPSH levels, and they reported that MT1 is uniformly expressed in all tested treatments and that MT2 is copper inducible in all 10 ecotypes. Moreover, they reported that ecotypes with higher levels of NPSH have an inducible tolerance mechanism. Thus, they concluded that the long-term quantitative differences in MT2 mRNA levels in the ecotypes are not due to differences

in the initial rate of increase but rather to differences in the ability to sustain the initial rate during a long-term exposure to copper.

The results obtained by the PCA based on a correlation matrix of different soils in relation to biochemical parameters showed that genotypes were clustered by response to Cu contamination and soil fertility. However, there were distinct patterns of response between the tested genotypes and between the harvests (Figs. 11, 12). During the first harvest there was a common pattern of response related to H_2O_2 concentration and the enzymes CAT and SOD activities, which were related to soils with high Cu content (Cambi VN2, Cambi VN3 and Ulti VN2) and also associated with P deficiency (Cambi C) for SMINIA 79101-3 genotype (Fig. 11). The APX activity was grouped only with Cambi C and Cambi VN2 treatments (Fig. 11A). Interestingly, the other soils treatments were clustered together, with the treatments Cambi VN1 and Ulti VN1 being primarily correlated with protein, AsA, MDA and NPSH. The principal component analysis promoted the separation of different treatments exclusively under high concentrations of Cu and P deficiency, suggesting that in biochemical terms, during the early stage of plant development, the genotype SMINIA 79101-3 only responded to situations of marked Cu toxicity or low availability of P, not presenting, in terms of multivariate analysis, sufficient differences to distinguish the other treatments (Figs. 11, 12).

On the other hand, SMIF 212-3 genotype showed a considerable sensibility in biochemical parameters, which resulted on the formation of four clusters in both harvests (Fig. 11B). For this genotype, differently of SMINIA 79101-3 response, MDA concentration, NPSH and SOD activity were the main parameters related to Cu toxicity and P deficiency at the first harvest. In addition, for both genotypes, at the first harvest, total protein concentration were related to treatments with suitable P levels and without high Cu in relation of biochemical parameters.

At the second harvest, not only the soil treatments relation changed, but also the relation among the biochemical parameters. In this view, for SMINIA 79101-3 genotype at the initial development stage, H_2O_2 concentration was not clustered with MDA, but near to the end of plant cycle these parameters were correlated. This pattern may be due to H_2O_2 function of signaling [68] and the insufficient action of antioxidant system to prevent biological damage, which resulted in high MDA concentrations at the end of the plant cycle. Conversely, both H_2O_2 and MDA concentrations were related with high Cu levels at the second harvest. Other interesting data change was observed in relation to total protein concentration, which turned to be more related to soil treatment with high concentrations of Cu in matured plants. This change in protein concentration is probably due to the stage of plant development, which

in plants grown in soils without or low toxicity of Cu was higher in relation to the other treatments during the first harvest, with plants with full growth and development, and gradually decreased during the plant cycle and the beginning of senescence. Already in plants grown under Cu toxicity, the initial growth was delayed in some cases even with reduced leaf expansion, which resulted in a higher protein concentration during the second harvest since these plants showed no signs of senescence or even maturation.

5. Conclusion

Vineyard soils with a long history of cupric fungicide application present toxic Cu levels to potato plants. Additionally, at higher concentrations of Cu, oxidative damage, as evidenced by increased lipid peroxidation and high H_2O_2 content, inhibited potato plant growth. However, to cope with Cu toxicity, the potato plants could activate various enzymatic and non-enzymatic antioxidants as a defense mechanism. This biochemical response as well as the Cu sensitivity of growth parameters greatly varied among the genotypes, with the P efficient genotypes being less sensitive to Cu.

Furthermore, the data suggests a similar response to P deficiency and Cu toxicity in terms of oxidative stress. In contrast, plant nutrition with high levels of P and K preserved plants from ROS damage. This study also provides evidence that antioxidants are not sufficient to prevent biological damage mediated by ROS at the highest Cu concentrations, which still result in deleterious effects.

Acknowledgments

The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação e Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa de Estado do Rio Grande do Sul (FAPERGS) for financial support.

References

[1]S. Khatun, M.B. Ali, E.J. Hahn, K.Y. Paek, Copper toxicity in *Withania somnifera*: Growth and antioxidant enzymes responses of *in vitro* grown plants, Environ. Exp. Bot. 64 (2008) 279-285.

[2]L. Wang, L. Yang, F. Yang, X. Li, Y. Song, X. Wang, X. Hu, Involvements of H_2O_2 and metallothionein in NO-mediated tomato tolerance to coppertoxicity, J. Plant Physiol. 167 (2010) 1298-1306.

[3]I. Yruela, Copper in plants, Braz. J. Plant Physiol. 17 (2005) 145-156.

[4]E.P. Seidel, A.C.S. Costa, M.C. Lana, Fitodisponibilidade de cobre e produção de matéria seca por plantas de milho em resposta à aplicação de dejetos de suínos, Rev. Bras. Ciênc. Solo, 33 (2009) 1871-1878.

[5]Thiele D, Metal-regulated transcription in eukaryotes, Nucl Acid Res 20. (1992) 1183–1191.

[6]T.F. Kagawa, B.H. Geierstanger, A.H.J. Wang, P.S. Ho, Covalent modification of guanine bases in double stranded DNA: the 1:2-AZ-DNA structure of dc(CACACG) in the presence of $CuCl_2$, J. Biol. Chem. 266 (1991) 20175–20184.

[7]E.P. Moiseeva, G.M. Almeida, G.D. Jones, M.M. Manson, Extended treatment with physiologic concentrations of dietary phytochemicals results in altered gene expression, reduced growth, and apoptosis of cancer cells, Mol. Cancer Ther. 6 (2007) 3071–3079.

[8]H.Y. Khan, H. Zubair, M.F. Ullah, A. Ahmad, S.M. Hadi, Oral administration of copper to rats leads to increased lymphocyte cellular DNA degradation by dietary polyphenols: implications for a cancer preventive mechanism, Bio.Metals. 24 (2011) 1169-1178.

[9]U.C. Gupta, S.C. Gupta, Trace element toxicity relationships to crop production and livestock and human health: implications for management, Com. Soil. Science Plant Analysis, 29 (1998) 1491-1522

[10]E. Sudo, M. Itouga, K. Yoshida-Hatanaka, Y. Ono, H. Sakakibara, Gene expression and sensitivity in response to copper stress in rice leaves, J. Exp. Bot. 59 (2008) 3465–3474.

[11]M.S.J. Warne, D. Heemsbergen, D. Stevens, M. Mclaughlin, G. Cozens, M. Whatmuff, K. Broos, G. Barry, M. Bell, D. Nash, D. Pritchard, N. Penney, Modeling the toxicity of copper and zinc salts to wheat in 14 soils, Environ. Toxicol. Chem. 27 (2008) 786-792.

[12]P. Sharma, R.S. Dubey, Lead toxicity in plants, Braz. J. Plant Physiol. 17 (2005) 35–52.

[13]S. Rossini Oliva, M.D. Mingorance, B. Vladés, E.O. Leidi, Uptake, localisation and physiological changes in response to copper excess in *Erica andevalensis*, Plant Soil, 328 (2010) 411-420.

[14]C. Parat, R. Chaussod, J. Lévêque, S. Dousset, F. Andreux, The relationship between copper accumulated in vineyard calcareous soils and soil organic matter and iron, Eur. J. Soil Sci. 53 (2002) 663-669.

[15]M. Arias, E. López, D. Fernández, B. Soto, Copper distribution and dynamics in acid vineyard soils treated with copper-based fungicides, Soil Sci. 169 (2004) 796-805.

[16]CQFSRS/SC- Comissão de Química e Fertilidade do Solo, Manual de adubação e calagem para os Estados do Rio Grande do Sul e de Santa Catarina, tenth ed., UFRGS, Porto Alegre, 2004.

[17]J.B. Jones Jr, The micronutrients, in: J.B. Jones Jr (ed), Plant nutrition manual, CRC Press, Boca Raton, 1998, p.55–76.

[18]X.Y. Guo, Y.B. Zuo, B.R. Wang, J.M. Li, Y.B. Ma, Toxicity and accumulation of copper and nickel in maize plants cropped on calcareous and acidic field soils, Plant Soil, 333 (2010) 365–373.

[19]R.X. Cao, L.Q. Lena, M. Chen, S.P. Singh, W.G. Harris, Phosphateinduced metal immobilization in a contaminated site, Environ. Pollut. 122 (2003) 19-28.

[20]Y.J. Lee, E.George, Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply, Plant Soil, 278 (2005) 361-370.

[21]C.-R. Zhao, T. Ikka, Y. Sawaki, Y. Kobayashi**,** Y. Suzuki**,** T. Hibino**,** S. Sato**,** N. Sakurai**,** D. Shibata, H. Koyama , Comparative transcriptomic characterization of aluminum, sodium chloride, cadmium and copper rhizotoxicities in *Arabidopsis thaliana*, BMC Plant Biology, 9 (2009) doi:10.1186/1471-2229-9-32

[22]L.A. Tabaldi, R. Ruppenthal, D. Cargnelutti, V.M. Morsch, L.B. Pereira, M.R.C. Schetinger, Effects of metal elements on acid phosphatase activity in cucumber (*Cucumis sativus* L.) seedlings, Environ. Exp. Bot. 59 (2007) 43-48.

[23]L.A. Tabaldi, Y.G. Castro, D. Cargnelutti, E.C. Skrebsky, J.F. Gonçalves, R. Rauber, L.V. Rossato, M.R.C. Schetinger, D.A. Bisognin, F.T. Nicoloso, Micronutrient concentration in potato clones with distinct physiological sensitivity to Al stress, C. Rural, 39 (2009), 379-385.

[24]EMBRAPA- Empresa Brasileira de Pesquisa Agropecuária, Manual de métodos de análise de solo, second ed., Centro Nacional de Pesquisa de Solos, Rio de Janeiro,1997.

[25]M.J. Tedesco, C. Gianello, C. Bissani, H. Bohnen, S.J. Volkweiss, Análise de solo, plantas e outros materiais, second ed., Universidade Federal do Rio Grande do Sul, Porto Alegre, 1995.

[26]US EPA. Method 1640: Determination of trace metal elements in ambient waters by on-line chelation preconcentration and inductive coupled plasma-mass spectrometry. U.S. Environmental Protection Agency, Office of Water, Engineering and Analysis Division, Washington, D.C. 1996.

[27]V. Chaignon, I. Sanchez-Neira, P. Herrmann, B. Jaillard, P. Hinsinger, Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area, Environmental Pollution123 (2003) 229-238.

[28]J. Murphy, J.P. Riley, A modified single solution method for determination of phosphate in natural waters, Anal. Chem. Acta, 27 (1962) 31-36.

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

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

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

[32]H. Aebi, Catalase *in vitro*, Methods Enzymol.105 (1984)121-126.

[33]S. Mishra, S. Srivastava, R.D. Tripathi, R. Govindarajan, S.V. Kuriakose, M.N.V. Prasad, Phytochelatin synthesis and resopsne of antioxidants during cadmium stress in *Bacopa monnieri* L, Plant. Physiol. Biochem. 44 (2006) 25-37.

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

[35]G.L. Ellman, Tissue sulfhydryl groups, Arch. Biochem. Biophys. 82 (1959) 70–77.

[36]M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem.72 (1976) 248-254.

[37]E. Girotto, Alterações fisiológicas e bioquímicas em plantas cultivadas em solos com acúmulo de cobre e zinco. Tese Curso de Doutorado do Programa de Pós-Graduação em Ciência do Solo. UFSM, 2010.

[38]R.N. Arteca, J.M. Arteca, Heavy-metal-induced ethylene production in *Arabidopsis thaliana*, J. Plant. Physiol.164 (2007) 1480-1488.

[39]W. Maksymiec, Signaling responses in plants to heavy metal stress, Acta Physiol. Plant, 29 (2007) 177-187.

[40]J. López-Bucio, A. Cruz-Ramírez, L. Herrera-Estrella, The role of nutrient availability in regulating root architecture, Curr. Opin. Plant Biol. 6 (2003) 280-287.

[41]G. Potters, T.P. Pasternak, Y. Guisez, K.J. Palme, M.A.K. Jansen, Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci. 12 (2007) 98-105.

[42]F.C. Lidon, M.G. Barreiro, F.S. Henriques, Interactions between biomass production and ethylene biosynthesis in copper-treated rice, J. Plant Nutr. 6 (1995) 1301-1314.

[43]L. Bailian, S.E. Mckeand, H.L. Allen, Genetic variation in nitrogen use efficiency of lobeolly pine seedlings, Forest Science, 37 (1991) 613-626.

[44]J.P. Lynch, K.M. Brown, Topsoil foraging – an architectural adaptation of plants to low phosphorus availability, Plant Soil, 237 (2001) 225-237.

[45]J.C.Fernandes, F.S. Henriques, Biochemical, physiological, and structural effects of excess copper in plants, Bot. Rev. 57 (1991) 246-273.

[46]C.P. Rooney, F.-J. Zhao, S.P. Mcgrath, Soil factors controlling the expression of copper toxicity to plants in a wide range of european soils, Environ. Toxicol. Chem. 25 (2006) 726–732.

[47]H. Lequeux, C. Hermans, S. Lutts, N.Verbruggen, Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile, Plant Physiol. Biochem. 48 (2010) 673-82.

[48]L.A. Calabrese, Toxicology rethinks its central belief-hormesis demands a reappraisal of the way risks are assessed, Nature 421 (2003) 691-692.

[49]S.L. Brown, R.L. Chaney, J.S. Angle, A.J.M. Baker, Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution, Soil. Sci. Soc. Am. J. 59 (1995) 125-133.

[50]M. Kaldorf, A.J. Kuhn, W.R. Schröder, U. Hildebrandt, H. Bothe, Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus, J. Plant Physiol. 154 (1999) 718-728.

[51]H. Marschner, Mineral Nutrition of High Plants, second ed., Academic Press, London, 2003.

[52]B.H. Adhikary, K.B. Karki, Effect of potassium on potato tuber production in acid soils of Malepatan,Pokhara, Nepal Agric. Res. J. 7(2006) 42-48.

[53]CONAMA – Conselho Nacional do Meio Ambiente. Resolução nº 420, de 28 de dezembro e 2009. http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=620/, Access january 2010.

[54]B.J. Alloway, Heavy metals in soils, Blackie Academic & Professional, London, 1995.

[55]X.-Q. Li, Molecular characterization and biotechnological improvement of the processing quality of potatoes, Can. J. Plant Sci. 88 (2008) 639-648.

[56]A. Badoni, J.S. Chauhan, Importance of potato micro tuber seed material for farmers of Uttarakhand Hills, Int. J. Sust. Agriculture, 2 (2010) 01-09.

[57]Perona, E., Bonilla I.; Mateo, P, Spatial and temporal changes in water quality in a Spanish river, Sci. Total Environ. 241(1999) 75-90.

[58]M.-L. Wu, Y.-S. Wang, Using chemometrics to evaluate anthropogenic effects in Daya Bay, China, Estuar. Coast. Shelf. Sci. 72 (2007) 732-742.

[59]W. Hou, X. Chen, G. Song, Q. Wang, C.C. Chang, Effect of copper and cadmium on heavy metal polluted water body restoration by duckweed (*Lemna minor*), *Plant Physiol. Biochem*. 2007; 45: 62–69.

[60]P.L. Gratão, A. Polle, P.J. Lea, R.A. Azevedo, Making the life of heavy metal-stressed plants a little easier, Funct. Plant. Biol. 32 (2005) 481-494.

[61]E. Islam, D. Liu, T. Li, X. Yang, X. Jin, Q. Mahmood, S. Tian, J. Li, Effect of Pb toxicity on leaves growth, physiology and ultrastructure in the two ecotypes of *Elsholtzia argyi*, J. Hazard Mater. 154 (2008) 914–926.

[62]A. Mithöfer, B. Schulze, W. Boland, Biotic and heavy metal stress response in plants: evidence for common signals, FEBS Letters, 566 (2004) 1-5.

[63]J.F. Briat, M.Lebrun, Plant responses to metal toxicity, C. R. Acad. Sci. Paris Life Sci.322 (1999) 43-54.

[64]H.P. Misra, I. Fridovich, The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase, J. Biol. Chem. 244 (1972) 6049-6055.

[65]Apel, K. and Hirt, H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55 (2004) 373–399.

[66]A.H. Kingston-Smith, H. Thomas, C.H. Foyer, Chlorophyll *a* fluorescence, enzyme and antioxidant analysis provide evidence for the operation of alternative electron sink during leaves senescence in a stay-green mutant of *Festuca pratensis*, Plant. Cell Environ. 20 (1997) 1323–1337.

[67]Y.K. Markovska, N.I. Gorinova, M.P. Nedkovska, K.M. Miteva, Cadmium-induced oxidative damage and antioxidant responses in *Brassica juncea* plants, Biol. Plant, 53 (2009) 151–154.

[68]L. Tamás, M. Simonovicová, J. Huttová, I. Mistrík, Aluminum stimulated hydrogen peroxide production of germinating barley seeds, Environ. Exp. Bot. 51 (2004) 281–288.

[69]R.K. Tewari, P. Kumar, N. Tewari, S. Srivastava, P.N. Sharma, Macronutrient deficiences and differential antioxidant responses: influence on the activity and expression of superoxide dismutase in maize, Plant Sci. 166 (2004) 687-694.

[70]R.K. Tewari, P. Kumar, P.N. Sharma, Oxidative stress and antioxidant responses in young leaves of mulberry plants grown under nitrogen, phosphorus or potassium deficiency, J. Integr. Plant Biol. 49 (2007) 313−322.

[71]M. Drążkiewicz, E. Skórzyńska-Polit, Z.Krupa, The redox state and activity of superoxide dismutase classes in *Arabidopsis thaliana* under cadmium or copper stress, Chemosphere. 67 (2007) 188–193.

[72]H. Zhang, F. Zhang, Y. Xia, G. Wang, Z. Shen, Excess copper induces production of hydrogen peroxide in the leaves of *Elsholtzia haichowensis* through apoplastic and symplastic CuZn-superoxide dismutase, J. Hazard. Mater. 178 (2010) 834-843.

[73]C.V. Attallah, E. Welchen, A.P. Martin, S.V. Spinelli, G. Bonnard, J.F. Palatnik, D.H. Gonzalez, Plants contain two SCO proteins that are differentially involved in cytochrome *c* oxidase function and copper and redox homeostasis, J. Exp. Bot. 2011, doi: 10.1093/jxb/err138.

[74]S. Verma, R.S. Dubey, Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants, Plant Sci. 164 (2003) 645-655.

[75]R.A. Fatima, M. Ahmad, Certain antioxidant enzymes of *Allium cepa* as biomarkers for the detection of toxic heavy metals in wastewater, Sci. Total. Environ. 346 (2005) 256-273.

[76]R. Mittler, Oxidative stress, antioxidants and stress tolerance, Trends Plant Sci. 7 (2002) 405-410.

[77]K. Apel, H. Hirt, Reactive oxygen species: metabolism, oxidative stress, and signal transduction, Annu. Rev. Plant. Biol. 55 (2004) 373-399.

[78]G. Queval, E. Issakidis-Bourguet, F.A. Hoeberichts, M. Vandorpe, B. Gakière, H. Vanacker, M. Miginiac-Maslow, F. Van Breusegem, G. Noctor, Conditional oxidative stress responses in the *Arabidopsis photore*- spiratory mutant cat2 demonstrate that redox state is a key modulator of daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H_2O_2-induced cell death, Plant J. 52 (2007) 640–657.

[79]S. Shigeoka, T. Ishikawa, M. Tamoi, Y. Miyagawa, T. Takeda, Y. Yabuta, K. Yoshimura, Regulation and function of ascorbate peroxidase isoenzymes, J. Exp. Bot. 53 (2002) 1305–1319.

[80]W.H. Sun, M. Duan, D.F. Shu, S. Yang, Q.W. Meng, Over-expression of StAPX in tobacco improves seed germination and increases early seedling tolerance to salinity and osmotic stresses, Plant Cell Reports, 29 (2010) 917-926.

[81]N. Smirnoff, The function and metabolism of ascorbic acid in plants, Ann. Bot. 78 (1996) 661–669.

[82]N. Horemans, C.H. Foyer, G. Potters, H. Asard, Ascorbate function and associated transport systems in plants, Plant Physiol. Biochem. 38 (2000) 531–540.

[83]M.B. Ali, N. Singh, A.M. Shohael, Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress, Plant Science, 171(2006) 147-154.

[84]C. Xiang, D.J. Oliver, Glutathione metabolic genes coordinately respond to havy metals and jasmonic acid in *Arabidopsis*, Plant Cell, 10 (1998) 1539-1550.

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

[86]G. Scarano, E. Morelli, Characterization of cadmium- and lead phytochelatin complexes formed in a marine microalga in response to metal exposure, *Biometals*, 15 (2002) 145–151

[87]A. Murphy, L. Taiz, Comparison of metallothionein cene expression and nonprotein thiols in ten *Arabidopsis* ecotypes. Correlation with copper tolerance, Plant Physiol. 109 (1995) 945-954.

COPPER EXCESS: THE EFFECTS OF PARTITIONING AND ACCUMULATION ON THE MINERAL NUTRITION OF POTATO GENOTYPES

ABSTRACT

The ecotoxicological effects of copper (Cu) are a global concern due to the intensive and long-term application of Cu-based fungicides, which may accumulate Cu in soil. Comparatively little is known about accumulation, translocation and Cu effects on other nutrients. This work aimed to study mineral nutrients distribution under Cu toxicity in potato genotypes differing in efficiency of use and response to phosphorus. Plants were grown under vineyard soils presenting different Cu levels (2.2, 5, 36.3, 67, 95.7, 270.5 and 320.70 mg kg^{-1}) during the fall and spring growing seasons. The increase of Cu concentration in plant tissues was dependent on external Cu concentrations, with the higher concentrations found in root and stolon tissues, and most of the Cu taken up by the plants was accumulated in tubers. During the fall growing season, plants pre-classified as NER (non-efficient but responsive to P) showed the highest sensitivity to Cu excess in growth and nutrition terms. The response to Cu toxicity included plants without expanded leaves and plants without tubers production, and genotypes pre-classified as ENR (P efficient and not responsive), were able to expand leaves and produce tubers in all tested soils. Tissue P and Cu concentrations showed high correlation to high Cu exposure in Cambisols, while Fe and K were more correlated in Ultisols. Our data suggests the use of middle and apex leaves tissues to investigate Cu toxicity in potato plants. This study also provides evidence of non-competitive uptake of Cu and Fe by potato plants.

Keywords: copper toxicity, heavy metals, mineral nutrition, phosphorus, *Solanum tuberosum*.

1. Introduction

Anthropogenic activities, such as mining, smelting and the intensive use of pesticides and herbicides, add copper (Cu) to the environment and might result in Cu accumulation in the soil [1]. Viniculture is an important agricultural activity that is widespread throughout the world; since the end of the 19th century, many countries have used a Bordeaux mixture ($CuSO_4$+Ca $(OH)_2$) to control the *Plasmopora viticola* fungus in vine-growing areas [2]. However, the intensive and long-term application of this fungicide has led to the accumulation of Cu in soils. Vineyard soils have been found to contain 50 to 1500 mg kg^{-1} of Cu, thus surpassing background values (5 to 30 mg kg^{-1}) by up to 300 fold [2]. Mirlean et al. [3] conducted a field investigation in southern Brazil and reported a maximum of 3200 mg kg^{-1} Cu concentration in vineyard soils. The high Cu content in soil can inhibit growth, generate reactive oxygen species (ROS), and can produce a disturbance of the biochemical and physiological processes, such as photosynthesis, enzyme activity, pigment synthesis, protein synthesis and cell division [4]. These disturbances can result in damages such as the peroxidation of membrane lipids, thus leading to ion leakage [5] and further accumulation of Cu in plant tissues. Cao and Hu [6] found an elevated Cu concentration in brown rice (15.5 mg kg^{-1}) when the concentration of Cu reached 101.2 mg kg^{-1} in a paddy soil, thus exceeding the maximum permissible concentration of Cu in grains of 10 mg kg^{-1} [7]. Similarly, Cu contamination in vineyard soils and the subsequent soil–grapevine–human transfer has become a growing public concern [8].

The susceptibility to Cu toxicity and its accumulation varies with both plant species and different types of soil [9]. Soil properties are also important factors that influence the bioavailability and toxicity of Cu [10]. Weng et al. [11] reported a decrease in Cu toxicity as the soil pH increased. Furthermore, the levels of OM (organic matter) and clay directly affect the Cu damages in plants. Rooney et al. [10] used single regressions performed between Cu toxicity threshold values and various soil properties to demonstrate that exchangeable calcium, soil cation exchange capacity, iron oxide concentration, soil pH, clay, and organic carbon content are the best predictors for Cu toxicity in barley and tomato plants. Furthermore, some elements present in the soil (such as phosphate) also affect the susceptibility of plants to Cu toxicity. The increase of P availability might precipitate heavy metals, thereby making the metals less available to plants and other soil organisms [12]. Thus, previous studies have indicated that Cu transport to the shoots is reduced in plants that are grown in contaminated soils containing an added phosphate [13]. The Pi availability is

particularly limited in highly weathered soil because of its fixation with Al and Fe oxides on the surface of clay minerals. Hence, P availability is one of the major factors limiting crop production of the potato (*Solanum tuberosum*) plant, which, with a total yield of 323 million tons, ranks fourth in world food production [7]. By stimulating the formation of tubers, thus accelerating the ripening and increased the incidence of large tubers, P is considered a conditioner for potato production [14].

Because of the low availability of this mineral nutrient, plants have evolved numerous adaptive mechanisms to acquire Pi from the soil. The P efficiency can be based on the superior ability to acquire P from the soil. With regard to the internal need for plant nutrients, efficiency is generally defined as the biomass produced per unit of nutrient applied to the soil; thus, P efficiency depends on two main components: acquisition and utilization efficiency [15]. The first component depends on the efficiencies of absorption and rooting, including increases in root proliferation, root branching, root hairs, association with vesicular-arbuscular mycorrhizae, regulation of Pi transporters and the production of exudates [16,17]. The second component depends on the efficiency of translocation and the conversion into biomass. Because a greater production of biomass dilutes the tissue metal, these components might mitigate those effects of Cu that are thought to be good for nutrition [18].

Interestingly, as P affects the Cu bioavailability and the consequent uptake, the Cu excess also affects the mineral nutrition in higher plants. Due to Cu essentiality and potential toxicity, plants have developed sophisticated mechanisms to tightly control the acquisition and distribution of copper in response to environmental fluctuations [9]. Recent studies with *Arabidopsis thaliana* allowed the characterization of the diverse families and components involved in metal uptake, such as metal-chelate reductases and plasma membrane transporters. Simultaneously, the emerging data on both intra and intercellular metal distribution, as well as on long-distance transport, contribute to the understanding of the metal homeostatic networks in plants. Some examples of plants' strategies to optimize copper utilization include the prioritization of the use of metals in essential versus dispensable processes, and the substitution of specific metalloproteins by other metal counterparts [9,18].

Our group has previously demonstrated that some potato genotypes have significant differences in P use and response (data not published). The genotypic differences in the response to P observed in potato plants and the strategies to maintain Cu homeostasis, as well as its effect on mineral nutrition, led us to hypothesize that P-efficient genotypes are less sensitive to Cu toxicity in growth and mineral parameters. Thus, the aim of this study was to characterize the distribution of mineral nutrients in potato genotypes (*Solanum tuberosum*)

affected by Cu toxicity; these potato genotypes differ in the efficiency of their use and in their response to phosphorus.

2. Materials and Methods

2.1 Plant materials and growth conditions

This study consisted of two experiments conducted with vineyard soils containing different Cu concentrations from the southern region of Brazil, and the studied soils received successive applications of Bordeaux mixture ($CuSO_4.5H_2O$ $Ca(OH)_2$). The effects of Cu toxicity on potato plant growth were evaluated in potato plants cultivated in Humic Cambisols and Ultisols in two different periods (the fall growing season and the spring growing season) with potato genotypes pre-classified according to efficiency and responsiveness to P.

During the fall growing season, the SMIE040-6RY (not efficient but responsive to P) and SMINIA793101-3 (efficient and not responsive to P) genotypes were used. Due to the contrasting response to Cu excess between the genotypes, a second experiment was proposed. The second experiment consisted of the SMIF212-3 and SMINIA793101 genotypes (both efficient and not responsive to P) to verify the differences in Cu toxicity between two genotypes with the same P response.

For both experiments, four soils were collected from vineyards located in Serra Gaúcha, and three soils were collected from vineyards in Campanha Gaúcha. Both of these regions are located in Rio Grande do Sul (RS) State. The concentrations of P, Cu, K, Zn and Fe of these soils are presented at table 1.

Serra Gaúcha soils were collected from vineyards at Embrapa Uva and Vinho experimental areas in Bento Gonçalves (RS), and these soils were classified as Humic Cambisols. The Cu in these soils was extracted by 0.01 mol L^{-1} EDTA-Na_2/1.0 mol L^{-1} ammonium acetate. Cu levels in the soils from this region were found to be 5.5, 95.7, 270.5 and 320.7 mg kg^{-1}, and these soils were named Cambi C, Cambi VN1, Cambi VN2 and Cambi VN3, respectively (VN indicates that the soils were collected from vineyards). For purposes of comparison, Cambi C was used as a control because it was collected under a native forest. Cambi C+PK was created by correcting the levels of P and K. In this treatment, P (55 mg kg^{-1}) and K (50 mg kg^{-1}) were added based on the results of the soil analysis and according to CQFS-RS/SC [19].

Soils collected from Campanha Gaúcha were from commercial vineyards located on a property in the municipality of Santana do Livramento (RS) and were classified as Ultisols. The Cu in these soils was extracted by 0.01 mol L^{-1} EDTA-Na_2/1.0 mol L^{-1} ammonium acetate. Cu levels in the soils from this region were found to be 2.2, 36.3 and 67.2 mg kg^{-1}, and these soils were named Ulti, Ulti VN1 and Ulti VN2, respectively. Ulti was used as a control because it was collected from a seedling production area with a history of no application of cupric fungicides.

For the assays, 3 kg of each soil was air-dried and placed in pots with a capacity of 5 kg. In each pot, one tuber with a diameter of 2 to 3 cm and an average weight of 8.4 g was sown. Throughout cultivation, the soil humidity was maintained at 80% of field capacity, which was determined with samples deformed in a tension table (1 MPa). Irrigation was performed daily with distilled water to replenish evapotranspired water, which was calculated by weighing the pots daily. Throughout the cultivation of potatoes, two applications of N totaling 70 mg kg^{-1} were applied to the soil. The experimental design was completely randomized with six replicates per treatment.

The experiments were conducted in a greenhouse from March to May (fall growing season) and from September to November (spring growing season) where potato cultivation was conducted in soils with increasing levels of Cu and similar pH values in water (raise the pH 6.0) and exchangeable K levels.

2.2 Cu concentration in plant tissue

Cu concentration was determined in the roots, stolons, tubers, and shoots. Dried plant tissues (between 0.01 and 0.25 g) were ground and digested with 5 ml of concentrated HNO_3. Sample digestion was performed in an open digestion system using a heating block Velp Scientific (Milano Italy) at 130ºC for 2 h. Plastic caps were fitted to the vessels to prevent losses by volatilization. The Cu 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.

2.3 Soil analysis

The soils were analyzed for particle size distribution of the soil constituents according to the pipette method [20]. The determination of pH was performed with water in a 1:1 ratio

according to the methodology proposed by Tedesco et al. [21]. The content of soil organic matter (OM) was analyzed by wet oxidation using potassium dichromate in a sulfuric acid medium (0.4 N), and the determination of OM was made by titration with 0.1 N ammonium ferrous sulfate according to Embrapa [20]. The total contents of Cu and Zn in the soil samples were extracted with the use of hydrogen peroxide (H_2O_2), nitric acid (HNO_3) and hydrochloric acid (HCl) according to method No. 3050b EPA [22]. The extraction of available Cu (CuEDTA) and Zn (ZnEDTA) was performed using 0.01 mol L^{-1} Na_2-EDTA/1.0 mol L^{-1} ammonium acetate with the pH level adjusted to 7.0 according to Chaignon et al. [2]. Both levels of Cu and Zn were measured using an atomic absorption spectrophotometer (GBC brand, model 932 AA).

Table 1. Chemical and physical properties of vineyard soils with application of Cu-based fungicides.

Parameters	Cambi C+PK	Cambi C	Cambi Vn1	Cambi Vn2	Cambi Vn3	Ulti	Ulti Vn1	Ulti Vn2
Sand, g kg^{-1}	34.6	346.0	298.0	345.0	320.0	675.0	661.0	705.0
Silt, g kg^{-1}	391.0	391.0	373.0	353.0	370.0	260.0	264.0	205.0
Clay, g kg^{-1}	263.0	263.0	329.0	302.0	310.0	65.0	75.0	90.0
pH H_2O	5.8	5.8	5.2	5.3	5.3	5.5	5.3	5.2
OM, g kg^{-1}	34.3	33.9	27.3	37.9	35.9	11.2	12.1	9.2
Al, cmol c kg-1	0.0	0.0	0.05	0.02	0.03	0.0	0.06	0.03
H+Al, cmol c kg-1	2.6	2.8	4.2	4.5	3.8	2.3	3.2	2.9
CECef, cmol c kg-1	8.8	8.6	6.0	8.0	8.0	2.4	2.2	1.6
CEC7, cmol c kg-1	11.5	11.4	10.2	12.5	11.8	4.6	5.4	4.4
V. %	76.0	75.4	58.9	64.4	67.4	50.6	40.2	34.9
CuEDTA. mg kg-1	5.0	5,0	95.7	270.5	320.7	2.2	36.3	67.0
Cutot. mg kg-1	29.0	29.8	183.0	408.3	490.3	11.3	51.6	73.1
ZnEDTA. mg kg-1	2.0	2.0	14.0	18.0	21.0	2.0	7.0	10.0
Zntot. mg kg-1	60.6	59.2	81.0	84.6	81.8	8.2	10.8	16.4
Fe oxalate (mg kg^{-1})	101.0	102.0	101.0	110.0	114.0	15.0	16.0	12.0
Mn mg kg^{-1}	280.0	270.0	210.0	210.0	180.0	90.0	85.0	87.0
P. mg kg-1	18.2	4.8	37.0	19.0	27.0	47.1	75.0	60.0
K. mg kg-1	130.0	110.9	260.0	100.0	110.0	129.0	100.0	110.0
Ca. cmolc kg-1	4.7	4.6	3.7	5.9	5.6	1.4	1.3	1.0
Mg. cmolc kg-1	3.7	3.7	1.6	1.9	2.1	0.6	0.6	0.4

The extraction of available P and exchangeable K was performed with the Mehlich 1 solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4). The concentration of P extracted by the Mehlich 1 solution was determined according to Murphy and Riley [23]. The concentration of exchangeable K was determined by flame emission spectroscopy. The exchangeable cations (Ca, Mg and Al^{3+}) were extracted with a 1 mol L^{-1} KCl solution [20]. The concentration of Al^{3+} was determined by an acid-base titration with a 0.0125 mol L^{-1} NaOH solution, and the concentrations of Ca and Mg were determined by atomic absorption spectroscopy (AAS) [21].

2.4 Biomass, Cu and mineral nutrient concentration determination

At the end of the cycle, the plants were collected and divided into shoot, tubers, root and stolon. In the first experiment, carried out during the fall growing season, the shoot part was subdivided into leaves and stems. During the spring growing season, the leaves and stems were subdivided as follows into three parts according to the position of the leaves on the stem: the apex part (from the 1st to 3rd leaves), basal part (the last two leaves) and middle part (all leaves found between the apex and basal parts). Subsequently, the plants were gently washed with distilled water. They were then oven-dried at 65 °C to a constant mass for the determination of biomass, as well as for the Cu and macro and micronutrient concentration. The dried plant tissues (0.01–0.1 g) were ground and digested with 4 ml of concentrated HNO_3. The sample decomposition was carried out using a heating block from Velp Scientifica (Milano, Italy). Heating was set at 130 °C for 2 h. Plastic caps were fitted to the vessels to prevent any losses by volatilization. The Cu concentration, as well as the Ca, K, Mg, P, Fe, Mn and Zn concentrations were determined by an 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.

2.5 Statistical analysis

The experiments were performed using a randomized design. The analyses of variance were computed on statistically significant differences determined based on appropriate F-tests. Results were presented as means ± SD of at least three independent replicates. The mean differences were compared using Tukey's test ($P<0.05$).

2.6 Multivariate analysis

Principal component analysis (PCA) was used to evaluate the relationship among variables and possible patterns in the data distribution obtained from different seasons during the potato cycle.

Initially, data from experiments cultivated in different seasons were transformed by ranking on a scale ranging from 1 to 10. The average value of the evaluated parameters corresponded to 5 on the scale with 1 being the lowest assessed value and 10 being the highest

assessed value. The average data were analyzed using CANOCO® statistical software (version 4.5, Fa. Biometris). The data matrix was submitted to PCA analysis to compound variables, thus providing information about the factors responsible for these patterns.

3. Results

3.1 Biomass, Cu concentration and content

The contamination of Cu in Ulti soils resulted in growth inhibition; there was a reduction of the shoot and the produced tubers dry weight for the SMIE040-6RY and SMIF 212-3 genotypes (Table 2). In contrast, the shoot dry weight of the SMINIA 793101-3 genotype was not affected by increased Cu concentrations during the spring growing season (Table 2). In addition, the Cambi soils with the highest levels of Cu (Cambi VN2 and Cambi VN3) reduced the shoot and produced tubers dry weights when compared with Cambi C+PK and Cambi C without fertilization in all tested genotypes (Table 2). Moreover, a significant difference was found between Cambi C and Cambi C+PK in their shoot dry weight and produced tubers. Interestingly, during the spring growing season, a smaller difference in shoot dry weight was observed in the plants cultivated in Cambi soils with or without PK fertilization; however, these plants had a larger difference in produced tubers (Table 2). Overall, compared with the other genotypes, the SMINIA 793101-3 genotype maintained higher values in shoot parameters, and it also produced tubers in Cu-contaminated soils in both of the growing seasons. Conversely, in relation to the other genotypes, the root and stolon dry weights were also higher in the SMINIA 793101-3 plants.

For the SMIE040-6RY genotypes, the response of stolon dry weight to Cu toxicity was negative in both Ulti VN1 and Ulti VN2. However, for the genotypes grown during the spring growing season (efficient but not responsive to P (ENR)) and for the SMINIA 793101-3 plants cultivated during the fall, in general there were no significant differences in the stolon fresh weight of control and the contaminated Ulti soils (Table 2). In the Cambi soils, the plants grown during the fall demonstrated no significant difference in stolon dry weight among the controls without PK addition and the other treatments with higher Cu concentrations (Cambi VN2 and Cambi VN3). In contrast, during the spring season, the stolon production for the SMINIA793101-3 and SMIF 212-3 genotypes was significantly reduced in Cambi VN3.

Table 2. Effect of increasing Cu level on shoot, tubers, stolon and root dry weight in two potato genotypes grown in vineyards soils.

	GENOTYPE	Soil	Shoot	Seed Tuber	Produced tuber	Stolon	Root
Fall growing season	SMIE040-6RY	Cambi C+PK	2.68 ± 0.28 aB	2.05 ± 0.23 bB	16.40 ± 5.01 aB	0.40 ± 0.11 aA	0.15 ± 0.01 cA
	SMIE040-6RY	Cambi C	1.10 ± 0.12 cB	1.54 ± 0.07 cB	8.72 ± 1.59 bA	0.18 ± 0.04 bB	0.09 ± 0.02 dB
	SMIE040-6RY	Cambi VN1	2.88 ± 0.59 aB	1.40 ± 0.54 cB	22.04 ± 1.57 aA	0.36 ± 0.14 abA	0.25 ± 0.02 aA
	SMIE040-6RY	Cambi VN2	0.56 ± 0.03 dB	2.27 ± 0.02 abA	1.61 ± 1.64 cB	0.18 ± 0.02 bB	0.19 ± 0.01 bA
	SMIE040-6RY	Cambi VN3	0.44 ± 0.02 eB	2.82 ± 0.03 aA	0.09 ± 0.20 cB	0.20 ± 0.04 bB	0.12 ± 0.02 cdB
	SMIE040-6RY	Ulti	2.32 ± 0.00 aA	1.03 ± 0.05 cB	8.92 ± 1.13 bB	0.46 ± 0.09 aA	0.10 ± 0.03 dB
	SMIE040-6RY	Ulti VN1	1.55 ± 0.05 bB	1.01 ± 0.00 cB	7.50 ± 0.83 bA	0.17 ± 0.08 bB	0.22 ± 0.03 aA
	SMIE040-6RY	Ulti VN2	0.60 ± 0.35 cdB	1.90 ± 0.30 abA	1.20 ± 0.70 cB	0.12 ± 0.04 cB	0.12 ± 0.02 cdB
	SMINIA793101-3	Cambi C+PK	5.60 ± 0.40 aA	3.20 ± 0.35 abA	24.81 ± 3.73 aA	1.17 ± 0.29 aA	0.20 ± 0.04 abA
	SMINIA793101-3	Cambi C	2.08 ± 0.19 cdA	4.11 ± 0.14 aA	11.07 ± 2.40 bcA	0.27 ± 0.02 cA	0.18 ± 0.04 cA
	SMINIA793101-3	Cambi VN1	4.00 ± 0.37 bA	2.47 ± 0.32 bA	23.48 ± 2.69 aA	0.63 ± 0.07 bA	0.14 ± 0.03 bcB
	SMINIA793101-3	Cambi VN2	1.75 ± 0.47 eA	1.01 ± 0.42 cB	5.21 ± 0.77 dA	0.31 ± 0.11 cA	0.16 ± 0.04 bcA
	SMINIA793101-3	Cambi VN3	1.60 ± 0.17 eA	2.15 ± 0.12 bB	6.02 ± 1.20 dA	0.45 ± 0.10 bcA	0.25 ± 0.04 aA
	SMINIA793101-3	Ulti	2.29 ± 0.15 cA	3.35 ± 0.10 abA	12.46 ± 0.67 bA	0.25 ± 0.03 cA	0.18 ± 0.03 bcA
	SMINIA793101-3	Ulti VN1	2.05 ± 0.06 dA	2.08 ± 0.01 bA	9.31 ± 2.00 cA	0.24 ± 0.17 cA	0.13 ± 0.04 cA
	SMINIA793101-3	Ulti VN2	1.28 ± 0.08 eA	2.08 ± 0.03 bA	4.92 ± 1.34 dA	0.31 ± 0.12 cA	0.19 ± 0.01 bA
Spring growing season	SMIF212-3	Cambi C+PK	2.56 ± 0.28 aB	6.87 ± 0.23 abA	24.50 ± 4.39 aA	0.45 ± 0.23 abA	0.58 ± 0.13 aA
	SMIF212-3	Cambi C	1.10 ± 0.59 cB	6.21 ± 0.07 bA	7.82 ± 1.59 cdA	0.32 ± 0.02 bA	0.19 ± 0.02 dB
	SMIF212-3	Cambi VN1	2.70 ± 0.59 aB	8.91 ± 0.54 aA	24.04 ± 1.57 aB	0.14 ± 0.01 cB	0.31 ± 0.02 bB
	SMIF212-3	Cambi VN2	0.71 ± 0.16 dB	4.96 ± 0.11 bcB	4.85 ± 1.93 dA	0.08 ± 0.02 dB	0.23 ± 0.01 cB
	SMIF212-3	Cambi VN3	0.93 ± 0.02 dB	3.72 ± 0.03 cB	5.12 ± 1.19 dA	0.09 ± 0.03 dB	0.15 ± 0.02 dB
	SMIF212-3	Ulti	2.73 ± 0.80 aA	6.41 ± 0.75 bA	11.45 ± 1.44 bA	0.50 ± 0.08 aA	0.41 ± 0.06 abA
	SMIF212-3	Ulti VN1	1.92 ± 0.19 bA	5.16 ± 0.14 bcA	9.13 ± 1.23 bcA	0.19 ± 0.05 bcA	0.17 ± 0.04 dB
	SMIF212-3	Ulti VN2	1.20 ± 0.20 cB	5.99 ± 0.15 bA	5.37 ± 1.19 dA	0.39 ± 0.08 abA	0.32 ± 0.04 bB
	SMINIA793101-3	Cambi C+PK	3.65 ± 0.40 aA	5.51 ± 0.35 bA	27.46 ± 3.17 aA	0.27 ± 0.02 dB	0.50 ± 0.08 bA
	SMINIA793101-3	Cambi C	2.32 ± 1.04 abA	6.50 ± 0.99 abA	9.87 ± 2.40 cA	0.72 ± 0.17 aA	0.70 ± 0.10 abA
	SMINIA793101-3	Cambi VN1	3.24 ± 0.87 abA	8.97 ± 0.82 aA	28.34 ± 1.75 aA	0.42 ± 0.02 bcA	0.45 ± 0.07 bcA
	SMINIA793101-3	Cambi VN2	2.03 ± 0.47 bA	6.30 ± 0.42 abA	6.11 ± 0.77 dA	1.15 ± 0.18 aA	0.33 ± 0.06 cA
	SMINIA793101-3	Cambi VN3	1.18 ± 0.21 cA	5.75 ± 0.16 bA	6.74 ± 0.78 dA	0.51 ± 0.05 bA	0.35 ± 0.03 cA
	SMINIA793101-3	Ulti	2.35 ± 0.39 bA	8.32 ± 0.34 aA	13.66 ± 0.67 bA	0.17 ± 0.06 eB	0.33 ± 0.05 cA
	SMINIA793101-3	Ulti VN1	2.05 ± 0.22 bA	5.95 ± 0.17 bA	9.40 ± 2.00 cA	0.38 ± 0.05 cA	0.49 ± 0.12 bA
	SMINIA793101-3	Ulti VN2	2.29 ± 0.34 bA	7.55 ± 0.29 abA	6.12 ± 1.34 dA	0.20 ± 0.09 deA	0.85 ± 0.04 aA

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Unlike the response observed in shoot dry weight, the root dry weight was not reduced with a Cu increment in the Ulti soils. Additionally, when compared with the Ulti control during the spring growing season, the root fresh weight was increased in the SMINIA793101-3 plants grown in Ulti VN2. In comparison, the root dry weight in the Cambi soils with a high Cu content (Cambi VN2 and Cambi VN3) only decreased significantly in relation to the Cambi C treatment grown during the spring (Table 2). Moreover, compared with the fall growing season, a greater root dry weight was observed during the spring.

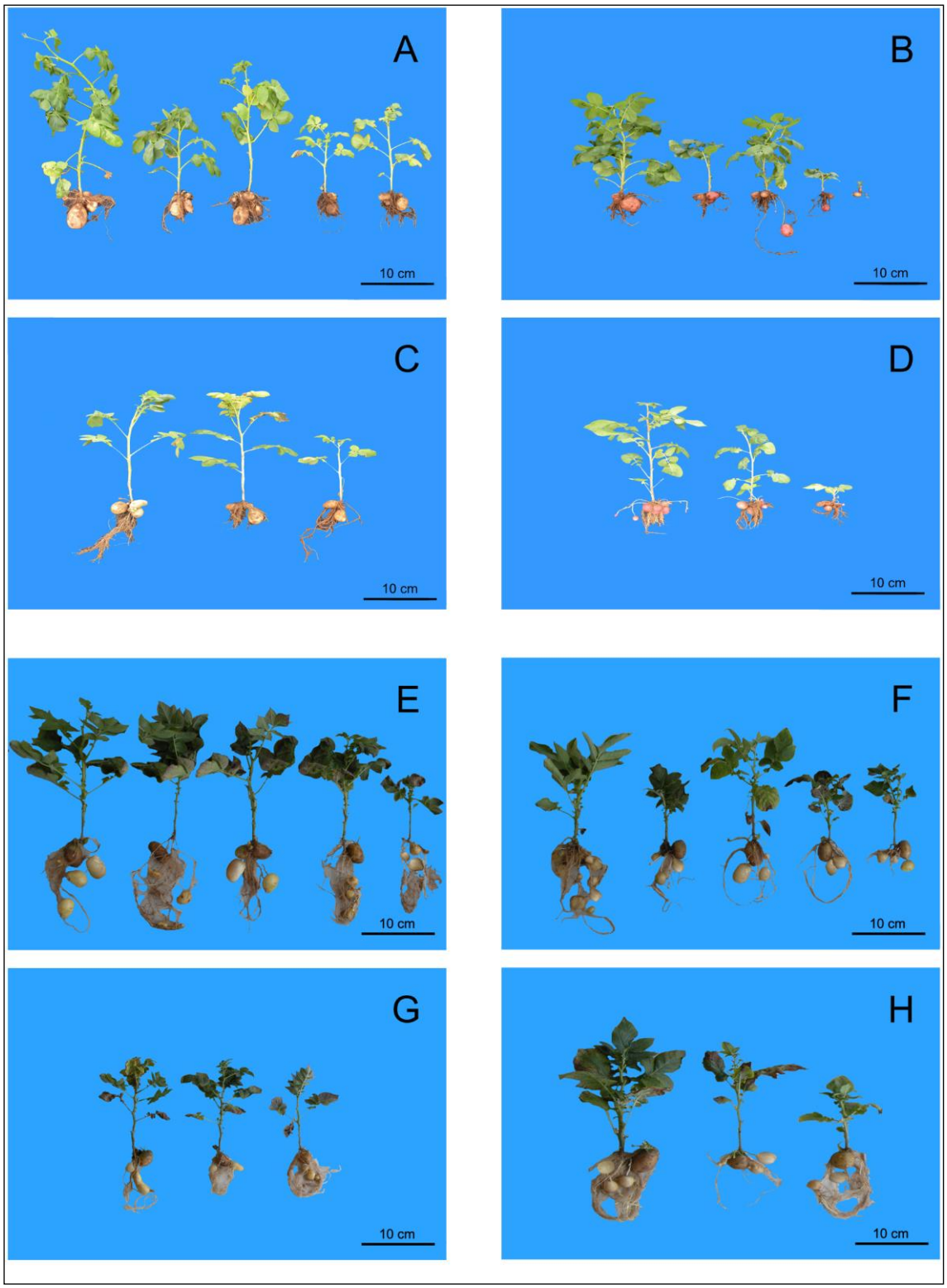

Fig. 1. Potato genotypes grown in vineyard soils during the fall and spring season. A and C SMINIA793101-3 genotype efficient e not responsive (ENR) grown in Cambi and Ulti soils respectively during the fall growing season; B and D, SMIE040-6RY genotype non-efficient and responsive (NER) grown in Cambi and Ulti soils respectively during the fall growing season; E and G, ENR SMINIA793101-3 genotype grown in Cambi and Ulti soils respectively during the spring growing season; F and H, SMIF 212-3 genotype ENR grown in Cambi and Ulti soils respectively during the spring growing season. Cambi soils followed the sequence: Cambi C+PK, Cambi C, Cambi VN1, Cambi VN2, Cambi VN3, from right to left. The Ulti soils followed the sequence: Ulti, Ulti VN1, Ulti VN2, from right to left.

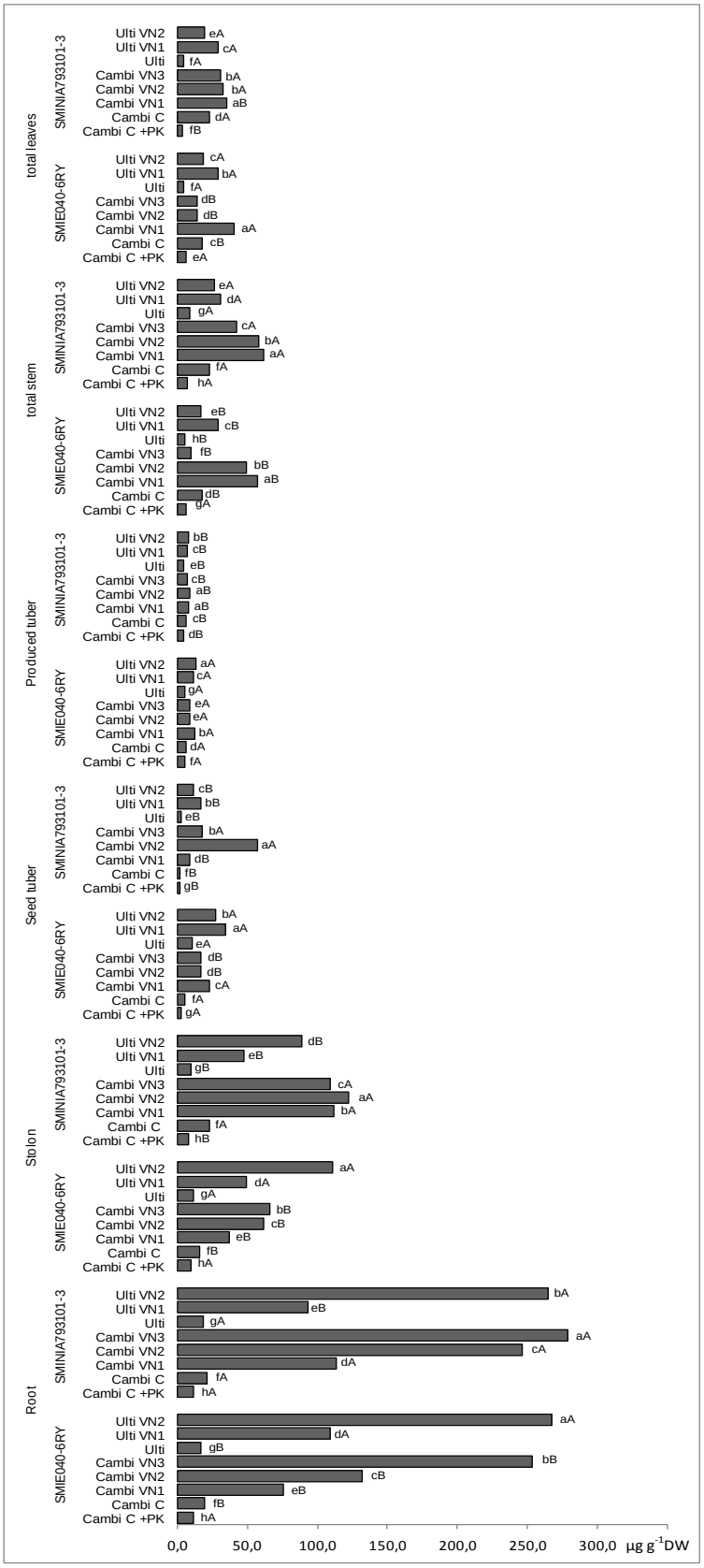

Fig. 2. Tissue Cu concentration in potato plants grown during fall growing season. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and harvesting ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and harvesting ($p < 0.05$).

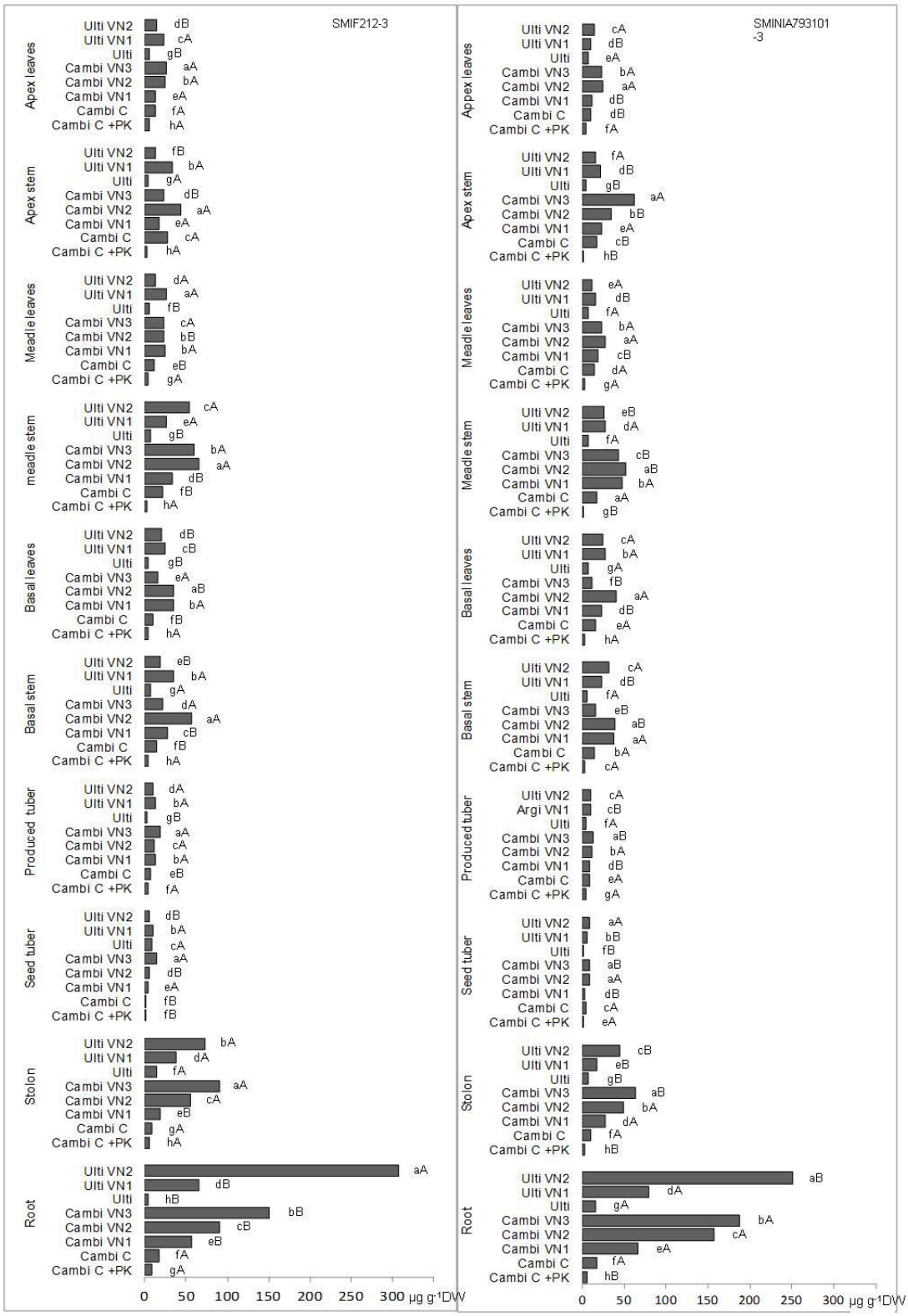

Fig. 3. Tissue Cu concentration in potato plants, grown during the spring growing season. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and harvesting ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and collect ($p < 0.05$).

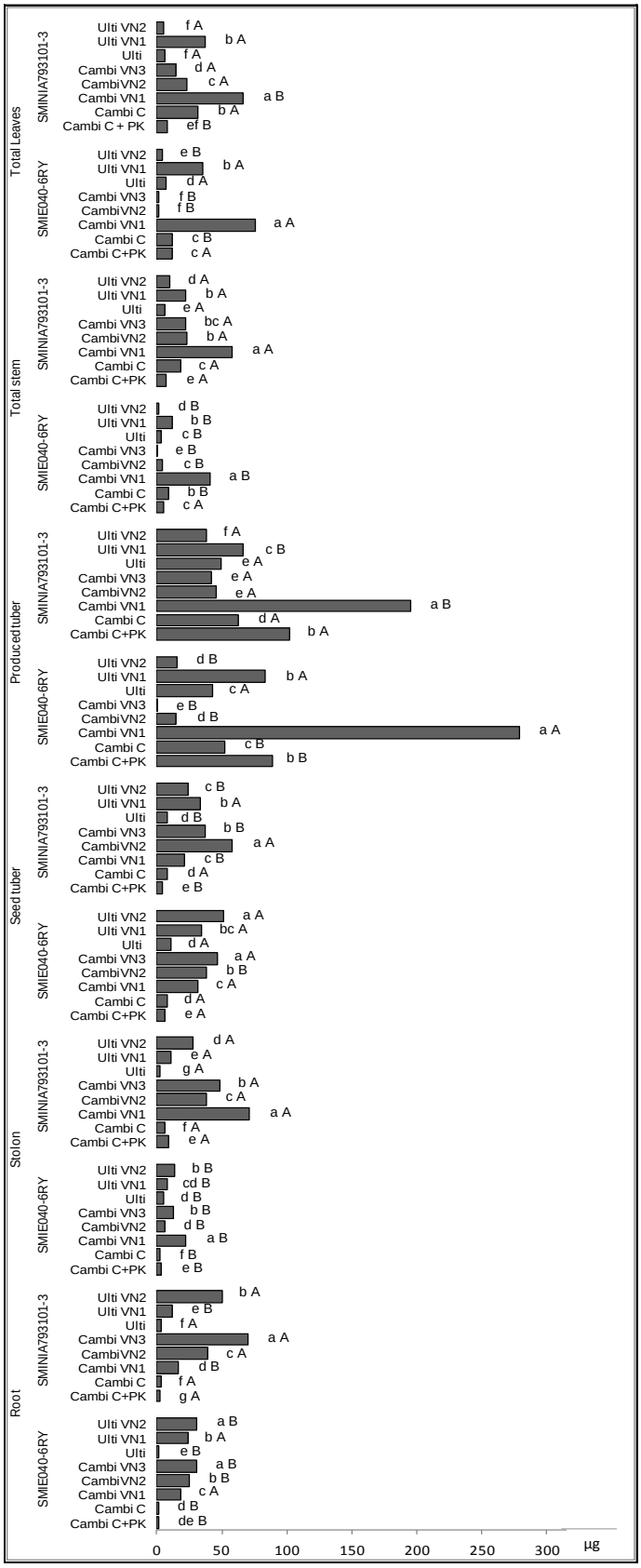

Fig. 4. Tisue Cu content in potato plants, grown during the fall growing season. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and harvesting ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and harvesting ($p < 0.05$).

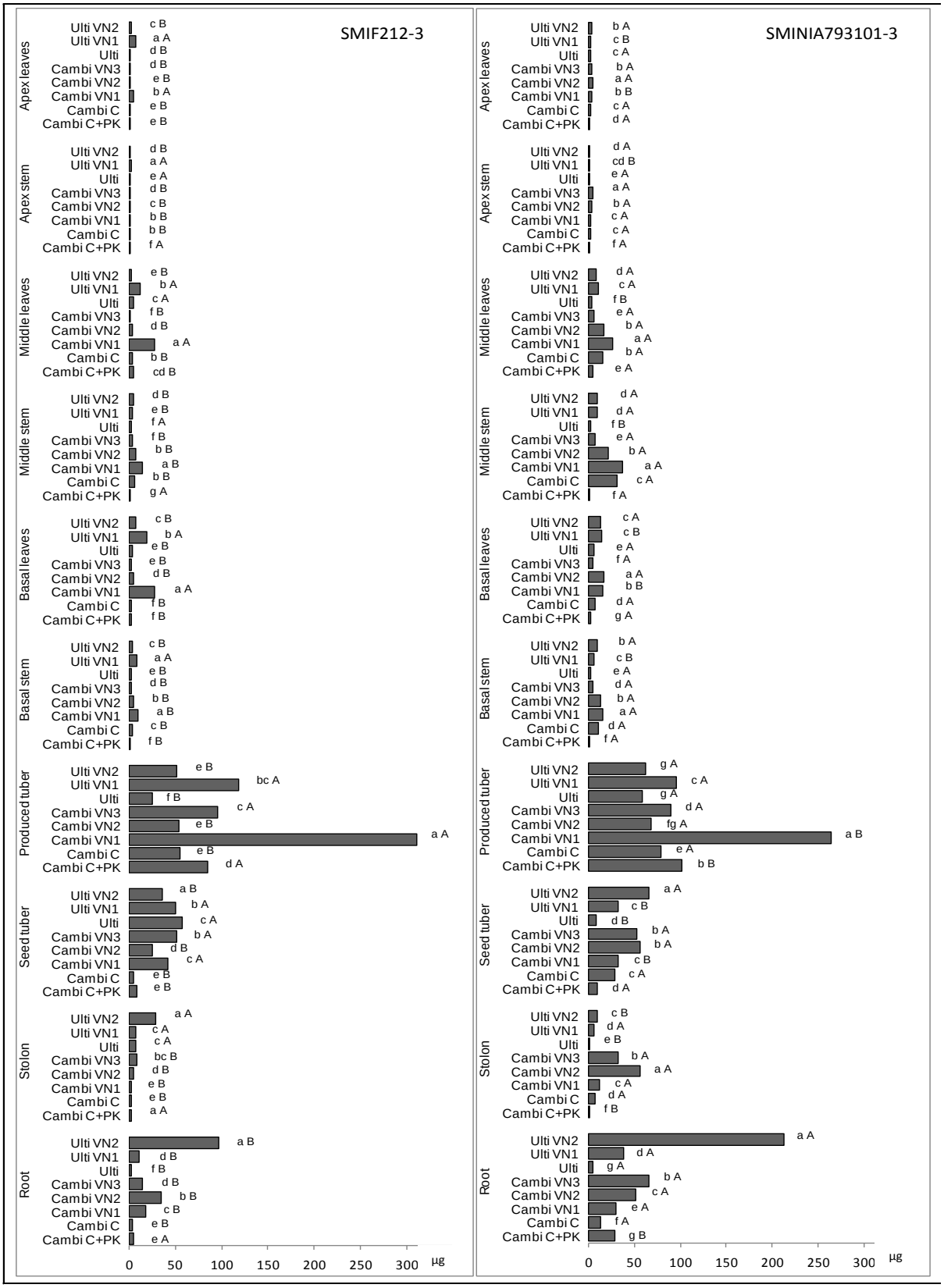

Fig. 5. Tissue Cu content in potato plants, grown during the spring growing season. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and harvesting ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and harvesting ($p < 0.05$).

When compared with the fall, the root production of the SMINIA793101-3 plants increased by 4.47 and 3.88 fold in the Ulti VN2 and Cambi C soils, respectively, during the spring.

The pattern of response to Cu toxicity in tuber dry weight was similar between the genotypes and growing seasons (Table 2). Thus, there were visual differences between the Cambi and Ulti soils with an excess of Cu (Fig 1). During the fall, SMIE040-6RY (not efficient but responsive to P (NER)) exhibited a critical response to Cu toxicity in Cambi soils VN2 and VN3; this toxicity produced plants without expanded leaves. The genotype showed thickening of the stem, thus giving a similar appearance to the bulb with violet staining, where there were dark green leaves in these same treatments. Interestingly, a large difference in the weight variation of the seed tuber was observed among the genotypes and growing seasons (Table 2). During the fall season, the SMINIA793101-3 plants absorbed much of the seed tuber in soils with high Cu content, whereas the seed tuber weight of the SMIE040-6RY plants was increased in the same soils. Conversely, in the soils with lower Cu concentrations, the SMIE040-6RY plants had the opposite response, which resulted in an absorption of the seed tuber. By the spring season, when compared with the initial seed tuber weight, there was an increase in the seed tuber weight regardless of the soil and genotype, with the lowest values being found in the Cambi VN2 and Cambi VN3 soils (Table 2).

In both growing seasons (fall and spring), the Cu concentration in roots, stolons and tubers (seed tubers and produced tubers) increased with the Cu contamination level (Figs. 2, 3, 4, 5), and the higher concentrations were found in the root tissue. However, most of the Cu absorbed by the plants was accumulated in the tubers (Figs. 4, 5). Remarkably, the root and stolon Cu concentrations were greater in Ulti VN2, Cambi VN2 and Cambi VN3 regardless of tested genotype and growing season, with similar values among these treatments. Overall, when compared with the other genotypes, the SMINIA793101-3 plants had the highest values of Cu concentration in their roots. However, when compared with the SMIE040-6RY and SMIF 212-3 plants, this genotype had lower values in the stolon tissue when it was grown in Ulti soils (Figs. 2, 3).

Compared with Cambi C, the concentrations of Cu in all of the tested tissues were lower in plants grown in Cambi C+PK (Figs 2, 3). Remarkably, during the fall growing season, the genotypes ENR (SMINIA793101-3) and NER (SMIE040-6RY) had a higher Cu concentration in seed tubers when compared with the produced tubers. Thus, plants grown in Cambi VN3 soil increased by 82% and 145% of the Cu concentration from seed tuber to produced tuber in SMIE040-6RY and SMINIA793101-3, respectively. However, in relation

to the seed tubers, both genotypes showed a greater concentration in produced tubers during the spring. During the spring, an increment of 130% and 41% was observed in SMIF 212-3 and SMINIA793101-3 plants grown in Cambi VN3, respectively. The content of the Cu in produced tubers was also higher during spring and reached values close to 300 mg in Cambi VN1. During the fall, the values were close to 200 mg when using the same treatment. During both seasons, the Ulti soil indicated lower values of Cu content; overall, the contents were lower than in Cambi C+PK (Figs. 4, 5).

The shoot tissues (leaves and stems) had higher Cu concentrations when compared with seed and produced tubers; however, the shoot tissues also had a remarkably lower Cu content. Additionally, the Cu content in the shoot tissues decreased from the basal to apex parts and demonstrated, in general, a greater accumulation in the leaves (Figs. 4, 5).

3.2 Ca, K, Mg and P tissue concentration

In the plants grown in Ulti soils, the root and stolon Ca concentrations were increased at Cu levels exceeding the control (Table 3). However, compared with the Cambi controls in SMIF 212-3, the Ca concentrations were reduced in Cambi VN3. Moreover, in relation to SMINIA793101-3 plants, the root, stolon, seed and produced tuber Ca concentrations were greater in the SMIF 212-3 and SMIE040-6RY grown in Ulti soils (Tables 3, 4). In general, the ENR plants increased their seed tuber Ca concentration with increasing Cu, whereas the NER plants decreased their seed tuber Ca concentration. However, in the spring the produced tuber Ca concentration of the ENR plants exhibited a distinct response. Compared with the Cambi controls, both genotypes exhibited a negative response to Cu in the Ulti soils with a Cu increment in Cambi VN3. Conversely, in the Ulti soils SMIF 212-3 decreased its produced tuber Ca concentration, whereas SMINIA793101-3 experienced an increase with the Cu increment (Table 4).

Interestingly, the shoot parts, stems and leaves had distinct responses to Cu contamination in the Ca concentration. During the fall season, the ENR plants increased their total stem Ca concentration in both of the soil types with a Cu increment; they also increased their Ca concentration in total leaves in the Cambi soils, with no significant difference in the Ulti soils. When grown in Cambi soils, the NER plants increased their total stem Ca concentration with a Cu increment while simultaneously decreasing their total leaves Ca concentration. The opposite response occurred in the Ulti soils. Compared with the Cambi controls, the ENR genotype, SMINIA793101-3, showed a reduction during the spring in the apex stem and leaves Ca concentrations followed by an increment in both the basal stem and

leaves of the Cambi VN3. The other ENR genotype, SMIF 212-3, had an overall increase in apex stem and leaves ca concentration; additionally, as Cu increased there was an increase in the middle stem and leaves Ca concentration in the Ulti soils. However, except in basal leaves, in the Cambi soils the Cu increment resulted in leaves Ca concentration decreases and stem increases.

With increasing Cu in Ulti soils, the tissue K concentration, except for the produced tubers of SMIF 212-3 plants, demonstrated a continuous increase in the root, stolon, seed and produced tubers. Notably, during the fall season the ENR plants showed a greater K concentration in the root and stolon tissues compared with the NER plants. However, during the spring a significant difference between the ENR plants occurred. Contrary to the results observed in the fall, during the spring season the SMINIA793101-3 plants showed the highest values of K concentration in the roots and stolons of plants grown in Ulti soils. Moreover, in contrast to the fall, during the spring the stolon K concentration in the SMINIA793101-3 genotype increased by 4 fold in the Ulti VN2. In the Cambi soils, the plants grown under addition of PK fertilization showed a greater K concentration when compared with Cambi C in the tested tissues. When comparing the fertilized control (Cambi C+PK) to the soil with a higher Cu content in Cambi soils (Cambi VN3), with the exception of root tissue harvested in the spring, Cu contamination decreased the root and stolon K concentration. A similar response was observed in the seed and produced tubers, as well as for the leaves and stem. Interestingly, when compared with the Cambi controls, the K concentration in the basal stem increased in both ENR genotypes grown in Cambi VN3. However, the opposite response occurred in the basal leaves K concentration.

In the SMINIA793101-3 plants grown in the Ulti soils, the Mg and P root, stolon, stem and leaves concentrations in both growing seasons were similarly affected by Cu contamination (Table 3, 5, 6). During the fall season, the ENR plants experienced decreased Mg and P concentrations in their root, stem and leaf tissues. Moreover, compared with the ENR plants, the NER plants showed greater values of these nutrients under high Cu levels. However, as the Cu increased the SMIF 212-3 and SMIE040-6RY plants grown in Ulti soils had increases in the Mg and P concentrations in the root and stolon. Overall, the SMIF 212-3 and SMIE040-6RY plants increased their P concentration in their seed, produced tubers, stems and leaves in response to the Cu contamination in the Ulti and Cambi soils. In contrast, the SMINIA793101-3 plants drastically reduced their P concentration in produced tubers, total stems, apex and basal stems and leaves in the Ulti soils, as well as in the stolon, root and total leaves in the Cambi soils. Conversely, in both seasons SMINIA793101-3 had lower P

values in the Cambi C+PK compared with the other genotypes.

Table 3. Root and stolon macronutrient concentrations of potato genotypes grown in vineyard soils during fall and spring growing seasons.

			Ca (µg/g)		K (µg/g)		Mg (µg/g)		P (µg/g)	
			Root							
Fall growing season	SMIE040-6RY	Cambi C +PK	9963 ±	29.74 eA	20682 ±	400.19 aA	6820 ±	61.99 aA	1300 ±	7.43 bA
	SMIE040-6RY	Cambi C	8159 ±	89.17 fA	10250 ±	74.68 bA	5440 ±	19.42 bB	1041 ±	10.57 eA
	SMIE040-6RY	Cambi VN1	6362 ±	38.89 gB	9848 ±	60.36 cB	2679 ±	18.16 gB	1025 ±	2.47 fA
	SMIE040-6RY	Cambi VN2	12607 ±	110.46 cB	9340 ±	207.41 dA	3288 ±	23.96 fB	1146 ±	13.34 dA
	SMIE040-6RY	Cambi VN3	16751 ±	58.22 bA	9854 ±	139.51 cA	4486 ±	11.75 cA	1528 ±	8.56 aA
	SMIE040-6RY	Ulti	11861 ±	50.12 dA	7350 ±	73.56 eA	3519 ±	21.25 eB	1025 ±	3.31 fA
	SMIE040-6RY	Ulti VN1	11701 ±	82.77 dA	3977 ±	43.20 fB	3621 ±	82.02 eA	921 ±	15.02 gA
	SMIE040-6RY	Ulti VN2	21800 ±	153.81 aA	9987 ±	89.81 cA	3984 ±	26.31 dA	1231 ±	17.24 cA
	SMINIA793101-3	Cambi C +PK	7347 ±	46.30 gB	9366 ±	196.25 bB	5911 ±	32.29 bB	854 ±	3.84 bB
	SMINIA793101-3	Cambi C	8666 ±	18.86 fA	3530 ±	20.82 gB	6389 ±	1.66 aA	891 ±	1.95 aB
	SMINIA793101-3	Cambi VN1	8343 ±	48.57 fA	10203 ±	198.19 aA	3128 ±	10.45 fA	811 ±	21.85 cB
	SMINIA793101-3	Cambi VN2	12990 ±	58.41 bA	3802 ±	71.17 fB	3528 ±	11.74 eA	641 ±	8.62 eB
	SMINIA793101-3	Cambi VN3	9742 ±	67.14 eB	5568 ±	39.06 cB	3632 ±	28.77 dB	554 ±	9.53 fB
	SMINIA793101-3	Ulti	11371 ±	48.93 cB	3741 ±	19.65 fB	3892 ±	16.26 cA	887 ±	5.54 aB
	SMINIA793101-3	Ulti VN1	10782 ±	52.01 dB	4284 ±	29.22 eA	2412 ±	21.04 gB	790 ±	5.16 dB
	SMINIA793101-3	Ulti VN2	14677 ±	28.52 aB	5300 ±	57.86 dB	3628 ±	18.79 dB	368 ±	6.55 gB
			Root							
Spring growing season	SMIF212-3	Cambi C +PK	7163 ±	17.03 eA	6312 ±	93.44 eA	7965 ±	17.19 cA	830 ±	14.75 eB
	SMIF212-3	Cambi C	14460 ±	24.33 ca	6641 ±	47.15 cA	8331 ±	9.32 aA	1220 ±	74.81 cA
	SMIF212-3	Cambi VN1	7107 ±	27.95 fA	8714 ±	159.35 bA	5052 ±	20.07 fA	1282 ±	12.92 cB
	SMIF212-3	Cambi VN2	17753 ±	197.33 bA	5782 ±	69.38 fB	7312 ±	34.25 eA	1313 ±	23.87 bB
	SMIF212-3	Cambi VN3	9430 ±	65.88 eB	10826 ±	235.62 aA	7937 ±	42.74 cA	1437 ±	6.60 aB
	SMIF212-3	Ulti	10424 ±	25.69 dA	1795 ±	30.37 hB	7886 ±	26.28 dA	559 ±	10.12 fB
	SMIF212-3	Ulti VN1	6797 ±	30.27 gA	3302 ±	126.59 gB	2668 ±	11.14 gB	1359 ±	30.82 bB
	SMIF212-3	Ulti VN2	18549 ±	161.72 aA	6401 ±	14.67 dA	8265 ±	183.57 bA	962 ±	4.91 dA
	SMINIA793101-3	Cambi C +PK	6573 ±	8.97 gB	5216 ±	45.51 eB	6045 ±	45.22 cB	965 ±	5.22 fA
	SMINIA793101-3	Cambi C	10687 ±	68.77 dB	3938 ±	42.53 fB	5100 ±	22.84 eB	798 ±	6.13 gB
	SMINIA793101-3	Cambi VN1	6727 ±	21.18 fB	5790 ±	50.74 dB	3641 ±	1.54 gB	1324 ±	4.31 eA
	SMINIA793101-3	Cambi VN2	15908 ±	30.72 cB	7467 ±	80.49 bA	5270 ±	38.73 dB	1811 ±	13.64 cA
	SMINIA793101-3	Cambi VN3	16196 ±	103.12 bA	10276 ±	104.34 aB	6713 ±	28.03 aB	3315 ±	12.17 aA
	SMINIA793101-3	Ulti	8889 ±	25.12 eB	5871 ±	156.06 dA	3978 ±	19.39 fB	2072 ±	12.56 bA
	SMINIA793101-3	Ulti VN1	5548 ±	29.57 hB	5175 ±	385.21 eA	3468 ±	19.12 hA	1676 ±	59.35 dA
	SMINIA793101-3	Ulti VN2	17590 ±	29.63 aB	6371 ±	214.47 cA	6408 ±	7.43 bB	585 ±	23.52 hB
			Stolon							
Fall growing season	SMIE040-6RY	Cambi C +PK	11815 ±	15.56 bA	18479 ±	225.78 aA	7475 ±	18.02 aA	1435 ±	3.53 cA
	SMIE040-6RY	Cambi C	9355 ±	30.44 cB	8904 ±	37.42 eA	5715 ±	29.36 bB	796 ±	6.34 gA
	SMIE040-6RY	Cambi VN1	7662 ±	37.62 eB	8491 ±	41.55 fA	2326 ±	16.21 gB	946 ±	11.86 fA
	SMIE040-6RY	Cambi VN2	7436 ±	37.58 fB	12754 ±	131.49 bA	2206 ±	9.53 hB	1990 ±	21.08 aA
	SMIE040-6RY	Cambi VN3	11782 ±	59.97 bA	12185 ±	75.02 cA	3315 ±	5.54 dB	1654 ±	7.07 bA
	SMIE040-6RY	Ulti	9058 ±	75.57 dA	8315 ±	170.26 gA	2943 ±	8.57 fA	1105 ±	12.92 eA
	SMIE040-6RY	Ulti VN1	9352 ±	62.17 cB	4069 ±	41.00 hA	2992 ±	27.48 eA	1181 ±	0.98 dA
	SMIE040-6RY	Ultii VN2	18840 ±	152.37 aA	11493 ±	100.59 dA	3461 ±	26.38 cA	1653 ±	6.61 bA
	SMINIA793101-3	Cambi C +PK	7267 ±	55.90 gB	10847 ±	103.64 aB	5439 ±	51.07 bB	745 ±	8.05 eB
	SMINIA793101-3	Cambi C	9645 ±	19.99 eA	4064 ±	14.85 dB	6851 ±	16.36 aA	793 ±	8.04 dA
	SMINIA793101-3	Cambi VN1	8267 ±	62.67 fA	6964 ±	34.88 bB	2555 ±	6.37 hA	882 ±	10.46 bB
	SMINIA793101-3	Cambi VN2	14154 ±	64.05 aA	4200 ±	40.89 cB	4590 ±	13.75 cA	852 ±	6.01 cB
	SMINIA793101-3	Cambi VN3	9693 ±	24.16 dB	3031 ±	26.27 gB	4262 ±	29.36 dA	539 ±	4.81 gB
	SMINIA793101-3	Ulti	6801 ±	23.69 hB	3100 ±	2.63 fB	2805 ±	20.23 fB	621 ±	9.84 fB
	SMINIA793101-3	Ulti VN1	10410 ±	46.27 cA	3623 ±	49.27 eB	2598 ±	26.25 gB	1097 ±	5.77 aB
	SMINIA793101-3	Ulti VN2	12050 ±	45.97 bB	4173 ±	15.41 cB	3230 ±	8.36 eB	440 ±	6.43 hB
			Stolon							
Spring growing season	SMIF212-3	Cambi C +PK	6573 ±	60.00 gA	19078 ±	45.51 aB	6045 ±	78.00 bA	1876 ±	5.22 cB
	SMIF212-3	Cambi C	11010 ±	8.48 cA	12202 ±	485.08 eB	5159 ±	13.10 dA	1304 ±	19.66 fB
	SMIF212-3	Cambi VN1	8328 ±	4.17 fA	13962 ±	63.57 cA	3373 ±	7.65 fA	1352 ±	10.20 eB
	SMIF212-3	Cambi VN2	18456 ±	2.27 aA	14039 ±	465.34 bA	7940 ±	17.15 aA	3170 ±	3.84 bA
	SMIF212-3	Cambi VN3	9430 ±	65.88 eB	10826 ±	235.62 fB	7937 ±	42.74 aA	1437 ±	6.60 dA
	SMIF212-3	Ulti	9919 ±	9.67 dA	4892 ±	272.38 gB	3949 ±	5.12 eA	1354 ±	19.08 eB
	SMIF212-3	Ulti VN1	4319 ±	22.11 hA	11501 ±	567.62 eB	2554 ±	12.81 gA	3287 ±	11.95 aB
	SMIF212-3	Ulti VN2	12012 ±	115.00 bA	13787 ±	74.57 dB	5668 ±	38.01 cA	999 ±	25.04 gB
	SMINIA793101-3	Cambi C +PK	5577 ±	8.97 fB	20099 ±	45.51 aA	5030 ±	45.22 bB	2232 ±	5.22 cA
	SMINIA793101-3	Cambi C	7191 ±	51.50 eB	19662 ±	382.31 bA	3232 ±	18.99 dB	2152 ±	0.64 dA
	SMINIA793101-3	Cambi VN1	8377 ±	47.85 cA	12373 ±	219.80 eB	2760 ±	12.05 fB	2031 ±	8.47 fA
	SMINIA793101-3	Cambi VN2	13446 ±	70.08 aB	11290 ±	95.63 fB	3410 ±	21.49 cB	2129 ±	15.00 eB
	SMINIA793101-3	Cambi VN3	11659 ±	20.04 bA	17061 ±	327.43 cA	6896 ±	10.38 aB	874 ±	6.94 hB
	SMINIA793101-3	Ulti	5120 ±	14.03 gB	10089 ±	117.53 gA	2878 ±	6.75 eB	2923 ±	21.94 bA
	SMINIA793101-3	Ulti VN1	2064 ±	9.17 hB	17121 ±	81.67 cA	1507 ±	9.54 hB	3875 ±	24.32 aA
	SMINIA793101-3	Ulti VN2	7727 ±	1.42 dB	16761 ±	113.00 dA	2435 ±	5.91 gB	1961 ±	11.60 gA

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital

letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Table 4. Seed tuber and produced tubers macronutrient concentrations of potato genotypes grown in vineyard soils during fall and spring growing seasons.

			Ca (µg/g)		K (µg/g)		Mg (µg/g)		P (µg/g)	
Seed tuber										
Fall growing season	SMIE040-6RY	Cambi C +PK	17060 ±	28.62 fA	74718 ±	293.71 aA	9404 ±	39.31 dA	5875 ±	23.68 dA
	SMIE040-6RY	Cambi C	18200 ±	77.38 dA	53848 ±	296.43 eA	10122 ±	57.75 cA	3292 ±	5.63 gA
	SMIE040-6RY	Cambi VN1	19388 ±	110.73 cA	66305 ±	984.52 bA	6571 ±	28.01 eA	3430 ±	14.36 fA
	SMIE040-6RY	Cambi VN2	12775 ±	24.19 gB	47292 ±	407.81 fB	4425 ±	26.10 gB	6354 ±	28.59 cA
	SMIE040-6RY	Cambi VN3	9642 ±	61.98 hB	46465 ±	399.98 fA	3881 ±	15.53 hB	6477 ±	15.39 bA
	SMIE040-6RY	Ulti	32483 ±	126.00 aA	56288 ±	711.31 dA	12579 ±	565.86 bA	4306 ±	198.08 eA
	SMIE040-6RY	Ulti VN1	22977 ±	124.82 bA	34131 ±	185.57 gA	13568 ±	29.54 aA	3080 ±	34.32 hB
	SMIE040-6RY	Ulti VN2	17711 ±	370.8 eA	60766 ±	940.92 cA	6382 ±	127.10 fA	8594 ±	130.74 aA
	SMINIA793101-3	Cambi C +PK	7060 ±	22.13 gB	41104 ±	586.94 cA	3059 ±	16.28 eB	2809 ±	6.08 gB
	SMINIA793101-3	Cambi C	5952 ±	38.15 hB	26139 ±	203.03 gB	3999 ±	20.54 dB	3078 ±	16.24 fB
	SMINIA793101-3	Cambi VN1	8647 ±	76.11 fB	42758 ±	382.70 bB	2929 ±	28.10 fB	3071 ±	4.44 fB
	SMINIA793101-3	Cambi VN2	19146 ±	35.42 aA	71037 ±	528.21 aA	7491 ±	10.92 aA	5300 ±	10.71 bB
	SMINIA793101-3	Cambi VN3	10590 ±	13.49 cA	36328 ±	493.07 dB	4008 ±	5.28 cA	3798 ±	24.64 eB
	SMINIA793101-3	Ulti	9178 ±	82.39 eA	30570 ±	513.93 fB	3979 ±	34.81 dB	4078 ±	33.95 dB
	SMINIA793101-3	Ulti VN1	11334 ±	89.79 bB	26758 ±	756.75 gB	7337 ±	18.59 bB	5338 ±	11.14 aA
	SMINIA793101-3	Ulti VN2	9878 ±	6.00 dB	34424 ±	489.75 eB	3036 ±	1.25 eB	4311 ±	12.46 cB
Seed tuber										
Spring growing season	SMIF212-3	Cambi C +PK	4057 ±	5.53 eB	34863 ±	125.30 aA	3647 ±	8.09 aA	1981 ±	22.18 fA
	SMIF212-3	Cambi C	4213 ±	19.43 dB	25596 ±	538.74 cA	3532 ±	22.26 bA	2112 ±	26.10 eA
	SMIF212-3	Cambi VN1	1959 ±	3.87 hA	25752 ±	318.34 cA	2145 ±	6.92 fA	1987 ±	11.16 fA
	SMIF212-3	Cambi VN2	4807 ±	5.97 bA	28538 ±	256.70 bA	3159 ±	3.79 da	3222 ±	25.27 aA
	SMIF212-3	Cambi VN3	5788 ±	26.19 aB	28552 ±	21.62 bB	3373 ±	20.28 cB	2802 ±	16.91 bA
	SMIF212-3	Ulti	3809 ±	17.27 fA	13501 ±	410.63 fB	1867 ±	7.00 gA	2596 ±	5.97 cA
	SMIF212-3	Ulti VN1	4675 ±	3.70 cA	24922 ±	62.37 dA	3375 ±	2.39 cA	2460 ±	21.19 dA
	SMIF212-3	Ulti VN2	3586 ±	21.79 gA	20974 ±	17.61 eB	2325 ±	20.25 eA	2447 ±	54.67 dA
	SMINIA793101-3	Cambi C +PK	4895 ±	2.85 cA	22836 ±	437.17 cB	3352 ±	4.73 bB	1757 ±	41.19 dB
	SMINIA793101-3	Cambi C	5736 ±	116.39 bA	15705 ±	34.78 gB	3051 ±	56.22 cB	971 ±	15.66 gB
	SMINIA793101-3	Cambi VN1	1638 ±	3.71 hB	21531 ±	285.96 dB	1489 ±	11.08 hB	1532 ±	9.90 fB
	SMINIA793101-3	Cambi VN2	2121 ±	31.16 gB	10629 ±	490.12 hB	1600 ±	24.33 gB	1888 ±	19.55 cB
	SMINIA793101-3	Cambi VN3	8334 ±	37.15 aA	32175 ±	191.94 aA	4272 ±	18.22 aA	2368 ±	44.86 aB
	SMINIA793101-3	Ulti	2246 ±	3.05 eB	18330 ±	101.74 fA	1709 ±	2.80 fB	2328 ±	7.99 aB
	SMINIA793101-3	Ulti VN1	2206 ±	0.85 fB	19138 ±	64.71 eB	2255 ±	3.35 dB	2102 ±	5.33 bB
	SMINIA793101-3	Ulti VN2	2527 ±	4.55 dB	24514 ±	205.58 bA	1892 ±	10.96 eB	1633 ±	19.60 eB
Produced tubers										
Fall growing season	SMIE040-6RY	Cambi C +PK	413 ±	2.66 dA	25311 ±	216.40 bA	1519 ±	14.35 cA	1619 ±	8.47 hA
	SMIE040-6RY	Cambi C	303 ±	1.72 eB	15718 ±	150.95 fA	1117 ±	5.29 gA	2063 ±	10.73 gA
	SMIE040-6RY	Cambi VN1	297 ±	2.24 fB	17812 ±	163.93 dA	1305 ±	11.53 eA	2516 ±	4.48 eA
	SMIE040-6RY	Cambi VN2	860 ±	5.37 bB	17187 ±	202.08 eB	1398 ±	12.22 dA	2658 ±	18.75 cA
	SMIE040-6RY	Cambi VN3	717 ±	4.39 cA	22731 ±	26.51 cA	1655 ±	10.76 bA	3357 ±	9.91 bA
	SMIE040-6RY	Ulti	200 ±	1.77 hA	14546 ±	152.58 gA	1262 ±	12.26 fA	2251 ±	14.91 fA
	SMIE040-6RY	Ulti VN1	266 ±	1.50 gA	13765 ±	73.33 hA	1118 ±	5.45 gA	2586 ±	15.69 dA
	SMIE040-6RY	Ulti VN2	2020 ±	8.47 aA	27160 ±	124.45 aA	1773 ±	6.20 aA	3720 ±	15.61 aA
	SMINIA793101-3	Cambi C +PK	258 ±	2.25 fB	18503 ±	81.93 cB	1031 ±	2.57 fB	1523 ±	14.86 hB
	SMINIA793101-3	Cambi C	376 ±	2.48 dA	13854 ±	89.57 fB	984 ±	2.88 gB	1785 ±	4.96 fB
	SMINIA793101-3	Cambi VN1	308 ±	2.66 eA	15697 ±	235.85 eB	1240 ±	4.65 bB	2294 ±	14.66 bB
	SMINIA793101-3	Cambi VN2	1682 ±	10.51 aA	18895 ±	66.88 bA	1376 ±	3.29 aB	2349 ±	23.20 aB
	SMINIA793101-3	Cambi VN3	566 ±	1.40 cB	15964 ±	154.94 dB	1079 ±	4.27 eB	1815 ±	9.04 eB
	SMINIA793101-3	Ulti	180 ±	1.79 hB	12907 ±	195.83 gB	1120 ±	10.65 dB	2073 ±	14.35 cB
	SMINIA793101-3	Ulti VN1	214 ±	1.65 gB	10855 ±	49.42 hB	898 ±	4.39 hB	2016 ±	5.83 dB
	SMINIA793101-3	Ulti VN2	898 ±	3.20 bB	19621 ±	165.45 aB	1158 ±	3.95 cB	1694 ±	6.44 gB
Produced tubers										
Spring growing season	SMIF212-3	Cambi C +PK	772 ±	30.39 fA	16398 ±	36.21 fB	1106 ±	55.97 fA	1290 ±	16.26 gB
	SMIF212-3	Cambi C	1364 ±	75.95 dA	18332 ±	420.20 dA	1590 ±	51.55 cA	1889 ±	4.40 eB
	SMIF212-3	Cambi VN1	720 ±	64.84 fA	19018 ±	25.37 cA	1520 ±	78.67 cdA	2627 ±	19.47 cA
	SMIF212-3	Cambi VN2	949 ±	109.64 eA	16992 ±	122.51 eB	1249 ±	73.89 efA	2791 ±	6.47 bB
	SMIF212-3	Cambi VN3	3159 ±	89.19 aA	22204 ±	31.00 bA	1857 ±	42.30 bA	1950 ±	4.89 dB
	SMIF212-3	Ulti	2595 ±	2.05 bA	26697 ±	210.09 aA	2851 ±	4.40 aA	2764 ±	23.90 bB
	SMIF212-3	Ulti VN1	926 ±	187.56 eA	14187 ±	109.83 hA	1288 ±	34.06 eA	3422 ±	5.67 aA
	SMIF212-3	Ulti VN2	1496 ±	10.22 cA	15467 ±	222.42 gB	1503 ±	15.86 dA	1637 ±	9.78 fB
	SMINIA793101-3	Cambi C +PK	362 ±	2.70 gB	20067 ±	348.12 aA	1157 ±	1.80 cA	1729 ±	13.08 gA
	SMINIA793101-3	Cambi C	786 ±	1.79 cB	12799 ±	146.74 fB	1131 ±	0.94 cB	1935 ±	12.11 fA
	SMINIA793101-3	Cambi VN1	654 ±	3.30 eB	16815 ±	117.85 cB	1343 ±	13.04 aB	2363 ±	18.43 dB
	SMINIA793101-3	Cambi VN2	677 ±	3.24 dB	17441 ±	44.13 bA	1160 ±	2.51 cB	2856 ±	2.24 cA
	SMINIA793101-3	Cambi VN3	2489 ±	84.28 aB	13447 ±	24.61 eB	1293 ±	27.73 bA	2106 ±	14.40 eA
	SMINIA793101-3	Ulti	527 ±	114.77 fB	14994 ±	69.55 dB	1054 ±	72.60 dB	3012 ±	15.21 bA
	SMINIA793101-3	Ulti VN1	600 ±	126.97 efB	14419 ±	154.11 dA	1060 ±	80.25 dB	3150 ±	4.04 aB
	SMINIA793101-3	Ulti VN2	914 ±	55.63 bB	19880 ±	93.60 aA	1152 ±	173.04 bcB	1743 ±	9.68 gA

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital

letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Table 5. Stem macronutrient concentrations of potato genotypes grown in vineyard soils during fall and spring growing seasons.

			Ca (µg/g)		K (µg/g)		Mg (µg/g)		P (µg/g)	
Total stem										
Fall season	SMIE040-6RY	Cambi C +PK	14580 ±	61.90 dA	39966 ±	501.52 aA	7184 ±	50.20 bB	706 ±	9.66 cA
	SMIE040-6RY	Cambi C	14635 ±	88.10 dA	5978 ±	106.53 gA	13298 ±	33.58 aB	608 ±	7.67 dB
	SMIE040-6RY	Cambi VN1	16100 ±	88.06 cA	12443 ±	121.38 fB	6542 ±	9.58 eB	565 ±	11.88 dA
	SMIE040-6RY	Cambi VN2	34141 ±	176.09 aA	3437 ±	33.86 hB	6955 ±	39.76 cB	760 ±	8.95 cA
	SMIE040-6RY	Cambi VN3	3518 ±	13.44 fA	13469 ±	128.83 eA	1488 ±	3.47 gA	2273 ±	10.63 aA
	SMIE040-6RY	Ulti	16823 ±	145.83 bA	2833 ±	38.62 bA	6861 ±	37.83 dB	539 ±	1.35 eA
	SMIE040-6RY	Ulti VN1	16735 ±	122.84 bA	1735 ±	13.57 cA	6935 ±	9.26 cB	614 ±	15.82 dA
	SMIE040-6RY	Ulti VN2	10700 ±	73.19 eB	15401 ±	69.75 dA	2698 ±	7.82 fB	1749 ±	5.17 bA
	SMINIA793101-3	Cambi C +PK	13005 ±	79.84 gB	39908 ±	196.36 aA	10263 ±	61.31 eA	514 ±	1.82 eB
	SMINIA793101-3	Cambi C	12127 ±	44.75 hB	3981 ±	30.12 dB	16386 ±	85.51 aA	640 ±	14.62 bA
	SMINIA793101-3	Cambi VN1	16202 ±	68.09 dA	17279 ±	115.90 bA	7530 ±	14.99 fA	451 ±	4.66 dB
	SMINIA793101-3	Cambi VN2	18874 ±	20.24 bB	3863 ±	47.93 eA	11985 ±	33.99 cA	627 ±	8.76 bB
	SMINIA793101-3	Cambi VN3	20011 ±	68.68 aB	5432 ±	199.30 cB	12300 ±	42.00 bB	876 ±	9.14 aB
	SMINIA793101-3	Ulti	13972 ±	22.62 fB	2754 ±	28.95 fB	11192 ±	8.43 dA	511 ±	8.99 eB
	SMINIA793101-3	Ulti VN1	14157 ±	84.03 eB	652 ±	5.59 gB	7371 ±	18.66 gA	583 ±	5.47 cB
	SMINIA793101-3	Ulti VN2	18569 ±	45.54 cA	5451 ±	39.04 cB	7159 ±	5.01 hA	224 ±	2.07 fB
Apex stem										
Spring season	SMIF212-3	Cambi C +PK	19906 ±	89.97 eA	30606 ±	269.86 bA	15122 ±	59.26 dA	1090 ±	3.44 gA
	SMIF212-3	Cambi C	14369 ±	100.00 hA	9529 ±	120.49 eA	16026 ±	103.96 cA	870 ±	10.20 hB
	SMIF212-3	Cambi VN1	17392 ±	81.03 gB	17587 ±	131.71 cA	11312 ±	39.74 gB	1403 ±	8.99 fB
	SMIF212-3	Cambi VN2	23530 ±	63.65 dA	2820 ±	44.80 gB	12672 ±	107.39 fA	1757 ±	3.37 cB
	SMIF212-3	Cambi VN3	17615 ±	58.30 fA	42526 ±	534.38 dA	17411 ±	42.69 bA	5239 ±	27.50 aA
	SMIF212-3	Ulti	24126 ±	98.56 cB	4379 ±	29.15 fA	14447 ±	101.15 eA	1459 ±	2.64 eB
	SMIF212-3	Ulti VN1	30184 ±	68.58 aA	2864 ±	33.71 gB	24935 ±	46.52 aA	2541 ±	7.34 bB
	SMIF212-3	Ulti VN2	25386 ±	375.40 bB	10518 ±	75.28 dB	12734 ±	77.10 fA	1635 ±	32.29 dA
	SMINIA793101-3	Cambi C +PK	19927 ±	195.34 dA	26756 ±	342.32 aB	6636 ±	44.26 fB	795 ±	15.02 hB
	SMINIA793101-3	Cambi C	14369 ±	31.69 fA	7429 ±	120.49 fB	14926 ±	106.96 aB	1008 ±	10.20 fA
	SMINIA793101-3	Cambi VN1	20806 ±	127.78 cA	16699 ±	162.55 cB	11487 ±	78.77 dA	1695 ±	6.82 eA
	SMINIA793101-3	Cambi VN2	19815 ±	69.25 dB	23239 ±	252.63 bA	9635 ±	31.86 eB	3300 ±	22.73 dA
	SMINIA793101-3	Cambi VN3	11630 ±	58.04 gB	13555 ±	169.86 dB	11540 ±	52.39 dB	4777 ±	19.16 aB
	SMINIA793101-3	Ulti	24983 ±	118.53 bA	2844 ±	14.86 gB	12648 ±	36.02 cB	3462 ±	5.05 cA
	SMINIA793101-3	Ulti VN1	16971 ±	24.52 eB	12554 ±	36.07 eA	13734 ±	79.83 bB	4396 ±	19.75 bA
	SMINIA793101-3	Ulti VN2	29784 ±	184.14 aA	23103 ±	162.57 bA	9345 ±	83.25 eB	809 ±	4.74 gB
Middle stem										
Spring season	SMIF212-3	Cambi C +PK	15794 ±	46.71 gA	26321 ±	303.76 aA	19080 ±	67.21 aA	935 ±	4.02 gA
	SMIF212-3	Cambi C	18084 ±	22.44 eA	11707 ±	136.26 cA	16903 ±	99.09 bA	1410 ±	9.13 cA
	SMIF212-3	Cambi VN1	12432 ±	34.05 hB	23499 ±	83.52 bA	11812 ±	39.93 dB	1001 ±	9.95 fB
	SMIF212-3	Cambi VN2	24124 ±	44.18 cA	2644 ±	7.47 fB	12627 ±	119.96 cA	1089 ±	13.29 eB
	SMIF212-3	Cambi VN3	24459 ±	68.68 bA	11718 ±	199.30 cB	11348 ±	42.00 eA	3772 ±	9.14 aB
	SMIF212-3	Ulti	17366 ±	89.44 fA	3212 ±	8.14 eA	10911 ±	59.06 gA	1279 ±	11.42 dB
	SMIF212-3	Ulti VN1	27008 ±	315.16 aA	5251 ±	11.79 dB	4139 ±	8.54 hB	612 ±	1.63 hA
	SMIF212-3	Ulti VN2	20281 ±	100.39 dA	1982 ±	3.13 gB	11043 ±	18.10 fA	2713 ±	8.24 bA
	SMINIA793101-3	Cambi C +PK	11862 ±	98.36 fB	16036 ±	270.94 aB	9600 ±	11.98 eB	645 ±	3.10 fB
	SMINIA793101-3	Cambi C	12282 ±	80.35 eB	3681 ±	14.40 gB	14989 ±	108.20 aB	610 ±	12.85 gB
	SMINIA793101-3	Cambi VN1	15655 ±	82.35 cA	10026 ±	37.16 eB	12891 ±	95.55 bA	1240 ±	10.97 eA
	SMINIA793101-3	Cambi VN2	15146 ±	207.54 dB	12656 ±	141.99 cA	10059 ±	150.29 dB	2217 ±	36.34 bA
	SMINIA793101-3	Cambi VN3	20050 ±	68.68 bB	13000 ±	199.30 bA	9030 ±	42.00 fB	4980 ±	9.14 aA
	SMINIA793101-3	Ulti	15085 ±	73.31 dB	1683 ±	24.42 hB	7862 ±	36.36 gB	1425 ±	8.49 dA
	SMINIA793101-3	Ulti VN1	20668 ±	129.07 aB	12099 ±	31.06 dA	11168 ±	94.79 cA	502 ±	12.12 hB
	SMINIA793101-3	Ulti VN2	8917 ±	40.90 gB	4242 ±	33.87 fA	9100 ±	30.49 fB	2044 ±	17.86 cB
Basal stem										
Spring season	SMIF212-3	Cambi C +PK	12713 ±	45.83 eA	22542 ±	88.28 bA	16853 ±	31.12 aA	832 ±	4.36 fA
	SMIF212-3	Cambi C	10011 ±	105.12 gA	10413 ±	83.80 eA	12125 ±	31.74 dB	1371 ±	3.87 bA
	SMIF212-3	Cambi VN1	6067 ±	38.91 hB	15428 ±	151.63 cA	8068 ±	25.18 gA	1316 ±	11.05 cA
	SMIF212-3	Cambi VN2	11839 ±	54.09 fA	4187 ±	72.43 fB	14036 ±	38.54 cA	1103 ±	19.19 eB
	SMIF212-3	Cambi VN3	21845 ±	11.82 aB	24899 ±	313.97 aB	15091 ±	101.81 bA	2397 ±	5.63 aA
	SMIF212-3	Ulti	17211 ±	112.21 bA	2520 ±	18.52 gA	11908 ±	67.79 eA	818 ±	3.48 gA
	SMIF212-3	Ulti VN1	15933 ±	20.79 cA	1248 ±	3.06 h B	3738 ±	3.62 hB	587 ±	6.26 hB
	SMIF212-3	Ulti VN2	13471 ±	178.18 dA	11993 ±	27.13 dA	8166 ±	52.76 fB	1245 ±	4.70 dA
	SMINIA793101-3	Cambi C +PK	10681 ±	86.92 dB	10072 ±	100.40 cB	13223 ±	40.45 aB	665 ±	2.47 fB
	SMINIA793101-3	Cambi C	8357 ±	32.90 gB	4197 ±	59.90 gB	12655 ±	106.70 bA	789 ±	4.14 eB
	SMINIA793101-3	Cambi VN1	9149 ±	69.42 eA	5731 ±	45.58 dB	8150 ±	65.03 dA	1044 ±	15.03 cB
	SMINIA793101-3	Cambi VN2	11105 ±	56.50 cB	10386 ±	77.33 bA	7986 ±	70.33 eB	1784 ±	4.64 bA
	SMINIA793101-3	Cambi VN3	34492 ±	238.44 aA	34375 ±	67.55 aA	6557 ±	66.65 gB	1023 ±	33.52 cB
	SMINIA793101-3	Ulti	8676 ±	54.33 fB	1445 ±	42.45 hB	5098 ±	28.25 hB	837 ±	21.50 dA
	SMINIA793101-3	Ulti VN1	5637 ±	31.25 hB	4779 ±	47.32 fA	6861 ±	22.44 fA	2299 ±	11.13 aA
	SMINIA793101-3	Ulti VN2	11547 ±	21.17 bB	5310 ±	24.90 eB	8971 ±	67.53 cA	379 ±	2.78 gB

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital

letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Table 6. Leaf macronutrient concentrations of potato genotypes grown in vineyard soils during fall and spring growing seasons.

			Ca (µg/g)	K (µg/g)	Mg (µg/g)	P (µg/g)
			Total Leaves			
Fall season	SMIE040-6RY	Cambi C +PK	23507 ± 137.51 dA	49637 ± 235.26 aA	4872 ± 27.62 eA	1440 ± 8.92 dA
	SMIE040-6RY	Cambi C	17043 ± 105.68 gB	16946 ± 174.71 dB	9039 ± 69.75 cB	1514 ± 9.44 cA
	SMIE040-6RY	Cambi VN1	20483 ± 97.55 eA	19177 ± 291.32 cB	3400 ± 17.90 gA	1193 ± 3.43 eA
	SMIE040-6RY	Cambi VN2	19209 ± 34.14 fB	6636 ± 200.11 fA	4148 ± 9.59 fB	1017 ± 7.92 fB
	SMIE040-6RY	Cambi VN3	4200 ± 33.61 hB	13120 ± 93.11 eA	2258 ± 34.90 hB	1991 ± 22.83 aA
	SMIE040-6RY	Ulti	35286 ± 100.77 bA	4795 ± 56.99 gB	12648 ± 84.53 bB	823 ± 12.74 hB
	SMIE040-6RY	Ulti VN1	34469 ± 145.85 cA	1561 ± 21.76 hB	23610 ± 62.81 aA	931 ± 6.53 gB
	SMIE040-6RY	Ulti VN2	37641 ± 224.88 aA	24505 ± 261.33 bA	6293 ± 75.15 dA	1773 ± 27.98 bA
	SMINIA793101-3	Cambi C +PK	20567 ± 77.38 dB	37002 ± 586.85 aB	4847 ± 9.14 gA	992 ± 15.26 dB
	SMINIA793101-3	Cambi C	19876 ± 83.25 eA	17909 ± 121.93 dA	11711 ± 39.01 cA	1206 ± 4.23 aB
	SMINIA793101-3	Cambi VN1	19596 ± 51.07 fB	29429 ± 326.70 bA	2591 ± 10.97 hB	961 ± 10.84 eB
	SMINIA793101-3	Cambi VN2	27099 ± 82.25 bA	5528 ± 64.83 gB	8790 ± 40.08 dA	1124 ± 7.48 bA
	SMINIA793101-3	Cambi VN3	23346 ± 125.85 cA	9030 ± 129.15 eB	7996 ± 28.65 eA	1050 ± 18.25 cB
	SMINIA793101-3	Ulti	27677 ± 225.55 aB	6221 ± 45.72 fA	14239 ± 17.65 bA	1146 ± 10.22 bA
	SMINIA793101-3	Ulti VN1	27744 ± 60.33 aB	2289 ± 39.70 hA	22954 ± 59.08 aB	991 ± 5.61 dA
	SMINIA793101-3	Ulti VN2	27653 ± 163.02 aB	18644 ± 155.57 cB	6074 ± 50.31 fB	613 ± 4.65 fA
			Apex leaves			
Spring season	SMIF212-3	Cambi C +PK	22592 ± 90.79 bA	13668 ± 154.20 bB	12783 ± 68.95 cA	2171 ± 23.82 dA
	SMIF212-3	Cambi C	13188 ± 129.65 gB	10690 ± 65.67 cB	12655 ± 109.77 cA	2053 ± 4.82 eA
	SMIF212-3	Cambi VN1	14888 ± 204.65 fB	13315 ± 298.42 bA	8252 ± 89.91 eA	1964 ± 15.77 fB
	SMIF212-3	Cambi VN2	21199 ± 90.99 dA	3204 ± 69.61 fB	14101 ± 37.25 bA	2320 ± 8.25 bB
	SMIF212-3	Cambi VN3	5115 ± 102.07 hB	20561 ± 171.46 aB	3623 ± 86.87 fB	4698 ± 3.78 aA
	SMIF212-3	Ulti	18569 ± 115.26 eB	5312 ± 6.33 eA	12744 ± 63.36 cB	2191 ± 8.30 dB
	SMIF212-3	Ulti VN1	22229 ± 32.30 cA	1434 ± 24.98 gB	23133 ± 40.72 aA	2290 ± 18.80 cB
	SMIF212-3	Ulti VN2	24641 ± 133.64 aB	8446 ± 59.22 dB	8444 ± 91.94 dA	1844 ± 36.95 gA
	SMINIA793101-3	Cambi C +PK	18640 ± 38.49 dB	21414 ± 174.16 aA	6467 ± 8.34 fB	1866 ± 10.14 eB
	SMINIA793101-3	Cambi C	14660 ± 28.74 gA	11008 ± 24.37 eA	9765 ± 7.14 bB	1894 ± 15.63 eB
	SMINIA793101-3	Cambi VN1	19496 ± 48.11 ca	9688 ± 116.03 fB	7957 ± 39.06 eB	2450 ± 14.33 dA
	SMINIA793101-3	Cambi VN2	17029 ± 256.20 eB	15696 ± 205.36 cA	8784 ± 74.04 dB	3316 ± 41.97 aA
	SMINIA793101-3	Cambi VN3	12232 ± 39.34 hA	18172 ± 244.56 bA	6233 ± 64.25 gA	3340 ± 21.89 aB
	SMINIA793101-3	Ulti	22791 ± 233.14 bA	4473 ± 26.16 hB	13528 ± 99.15 aA	2878 ± 5.19 bA
	SMINIA793101-3	Ulti VN1	15459 ± 58.41 fB	7461 ± 58.08 gA	8992 ± 44.14 cB	2829 ± 6.34 cA
	SMINIA793101-3	Ulti VN2	25541 ± 147.33 aA	13422 ± 89.34 dA	6307 ± 41.39 gB	1791 ± 20.01 fB
			Middle leaves			
Spring season	SMIF212-3	Cambi C +PK	22154 ± 56.78 dA	17548 ± 644.16 aB	14463 ± 103.32 eA	1733 ± 10.20 eA
	SMIF212-3	Cambi C	22103 ± 149.22 dA	6712 ± 67.03 dA	17946 ± 10.79 aA	1245 ± 8.71 fA
	SMIF212-3	Cambi VN1	20754 ± 130.41 eA	18216 ± 297.14 aA	11003 ± 57.69 fA	1796 ± 7.00 dB
	SMIF212-3	Cambi VN2	23602 ± 158.56 bA	4573 ± 45.27 eB	16273 ± 25.94 cA	1962 ± 10.94 cB
	SMIF212-3	Cambi VN3	20403 ± 74.23 fA	14053 ± 58.13 bB	9607 ± 16.89 gA	1938 ± 15.22 cB
	SMIF212-3	Ulti	22810 ± 25.63 cB	2362 ± 28.92 gB	15502 ± 48.55 dA	2129 ± 19.04 bA
	SMIF212-3	Ulti VN1	20614 ± 146.51 eA	3377 ± 23.01 fB	16921 ± 39.73 bA	2175 ± 7.86 aA
	SMIF212-3	Ulti VN2	27329 ± 93.07 aA	8481 ± 233.37 cB	11002 ± 6.41 fA	1040 ± 7.44 gA
	SMINIA793101-3	Cambi C +PK	20502 ± 136.07 bB	22795 ± 267.72 bA	8730 ± 51.22 eB	1078 ± 5.02 fB
	SMINIA793101-3	Cambi C	18584 ± 80.84 dB	5915 ± 32.64 fB	16611 ± 55.81 aB	889 ± 4.91 gB
	SMINIA793101-3	Cambi VN1	18112 ± 81.94 eB	10844 ± 45.89 cB	8533 ± 77.68 fB	1849 ± 16.28 dA
	SMINIA793101-3	Cambi VN2	18870 ± 31.40 cdB	9013 ± 80.27 eA	10771 ± 65.70 dB	2245 ± 17.89 bA
	SMINIA793101-3	Cambi VN3	8382 ± 27.46 fB	24946 ± 140.11 aA	5455 ± 32.83 hB	5096 ± 41.08 aA
	SMINIA793101-3	Ulti	24810 ± 208.11 aA	2754 ± 39.37 gA	14064 ± 42.90 bB	1755 ± 3.05 eB
	SMINIA793101-3	Ulti VN1	19061 ± 130.15 cB	5913 ± 66.81 fA	12266 ± 73.30 cB	1987 ± 8.83 cB
	SMINIA793101-3	Ulti VN2	18591 ± 124.93 dB	9338 ± 67.36 dA	6083 ± 142.95 gB	743 ± 8.96 hB
			Basal leaves			
Spring season	SMIF212-3	Cambi C +PK	21738 ± 106.58 hB	34044 ± 367.76 aA	13735 ± 104.69 eA	1597 ± 12.41 cA
	SMIF212-3	Cambi C	23486 ± 94.33 fA	10041 ± 86.38 dA	14734 ± 111.83 dB	1086 ± 13.71 eA
	SMIF212-3	Cambi VN1	22646 ± 125.40 gB	22180 ± 147.25 bA	11357 ± 89.83 hA	1319 ± 7.44 dB
	SMIF212-3	Cambi VN2	30598 ± 102.43 bA	6218 ± 36.66 fB	19087 ± 60.19 bA	2267 ± 45.33 aA
	SMIF212-3	Cambi VN3	31533 ± 137.20 aA	13129 ± 432.95 cB	11990 ± 20.90 gA	1580 ± 29.30 cA
	SMIF212-3	Ulti	24765 ± 99.12 dB	4205 ± 57.72 gA	16749 ± 93.94 cA	1884 ± 16.98 bA
	SMIF212-3	Ulti VN1	24548 ± 113.09 eA	2150 ± 41.23 hB	22767 ± 138.15 aA	2286 ± 23.67 aB
	SMIF212-3	Ulti VN2	28540 ± 150.98 cA	8888 ± 17.38 eB	12230 ± 141.73 fA	968 ± 18.69 fA
	SMINIA793101-3	Cambi C +PK	23889 ± 183.97 cA	28656 ± 595.26 aB	11352 ± 28.14 dB	1037 ± 5.31 fB
	SMINIA793101-3	Cambi C	21688 ± 173.02 fB	7213 ± 133.91 eB	17688 ± 172.94 aA	1141 ± 11.90 eB
	SMINIA793101-3	Cambi VN1	23207 ± 492.94 cA	14077 ± 117.36 cB	10661 ± 191.15 fB	2316 ± 43.44 bA
	SMINIA793101-3	Cambi VN2	22114 ± 166.57 eB	10731 ± 108.24 dA	10386 ± 35.93 gB	1804 ± 17.06 cB
	SMINIA793101-3	Cambi VN3	16133 ± 112.34 gB	25152 ± 313.70 bA	5608 ± 53.21 hB	1158 ± 13.39 eB
	SMINIA793101-3	Ulti	31083 ± 280.34 aA	2420 ± 21.94 gB	14791 ± 191.85 cB	1585 ± 44.57 dB
	SMINIA793101-3	Ulti VN1	23144 ± 228.28 dB	6255 ± 91,04 fA	16946 ± 128.56 bB	2780 ± 24.83 aA
	SMINIA793101-3	Ulti VN2	28142 ± 259.54 bA	11705 ± 341.55 dA	10855 ± 40.10 eB	711 ± 1.73 gB

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital

letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

3.3 Fe, Mn and Zn tissue concentration

In the plants grown during the fall season, with the exception of the SMINIA793101-3 root Mn concentration, the root and stolon Fe, Mn and Zn concentrations were increased in the Ulti VN2 compared with the Ulti control. Overall, these concentrations were greater in the NER plants than in the ENR plants (Table 7). Moreover, when compared with the Ulti control, the root Mn concentration of the SMIE040-6RY plants and stolon Mn concentration of both genotypes decreased in Ulti VN1. During the spring, the root Fe, Mn and Zn concentrations of the SMIF 212-3 plants were positively affected by the Cu contamination in Ulti soils (Table 7). The opposite response was observed in the root Fe and Mn concentrations of the SMINIA793101-3 genotype; however, there was an increase in the root Zn concentration in Ulti VN2. Cu contamination in the Ulti soils increased the stolon concentrations of Zn during the spring. In addition, the stolon Mn concentration decreased in both tested genotypes. The SMIF 212-3 genotype had greater Fe, Mn and Zn concentrations in the stolon compared with SMINIA793101-3 in the Ulti soils, and it was the only genotype with a decrease in the Fe concentration of the stolon tissue (Table 7).

In the Cambi soils, the responses to the Cu increase in the root and stolon Fe, Mn and Zn concentrations varied greatly among the genotypes and growing seasons. During the fall, the Fe and Zn concentrations in the root tissue increased for both genotypes with Cu contamination. However, in the stolon tissue, the Mn and Zn decreased as Fe in relation to Cambi C+PK. During the spring, both the root and stolon Zn concentrations increased at high Cu levels (Cambi VN2 and Cambi VN3) in the tested genotypes. Additionally, compared with Cambi C, the Fe concentrations increased in the roots collected in Cambi VN3 and decreased in the stolon tissues that received the same treatment. Compared with Cambi C, the fertilized treatment (Cambi C+PK) exhibited another remarkable difference. Thus, for the SMINIA793101-3 genotype the Cambi C+PK conferred a greater root Fe and Mn concentration and a lower root Zn concentration than Cambi C in both seasons (Table 7).

Table 7. Root and stolon micronutrient concentrations of potato genotypes grown in vineyard soils during fall and spring growing seasons.

			Fe (µg/g)	Mn (µg/g)	Zn (µg/g)
		Root			
Fall growing season	SMIE040-6RY	Cambi C +PK	5184 ± 7.38 d A	742 ± 0.56 b A	35 ± 0.30 g A
	SMIE040-6RY	Cambi C	3822 ± 44.50 e A	313 ± 3.57 e A	157 ± 0.71 d A
	SMIE040-6RY	Cambi VN1	5363 ± 20.73 c B	294 ± 1.25 f B	165 ± 0.91 c B
	SMIE040-6RY	Cambi VN2	6118 ± 38.64 b A	259 ± 2.03 g A	87 ± 0.70 e B
	SMIE040-6RY	Cambi VN3	11286 ± 58.86 a A	497 ± 1.59 c A	156 ± 1.14 d A
	SMIE040-6RY	Ulti	2221 ± 15.06 g A	392 ± 3.55 d A	56 ± 0.29 f A
	SMIE040-6RY	Ulti VN1	2841 ± 103.48 f A	210 ± 3.19 h A	230 ± 3.72 b A
	SMIE040-6RY	Ulti VN2	2728 ± 98.23 f B	1059 ± 7.69 a A	542 ± 2.87 a A
	SMINIA793101-3	Cambi C +PK	3298 ± 27.52 d B	292 ± 2.04 c B	34 ± 0.25 h A
	SMINIA793101-3	Cambi C	2870 ± 14.77 e B	198 ± 0.81 e B	66 ± 0.60 f B
	SMINIA793101-3	Cambi VN1	6049 ± 80.89 a A	319 ± 3.38 b A	170 ± 0.94 c A
	SMINIA793101-3	Cambi VN2	4556 ± 32.73 b B	167 ± 1.04 g B	149 ± 0.69 d A
	SMINIA793101-3	Cambi VN3	3445 ± 22.46 c B	141 ± 0.83 h B	126 ± 1.20 e B
	SMINIA793101-3	Ulti	1886 ± 8.50 g B	391 ± 2.04 a A	56 ± 0.26 g A
	SMINIA793101-3	Ulti VN1	2620 ± 12.06 f B	175 ± 1.67 f B	233 ± 1.29 b A
	SMINIA793101-3	Ulti VN2	3465 ± 30.10 c A	236 ± 1.57 d B	297 ± 1.40 a B
		Root			
Spring growing season	SMIF212-3	Cambi C +PK	2340 ± 0.76 e A	125 ± 0.12 e B	45 ± 0.65 g B
	SMIF212-3	Cambi C	7049 ± 8.69 b A	372 ± 0.28 b A	76 ± 0.57 f A
	SMIF212-3	Cambi VN1	3348 ± 5.00 d B	127 ± 0.68 d B	100 ± 0.07 c B
	SMIF212-3	Cambi VN2	4193 ± 10.97 c B	113 ± 0.42 g B	98 ± 0.88 d B
	SMIF212-3	Cambi VN3	10399 ± 10.93 a A	507 ± 0.71 a A	80 ± 0.36 e B
	SMIF212-3	Ulti	100 ± 6.43 h B	116 ± 0.68 f B	257 ± 0.59 b A
	SMIF212-3	Ulti VN1	1341 ± 6.90 g B	90 ± 0.64 h B	257 ± 0.39 b A
	SMIF212-3	Ulti VN2	2010 ± 12.08 h B	342 ± 6.42 c B	735 ± 7.75 a A
	SMINIA793101-3	Cambi C +PK	19091 ± 18.66 a B	973 ± 0.81 a A	51 ± 0.39 g A
	SMINIA793101-3	Cambi C	5863 ± 94.88 e B	340 ± 1.99 c B	70 ± 0.21 f B
	SMINIA793101-3	Cambi VN1	9364 ± 22.64 b A	352 ± 0.29 b A	113 ± 0.72 d A
	SMINIA793101-3	Cambi VN2	7308 ± 11.59 c A	284 ± 2.06 d A	108 ± 1.50 e A
	SMINIA793101-3	Cambi VN3	2334 ± 16.03 f B	94 ± 0.44 h B	117 ± 1.42 c A
	SMINIA793101-3	Ulti	7243 ± 1.30 d A	163 ± 0.57 e A	104 ± 0.64 e B
	SMINIA793101-3	Ulti VN1	1774 ± 4.16 g A	116 ± 0.33 g A	154 ± 0.24 b B
	SMINIA793101-3	Ulti VN2	2316 ± 2.25 f A	159 ± 0.23 f A	406 ± 1.13 a B
		Stolon			
Fall growing season	SMIE040-6RY	Cambi C +PK	3790 ± 14.78 b B	739 ± 0.96 a A	133 ± 0.26 f A
	SMIE040-6RY	Cambi C	1467 ± 8.16 d B	246 ± 1.26 e B	256 ± 1.18 d A
	SMIE040-6RY	Cambi VN1	7752 ± 28.82 a B	512 ± 2.31 c B	404 ± 0.95 b A
	SMIE040-6RY	Cambi VN2	1051 ± 12.76 g B	74 ± 0.61 h B	97 ± 0.16 g B
	SMIE040-6RY	Cambi VN3	2029 ± 19.90 c B	112 ± 1.09 g B	68 ± 0.26 h B
	SMIE040-6RY	Ulti	649 ± 9.81 h A	254 ± 0.85 d A	201 ± 0.38 e A
	SMIE040-6RY	Ulti VN1	1275 ± 12.49 e A	239 ± 2.56 f A	475 ± 0.99 a A
	SMIE040-6RY	Ulti VN2	1194 ± 23.12 f A	655 ± 5.03 b A	348 ± 3.30 c B
	SMINIA793101-3	Cambi C +PK	4772 ± 38.68 c A	370 ± 3.44 b B	92 ± 0.33 h B
	SMINIA793101-3	Cambi C	3220 ± 18.83 d A	260 ± 0.89 e A	194 ± 1.31 f B
	SMINIA793101-3	Cambi VN1	18320 ± 118.45 a A	804 ± 4.52 a A	261 ± 1.75 e B
	SMINIA793101-3	Cambi VN2	16869 ± 58.81 b A	353 ± 1.60 c A	231 ± 0.26 d A
	SMINIA793101-3	Cambi VN3	2451 ± 19.38 e A	138 ± 0.72 h A	239 ± 0.37 c A
	SMINIA793101-3	Ulti	578 ± 3.03 h B	190 ± 1.04 f B	128 ± 0.22 g B
	SMINIA793101-3	Ulti VN1	990 ± 9.94 f B	147 ± 1.29 g B	388 ± 0.97 b B
	SMINIA793101-3	Ulti VN2	977 ± 1.96 g B	301 ± 1.63 d B	495 ± 1.75 a A
		Stolon			
Spring growing season	SMIF212-3	Cambi C +PK	19091 ± 49.00 a B	243 ± 0.81 b A	32 ± 0.40 g A
	SMIF212-3	Cambi C	1965 ± 1.45 c B	92 ± 0.09 f B	24 ± 0.14 h B
	SMIF212-3	Cambi VN1	991 ± 1.42 f A	41 ± 0.40 h B	46 ± 0.31 f B
	SMIF212-3	Cambi VN2	856 ± 0.31 g B	46 ± 0.02 g B	113 ± 0.40 b A
	SMIF212-3	Cambi VN3	10399 ± 10.93 b A	507 ± 0.05 a A	80 ± 0.40 e A
	SMIF212-3	Ulti	1189 ± 1.18 d A	161 ± 0.37 c A	107 ± 0.19 c A
	SMIF212-3	Ulti VN1	1152 ± 1.78 e A	99 ± 0.28 e A	88 ± 0.16 d A
	SMIF212-3	Ulti VN2	452 ± 12.00 h B	134 ± 2.40 d A	243 ± 0.60 a A
	SMINIA793101-3	Cambi C +PK	20099 ± 18.66 a A	200 ± 0.81 a B	30 ± 0.40 h A
	SMINIA793101-3	Cambi C	2397 ± 64.65 b A	115 ± 1.67 c A	45 ± 0.38 e A
	SMINIA793101-3	Cambi VN1	889 ± 11.13 e B	45 ± 0.34 g A	87 ± 0.73 c A
	SMINIA793101-3	Cambi VN2	1614 ± 9.56 d A	69 ± 0.51 f A	92 ± 0.30 b B
	SMINIA793101-3	Cambi VN3	2111 ± 11.54 c B	143 ± 0.05 b B	58 ± 0.32 d B
	SMINIA793101-3	Ulti	388 ± 0.18 g B	107 ± 0.13 d B	40 ± 0.22 g B
	SMINIA793101-3	Ulti VN1	242 ± 1.71 h B	26 ± 0.15 h B	41 ± 0.13 f B
	SMINIA793101-3	Ulti VN2	610 ± 0.46 f A	76 ± 0.03 e B	134 ± 0.20 a B

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital

letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Table 8. Seed tuber and produced tubers micronutrient concentrations of potato genotypes grown in vineyard soils during fall and spring growing seasons.

			Fe (µg/g)	Mn (µg/g)	Zn (µg/g)
			seed tuber		
Fall growing season	SMIE040-6RY	Cambi C +PK	469 ± 2.60 dA	274 ± 1.04 cA	6 ± 0.25 hB
	SMIE040-6RY	Cambi C	1103 ± 12.13 aA	259 ± 1.25 dA	46 ± 0.10 dA
	SMIE040-6RY	Cambi VN1	1028 ± 4.86 bA	199 ± 0.62 fB	55 ± 0.12 cA
	SMIE040-6RY	Cambi VN2	206 ± 3.67 fB	49 ± 1.00 gB	9 ± 0.38 gB
	SMIE040-6RY	Cambi VN3	144 ± 0.28 gB	31 ± 2.00 hA	10 ± 0.27 fB
	SMIE040-6RY	Ulti	1167 ± 52.64 aA	484 ± 19.17 bA	34 ± 1.41 eA
	SMIE040-6RY	Ulti VN1	690 ± 6.20 cA	254 ± 0.64 eA	105 ± 0.79 aA
	SMIE040-6RY	Ulti VN2	238 ± 9.12 eA	552 ± 12.33 aA	63 ± 1.41 bA
	SMINIA793101-3	Cambi C +PK	327 ± 2.69 eB	54 ± 0.20 eB	14 ± 0.26 eA
	SMINIA793101-3	Cambi C	206 ± 2.02 fB	42 ± 0.26 fB	10 ± 0.11 gB
	SMINIA793101-3	Cambi VN1	440 ± 6.09 bB	78 ± 0.63 dB	31 ± 0.30 cB
	SMINIA793101-3	Cambi VN2	1007 ± 13.49 aA	86 ± 1.00 cA	54 ± 0.25 aA
	SMINIA793101-3	Cambi VN3	372 ± 7.43 cA	29 ± 1.00 gA	13 ± 0.19 fA
	SMINIA793101-3	Ulti	383 ± 2.74 cB	97 ± 0.70 bB	13 ± 0.31 fB
	SMINIA793101-3	Ulti VN1	355 ± 3.62 dB	98 ± 3.00 bB	35 ± 0.34 bB
	SMINIA793101-3	Ulti VN2	166 ± 1.09 gB	122 ± 3.00 aB	21 ± 0.35 dB
			seed tuber		
Spring growing season	SMIF212-3	Cambi C +PK	178 ± 35.50 fA	25 ± 0.13 dB	6 ± 0.68 gB
	SMIF212-3	Cambi C	333 ± 1.14 cB	22 ± 0.07 eB	5 ± 0.58 hB
	SMIF212-3	Cambi VN1	233 ± 0.73 eB	12 ± 0.03 gB	8 ± 0.43 fB
	SMIF212-3	Cambi VN2	114 ± 0.75 hB	12 ± 0.06 gA	11 ± 0.36 dB
	SMIF212-3	Cambi VN3	377 ± 0.43 bA	18 ± 0.03 fB	10 ± 0.24 eA
	SMIF212-3	Ulti	322 ± 1.06 dA	74 ± 0.08 aA	32 ± 0.37 aA
	SMIF212-3	Ulti VN1	400 ± 0.27 aA	27 ± 0.02 cA	14 ± 0.26 bA
	SMIF212-3	Ulti VN2	160 ± 9.00 gB	35 ± 0.88 bA	12 ± 0.15 cB
	SMINIA793101-3	Cambi C +PK	699 ± 3.29 bB	29 ± 0.36 bA	11 ± 0.08 deA
	SMINIA793101-3	Cambi C	1918 ± 30.71 aA	50 ± 1.09 aA	11 ± 0.71 eA
	SMINIA793101-3	Cambi VN1	358 ± 2.33 dA	15 ± 0.05 gA	12 ± 0.13 dA
	SMINIA793101-3	Cambi VN2	582 ± 1.42 cA	7 ± 0.19 hB	14 ± 0.09 bA
	SMINIA793101-3	Cambi VN3	264 ± 2.74 gB	26 ± 0.24 cA	11 ± 0.74 eA
	SMINIA793101-3	Ulti	131 ± 0.08 hB	20 ± 0.02 eB	7 ± 0.48 fB
	SMINIA793101-3	Ulti VN1	290 ± 0.09 eB	18 ± 0.01 fB	14 ± 0.03 cA
	SMINIA793101-3	Ulti VN2	279 ± 1.08 fA	23 ± 0.13 dB	16 ± 0.33 aA
			produced tuber		
Fall growing season	SMIE040-6RY	Cambi C +PK	44 ± 0.55 eB	13 ± 0.11 cA	20 ± 0.08 fA
	SMIE040-6RY	Cambi C	264 ± 1.82 aA	38 ± 0.08 bA	32 ± 0.25 cA
	SMIE040-6RY	Cambi VN1	123 ± 1.70 bB	10 ± 0.07 eA	35 ± 0.07 bA
	SMIE040-6RY	Cambi VN2	120 ± 1.75 bA	9 ± 0.07 gB	25 ± 0.25 dA
	SMIE040-6RY	Cambi VN3	56 ± 0.63 dA	10 ± 0.40 fA	23 ± 0.05 eA
	SMIE040-6RY	Ulti	62 ± 1.22 cA	11 ± 0.40 dA	18 ± 0.45 gA
	SMIE040-6RY	Ulti VN1	39 ± 0.58 fB	10 ± 0.07 fA	35 ± 0.51 bA
	SMIE040-6RY	Ulti VN2	57 ± 0.12 dA	119 ± 0.51 aA	48 ± 0.52 aA
	SMINIA793101-3	Cambi C +PK	99 ± 1.13 bA	11 ± 0.30 cB	13 ± 0.57 eB
	SMINIA793101-3	Cambi C	46 ± 0.88 eB	10 ± 0.20 eB	19 ± 0.07 cB
	SMINIA793101-3	Cambi VN1	141 ± 2.13 aA	12 ± 1.00 bA	20 ± 0.19 bB
	SMINIA793101-3	Cambi VN2	51 ± 0.42 dB	11 ± 0.40 cA	23 ± 0.56 aB
	SMINIA793101-3	Cambi VN3	45 ± 0.45 eB	8 ± 0.40 gB	19 ± 0.18 dB
	SMINIA793101-3	Ulti	55 ± 0.63 cB	10 ± 0.50 dB	12 ± 0.08 fB
	SMINIA793101-3	Ulti VN1	41 ± 0.72 fA	9 ± 0.20 fB	20 ± 0.07 bB
	SMINIA793101-3	Ulti VN2	41 ± 0.22 fB	19 ± 0.10 aB	20 ± 0.17 bB
			produced tuber		
Spring growing season	SMIF212-3	Cambi C +PK	217 ± 6.95 bA	9 ± 0.38 eA	14 ± 0.65 eB
	SMIF212-3	Cambi C	101 ± 8.68 dB	9 ± 1.15 dB	16 ± 0.87 dB
	SMIF212-3	Cambi VN1	63 ± 0.77 gB	7 ± 0.29 fB	21 ± 0.48 bcB
	SMIF212-3	Cambi VN2	96 ± 2.68 eA	6 ± 0.89 gB	20 ± 0.62 cB
	SMIF212-3	Cambi VN3	217 ± 0.90 bA	14 ± 0.67 bA	20 ± 0.12 cB
	SMIF212-3	Ulti	358 ± 0.20 aA	34 ± 0.06 aA	8 ± 0.29 fB
	SMIF212-3	Ulti VN1	114 ± 2.42 cA	11 ± 1.13 cA	30 ± 0.48 aA
	SMIF212-3	Ulti VN2	86 ± 5.00 fA	2 ± 0.05 hB	21 ± 0.12 bA
	SMINIA793101-3	Cambi C +PK	68 ± 0.20 dB	8 ± 0.02 eB	17 ± 0.27 dA
	SMINIA793101-3	Cambi C	980 ± 8.68 aA	28 ± 1.00 aA	20 ± 0.62 bA
	SMINIA793101-3	Cambi VN1	125 ± 11.00 cA	12 ± 0.07 bA	21 ± 0.39 bA
	SMINIA793101-3	Cambi VN2	68 ± 30.00 dB	10 ± 0.05 cA	25 ± 0.69 aA
	SMINIA793101-3	Cambi VN3	193 ± 1.00 bB	11 ± 0.74 bB	26 ± 0.50 aA
	SMINIA793101-3	Ulti	46 ± 1.17 fB	9 ± 0.65 dB	15 ± 0.94 eA
	SMINIA793101-3	Ulti VN1	52 ± 0.65 eB	8 ± 1.01 dB	22 ± 1.07 bB
	SMINIA793101-3	Ulti VN2	77 ± 13.44 dA	10 ± 1.41 bcA	20 ± 0.41 cB

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital

letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

During the fall, the Fe, Mn and Zn concentrations in seed and produced tubers of both the NER and ENR plants had a similar response to Cu excess when cultivated in the Ulti soils. Conversely, the Fe concentration decreased in both tissues and genotypes, whereas the Mn and Zn increased (Table 8). Moreover, during the spring season, the SMINIA793101-3 plants grown in Ulti VN2 had increased Fe, Mn and Zn concentrations in their seed and produced tubers compared with the Ulti control. During the same season, the SMIF 212-3 plants grown in Ulti soils with high Cu levels showed a decrease in the Fe and Mn concentrations in their seed and produced tubers and seed tuber Zn concentration.

In the Cambi soils during the fall season, the NER plants showed a continuous decrease of the Fe, Mn and Zn concentration in the seed tuber tissue with increasing Cu treatments; however, in the ENR plants, there was only a decrease in the Mn seed tuber concentration in the Cambi VN3. During the spring season, a Cu increment decreased the Mn concentrations of the seed tubers in both genotypes. Overall, the SMINIA793101-3 plants had significantly greater Fe, Mn and Fe concentrations when compared with the seed tuber tissue of SMIF 212-3 (Table 8). Moreover, SMIF 212-3 increased its Fe, Mn and Zn concentrations in the tissue of produced tubers. However, SMINIA793101-3 plants decreased both Fe and Mn concentrations (Table 8).

The total stem Fe concentration varied greatly among the soil treatments and genotypes. The NER genotype SMIE040-6RY showed a continuous increase with a Cu increment in Ulti soils. Compared with the Ulti control, the ENR plants, SMIF 212-3 and SMINIA793101-3, decreased their stem Fe concentration (apex, middle and basal parts) in Ulti VN2. Remarkably, in Ulti VN1, only SMINIA793101-3 plants showed a continuous decrease in Fe concentration. Conversely, the SMIF 212-3 genotype had a greater Fe concentration in Ulti VN1 than the Ulti control in middle and basal stems; additionally, this increase was accompanied by a significant decrease in Ulti VN2. Moreover, in the leaf tissues, regardless of the tested genotype, growing season or leaves development stage, the Cu increment resulted in a decrease of the Fe concentration in Ulti VN1 and an increase in the Ulti VN2 when compared with the Ulti control.

During the fall season, both of the ENR and NER plants grown in the Cambi C+PK treatment had a lower Fe concentration in their stem tissue when compared with Cambi C. In addition, for the tested genotypes during the fall, both the Cambi C+PK and Cambi C showed stem Fe concentration values lower than Cambi VN2 and Cambi VN3. Interestingly, for the leaf tissues, Cambi C+PK soil conferred a higher leaf Fe concentration in relation to Cambi C

during the fall season. Moreover, during the fall season, plants grown in Cambi C+PK had a greater Fe concentration in their leaf tissues when compared with Cambi VN2 and Cambi VN3; however, they had a lower Fe concentration when compared with Cambi VN1. During the spring season, in stem tissues, only the basal part of SMINIA793101-3 stem had a greater Fe concentration in Cambi VN3 when compared with Cambi C and Cambi C+PK. Overall, the higher Fe concentration occurred in plants grown in Cambi C soil. In leaves, the top values of the Fe concentration varied according to the leaves development stage. In young leaves (apex), as in the development leaves (middle), the Fe concentration increased with Cu contamination, with the higher values found in Cambi VN1 and Cambi VN3. In older leaves (basal), the Fe concentration decreased in Cambi VN3 when compared with Cambi VN2. Furthermore, the Fe concentration values were significantly lower when compared with Cambi C+PK. Under high Cu levels, the leaf tissues of both soil types (Cambi and Ulti) indicated a greater concentration of Fe. Thus, overall, the Cambi soils conferred higher leaf Fe concentrations when compared with the Ulti soils.

The concentrations of Mn and Zn in shoot parts (leaves and stems) greatly varied among the treatments, genotypes and tissue development stage. During the fall season, SMIE040-6RY (NER) indicated a continuous increase of Mn concentration in the stem tissues with Cu contamination in Ulti soils. Conversely, the Zn concentration in the stem tissues of this genotype was also increased in Ulti VN1; additionally, there was a remarkable decrease in Ulti VN2. However, when compared with the Ulti control, SMINIA793101-3 (ENR) increased both the Mn and Zn concentrations in stem tissues for plants grown in Ulti VN2. Furthermore, during the fall season, the leaf tissues in both NER and ENR plants increased Mn and Zn concentrations in Ulti VN2 and decreased Mn concentration in Ulti VN1 when compared with the Ulti control. During the spring, both ENR genotypes SMIF 212-3 and SMINIA793101-3 showed a distinct pattern of Mn and Zn concentrations in response to the Cu increment for each tissue part tested in the Ulti soils. Both the stem and the leaves from the apex increased in Mn concentration, whereas the middle parts decreased. The SMIF 212-3 plants showed an increase in Mn and Zn concentrations for both the stem and leaves of the basal part in Ulti VN2 when compared with the Ulti control. However, in Ulti VN2, SMINIA793101-3 had an increase in Mn and Zn concentrations in the basal leaves and an increase in Zn in the basal stem; additionally, the Mn concentration decreased when compared with the Ulti control. In the Cambi soils, compared with Cambi C+PK, the response to the Cu contamination in Cambi VN3 was positive in the Zn concentration in the total stem and negative in the total leaves for both genotypes (Table 9, 10). However, during the fall season,

the Mn concentration differed between the genotypes with the Cu increment. In the fall, the Mn concentrations in SMIE040-6RY NER decreased in both the stem and total leaves, whereas the Mn concentration of SMINIA793101-3 ENR increased in the total leaves tissue (Table 9, 10). Overall, during the spring season, the tested genotypes increased in Zn concentration in leaves tissues (Apex, middle and basal) with a Cu increment in Cambi soils (Table 10). A similar response was observed in the stem tissues, with the exception of the basal parts for both the SMIF 212-3 and SMINIA793101-3 plants (Table 9). Furthermore, with the exception of middle stem tissue of SMINIA793101-3 plants, the Mn concentrations decreased with a Cu increment in the Cambi soil (Table 9, 10).

Table 9. Stem micronutrient concentrations of potato genotypes grown in vineyard soils during fall and spring growing seasons.

			Fe (µg/g)	Mn (µg/g)	Zn (µg/g)
Fall season					
Total stem	SMIE040-6RY	Cambi C +PK	97 ± 0.30 d B	533 ± 3.52 b A	162 ± 0.89 g A
	SMIE040-6RY	Cambi C	121 ± 2.89 c B	374 ± 2.37 c A	400 ± 1.69 c A
	SMIE040-6RY	Cambi VN1	189 ± 4.42 b A	558 ± 1.90 a A	842 ± 5.01 a A
	SMIE040-6RY	Cambi VN2	681 ± 7.08 a A	261 ± 1.54 e A	383 ± 2.42 d B
	SMIE040-6RY	Cambi VN3	197 ± 3.48 b B	30,0 ± 0.07 g B	260 ± 0.30 e B
	SMIE040-6RY	Ulti	51 ± 1.02 g B	221 ± 0.79 f B	176 ± 1.26 f A
	SMIE040-6RY	Ulti VN1	73 ± 0.70 f A	228 ± 6.00 f A	829 ± 10.93 b A
	SMIE040-6RY	Ulti VN2	87 ± 1.26 e B	327 ± 2.65 d A	143 ± 0.87 h B
	SMINIA793101-3	Cambi C +PK	108 ± 0.44 f A	298 ± 2.01 b B	133 ± 1.02 g B
	SMINIA793101-3	Cambi C	201 ± 2.97 d A	234 ± 1.27 d B	274 ± 0.72 f B
	SMINIA793101-3	Cambi VN1	150 ± 1.34 e B	446 ± 0.53 a B	513 ± 1.20 a B
	SMINIA793101-3	Cambi VN2	343 ± 3.05 a B	228 ± 3.00 e B	421 ± 4.67 d A
	SMINIA793101-3	Cambi VN3	300 ± 0.70 b A	200 ± 1.98 f A	300 ± 1.70 e A
	SMINIA793101-3	Ulti	67 ± 0.75 g A	227 ± 2.00 e A	135 ± 0.67 g B
	SMINIA793101-3	Ulti VN1	62 ± 0.18 h B	129 ± 0.50 g B	478 ± 2.85 b B
	SMINIA793101-3	Ulti VN2	251 ± 7.74 c A	293 ± 0.56 c B	468 ± 0.73 c A
Spring season					
Apex stem	SMIF212-3	Cambi C +PK	152 ± 9.66 c A	122 ± 0.45 d A	38 ± 1.00 f A
	SMIF212-3	Cambi C	150 ± 1.39 c A	80 ± 0.15 e A	50 0.70 d A
	SMIF212-3	Cambi VN1	87 ± 0.57 e B	79 ± 0.03 e A	177 ± 1.49 c A
	SMIF212-3	Cambi VN2	93 ± 0.40 d A	38 ± 0.17 f A	267 ± 1.61 a A
	SMIF212-3	Cambi VN3	70 ± 0.13 f A	24 ± 0.09 g B	46 ± 1.30 e B
	SMIF212-3	Ulti	553 ± 0.05 a A	149 ± 1.62 b A	17 ± 0.88 h B
	SMIF212-3	Ulti VN1	144 ± 0.60 c A	132 ± 0.62 c A	34 ± 1.52 g B
	SMIF212-3	Ulti VN2	203 ± 15.00 b A	356 ± 3.82 a A	229 ± 0.72 b A
	SMINIA793101-3	Cambi C +PK	64 ± 0.45 f B	83 ± 0.17 c B	16 ± 0.71 h B
	SMINIA793101-3	Cambi C	118 ± 1.39 a B	54 ± 0.15 f B	34 ± 0.72 f B
	SMINIA793101-3	Cambi VN1	105 ± 0.37 b A	60 ± 0.45 e B	39 ± 0.54 e B
	SMINIA793101-3	Cambi VN2	91 ± 0.45 c A	35 ± 0.15 h B	163 ± 0.53 b B
	SMINIA793101-3	Cambi VN3	48 ± 0.19 g B	39 ± 0.29 g A	373 ± 0.30 a A
	SMINIA793101-3	Ulti	88 ± 0.25 d B	110 ± 0.93 b B	22 ± 1.92 g A
	SMINIA793101-3	Ulti VN1	88 ± 0.11 d B	67 ± 0.49 d B	42 ± 0.88 d A
	SMINIA793101-3	Ulti VN2	70 ± 0.25 e B	232 ± 1.20 a B	110 ± 0.88 c B
Middle stem	SMIF212-3	Cambi C +PK	81 ± 0.32 e A	159 ± 0.43 c A	125 ± 1.90 g A
	SMIF212-3	Cambi C	147 ± 2.81 b A	95 ± 0.42 e A	134 ± 0.27 f B
	SMIF212-3	Cambi VN1	65 ± 1.37 f B	53 ± 0.22 f B	243 ± 0.32 e A
	SMIF212-3	Cambi VN2	93 ± 0.75 d A	44 ± 0.30 h B	459 ± 1.70 c A
	SMIF212-3	Cambi VN3	47 ± 0.70 g A	86 ± 0.25 f B	384 ± 1.70 d A
	SMIF212-3	Ulti	117 ± 0.19 c A	171 ± 1.04 b A	87 ± 1.41 h B
	SMIF212-3	Ulti VN1	942 ± 23.00 a A	428 ± 4.08 a A	597 ± 5.80 a A
	SMIF212-3	Ulti VN2	81 ± 0.66 e A	123 ± 0.25 d A	480 ± 2.18 b A
	SMINIA793101-3	Cambi C +PK	49 ± 0.98 e B	80 ± 0.09 d B	79 ± 1.09 g B
	SMINIA793101-3	Cambi C	90 ± 0.28 a B	72 ± 0.51 e B	145 ± 0.73 e A
	SMINIA793101-3	Cambi VN1	85 ± 0.70 b A	70 ± 0.71 e A	218 ± 1.09 c B
	SMINIA793101-3	Cambi VN2	43 ± 0.42 f B	49 ± 0.70 g A	292 ± 3.70 b B
	SMINIA793101-3	Cambi VN3	25 ± 0.70 g B	95 ± 0.25 c A	300 ± 1.70 a B
	SMINIA793101-3	Ulti	70 ± 0.82 c B	171 ± 1.16 a A	136 ± 0.91 f A
	SMINIA793101-3	Ulti VN1	52 ± 0.42 d B	160 ± 1.25 b B	292 ± 3.98 b B
	SMINIA793101-3	Ulti VN2	43 ± 0.13 f B	57 ± 0.22 f B	157 ± 1.12 d B
Basal stem	SMIF212-3	Cambi C +PK	121 ± 15.99 c B	130 ± 0.75 c A	182 ± 0.18 f B
	SMIF212-3	Cambi C	188 ± 44.75 a A	52 ± 1.16 d B	113 ± 0.48 h B
	SMIF212-3	Cambi VN1	143 ± 22.84 b A	33 ± 0.82 f B	206 ± 0.77 e B
	SMIF212-3	Cambi VN2	75 ± 13.79 f A	23 ± 0.41 h B	311 ± 0.09 c A
	SMIF212-3	Cambi VN3	95 ± 0.83 e B	29 ± 0.22 g B	156 ± 0.95 g B
	SMIF212-3	Ulti	96 ± 0.77 e A	189 ± 0.84 b A	291 ± 3.45 d A
	SMIF212-3	Ulti VN1	102 ± 7.91 d A	41 ± 0.23 e B	672 ± 0.84 a A
	SMIF212-3	Ulti VN2	73 ± 2.00 f A	201 ± 2.35 a A	363 ± 5.08 b B
	SMINIA793101-3	Cambi C +PK	178 ± 0.83 b A	94 ± 0.30 c B	217 ± 0.67 f A
	SMINIA793101-3	Cambi C	167 ± 2.93 c B	59 ± 0.56 d A	198 ± 1.72 g A
	SMINIA793101-3	Cambi VN1	60 ± 0.58 e B	52 ± 0.35 e A	364 ± 1.42 b A
	SMINIA793101-3	Cambi VN2	79 ± 0.41 d A	44 ± 0.37 g A	300 ± 1.86 c B
	SMINIA793101-3	Cambi VN3	243 ± 0.53 a A	49 ± 0.60 f A	170 ± 1.42 h A
	SMINIA793101-3	Ulti	86 ± 41.65 d B	145 ± 0.81 a B	227 ± 0.42 e B
	SMINIA793101-3	Ulti VN1	51 ± 13.25 f B	47 ± 0.97 f A	267 ± 0.71 d B
	SMINIA793101-3	Ulti VN2	37 ± 32.16 g B	114 ± 2.04 b B	507 ± 0.63 a A

Data represent the mean S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

Table 10. Leaves micronutrient concentrations of potato genotypes grown in vineyard soils during fall and spring growing seasons.

			Fe (µg/g)	Mn (µg/g)	Zn (µg/g)
Fall season					
Total leaves	SMIE040-6RY	Cambi C +PK	604 ± 0.70 bA	717 ± 3.97 bA	38 ± 0.04 eB
	SMIE040-6RY	Cambi C	188 ± 4.97 fB	517 ± 4.18 eA	48 ± 0.47 cA
	SMIE040-6RY	Cambi VN1	797 ± 4.89 aA	548 ± 4.07 dA	101 ± 0.52 aA
	SMIE040-6RY	Cambi VN2	327 ± 1.55 dA	516 ± 5.00 eA	41 ± 0.44 dB
	SMIE040-6RY	Cambi VN3	178 ± 2.92 fgB	150 ± 1.61 fB	34 ± 1.48 fA
	SMIE040-6RY	Ulti	244 ± 1.30 eA	583 ± 2.00 cA	35 ± 0.72 fA
	SMIE040-6RY	Ulti VN1	177 ± 1.75 gA	547 ± 3.35 dA	84 ± 0.59 bA
	SMIE040-6RY	Ulti VN2	340 ± 5.36 cA	1780 ± 10.19 aA	99 ± 1.89 aA
	SMINIA793101-3	Cambi C +PK	233 ± 0.15 cB	353 ± 0.55 gB	40 ± 0.25 cA
	SMINIA793101-3	Cambi C	208 ± 1.78 dA	320 ± 1.54 hB	35 ± 0.25 dB
	SMINIA793101-3	Cambi VN1	335 ± 1.95 aB	460 ± 2.76 bB	63 ± 1.08 aB
	SMINIA793101-3	Cambi VN2	210 ± 2.71 dB	421 ± 3.13 eB	55 ± 0.47 bA
	SMINIA793101-3	Cambi VN3	272 ± 1.28 bA	360 ± 1.62 fA	34 ± 0.60 eA
	SMINIA793101-3	Ulti	178 ± 0.72 eB	446 ± 2.00 cB	36 ± 0.25 dA
	SMINIA793101-3	Ulti VN1	174 ± 2.53 fA	426 ± 0.98 dB	65 ± 0.33 aB
	SMINIA793101-3	Ulti VN2	239 ± 5.20 cB	720 ± 3.72 aB	55 ± 0.18 bB
Spring season					
Apex leaves	SMIF212-3	Cambi C +PK	148 ± 1.04 dB	127 ± 1.05 cA	14 ± 0.26 gB
	SMIF212-3	Cambi C	145 ± 1.62 dB	89 ± 0.70 dA	40 ± 0.05 bA
	SMIF212-3	Cambi VN1	211 ± 1.35 bA	60 ± 0.92 eB	20 ± 0.72 fA
	SMIF212-3	Cambi VN2	136 ± 0.78 fA	60 ± 0.21 fB	22 ± 0.44 eB
	SMIF212-3	Cambi VN3	162 ± 2.60 cB	24 ± 0.39 gB	40 ± 0.32 bA
	SMIF212-3	Ulti	139 ± 0.29 eA	129 ± 0.67 cB	53 ± 0.35 aA
	SMIF212-3	Ulti VN1	105 ± 0.39 gB	259 ± 0.61 bA	32 ± 2.00 cA
	SMIF212-3	Ulti VN2	231 ± 1.16 aA	450 ± 2.86 aA	28 ± 0.59 dA
	SMINIA793101-3	Cambi C +PK	177 ± 2.83 bA	127 ± 8.00 cA	17 ± 0.61 cA
	SMINIA793101-3	Cambi C	151 ± 1.53 dA	68 ± 0.02 gB	17 ± 0.26 bcB
	SMINIA793101-3	Cambi VN1	143 ± 1.86 eB	79 ± 0.04 fA	14 ± 0.13 eB
	SMINIA793101-3	Cambi VN2	123 ± 1.56 fB	98 ± 0.59 dA	41 ± 0.23 aA
	SMINIA793101-3	Cambi VN3	185 ± 2.06 aA	57 ± 0.59 hA	27 ± 0.46 bB
	SMINIA793101-3	Ulti	126 ± 0.55 gB	173 ± 1.81 bA	19 ± 0.90 cB
	SMINIA793101-3	Ulti VN1	113 ± 0.31 hA	84 ± 0.39 eB	15 ± 0.34 dB
	SMINIA793101-3	Ulti VN2	167 ± 0.66 cB	251 ± 0.96 aB	26 ± 0.48 bB
Middle leaves	SMIF212-3	Cambi C +PK	122 ± 1.69 eA	172 ± 1.66 bA	15 ± 0.29 fA
	SMIF212-3	Cambi C	155 ± 0.44 cA	117 ± 0.07 eB	16 ± 0.89 eB
	SMIF212-3	Cambi VN1	145 ± 1.90 dA	79 ± 0.38 fB	25 ± 0.39 bA
	SMIF212-3	Cambi VN2	158 ± 0.56 bA	68 ± 0.17 gB	21 ± 0.41 cB
	SMIF212-3	Cambi VN3	208 ± 0.39 aB	47 ± 0.09 hB	21 ± 0.13 cB
	SMIF212-3	Ulti	119 ± 0.86 fA	167 ± 0.63 cB	10 ± 0.34 gA
	SMIF212-3	Ulti VN1	100 ± 4.20 gB	119 ± 0.43 dB	18 ± 0.20 dA
	SMIF212-3	Ulti VN2	123 ± 0.47 eB	493 ± 0.29 aA	40 ± 0.46 aA
	SMINIA793101-3	Cambi C +PK	117 ± 0.69 gB	145 ± 0.80 eB	9 ± 0.42 fB
	SMINIA793101-3	Cambi C	136 ± 12.05 dB	178 ± 4.00 cA	19 ± 0.42 cA
	SMINIA793101-3	Cambi VN1	142 ± 3.39 cB	81 ± 0.71 gA	13 ± 0.35 eB
	SMINIA793101-3	Cambi VN2	118 ± 0.47 gB	187 ± 1.21 bA	30 ± 0.39 bA
	SMINIA793101-3	Cambi VN3	228 ± 0.70 aA	75 ± 0.28 hA	35 ± 0.62 aA
	SMINIA793101-3	Ulti	120 ± 0.85 fA	310 ± 1.15 aA	30 ± 0.10 bA
	SMINIA793101-3	Ulti VN1	129 ± 1.43 eA	136 ± 0.93 fA	19 ± 0.43 cA
	SMINIA793101-3	Ulti VN2	169 ± 1.33 bA	153 ± 3.88 dB	15 ± 0.53 dB
Basal leaves	SMIF212-3	Cambi C +PK	193 ± 6.20 cB	177 ± 0.87 bB	26 ± 0.70 cB
	SMIF212-3	Cambi C	283 ± 1.81 aA	115 ± 0.40 eB	16 ± 0.68 eB
	SMIF212-3	Cambi VN1	263 ± 0.30 bA	79 ± 0.55 gB	22 ± 0.54 dA
	SMIF212-3	Cambi VN2	157 ± 2.47 eB	91 ± 0.21 fB	37 ± 0.39 bB
	SMIF212-3	Cambi VN3	170 ± 1.43 dB	61 ± 0.14 hB	26 ± 0.65 cB
	SMIF212-3	Ulti	125 ± 2.70 fB	163 ± 0.92 cB	15 ± 0.23 eB
	SMIF212-3	Ulti VN1	105 ± 1.38 gB	156 ± 0.68 dB	25 ± 0.63 cB
	SMIF212-3	Ulti VN2	173 ± 8.00 dA	462 ± 10.40 aB	107 ± 1.96 aA
	SMINIA793101-3	Cambi C +PK	519 ± 2.41 aA	221 ± 0.92 dA	29 ± 0.36 dA
	SMINIA793101-3	Cambi C	234 ± 1.45 dB	182 ± 1.49 fA	27 ± 0.20 dA
	SMINIA793101-3	Cambi VN1	143 ± 3.69 gB	102 ± 1.69 hA	13 ± 0.18 fB
	SMINIA793101-3	Cambi VN2	394 ± 2.08 bA	572 ± 4.92 aA	82 ± 0.85 aA
	SMINIA793101-3	Cambi VN3	277 ± 3.29 cA	155 ± 1.03 gA	47 ± 1.08 cA
	SMINIA793101-3	Ulti	161 ± 0.97 fA	306 ± 2.02 cA	22 ± 0.36 eA
	SMINIA793101-3	Ulti VN1	206 ± 0.59 eA	217 ± 0.72 eA	53 ± 0.25 bA
	SMINIA793101-3	Ulti VN2	145 ± 0.36 gB	315 ± 0.51 bA	46 ± 0.68 cB

Data represent the mean±S.D. of six different replicates. Different lowercase letters indicate significant differences between Cu levels in the same potato genotype and growing season ($p < 0.05$). Different capital letters indicate significant differences between potato genotypes at the same Cu level and growing season ($p < 0.05$).

The principal component (PC) analysis for mineral nutrition parameters showed an absence of pattern response to Cu toxicity and was able to generate clusters by multiple correlations in roots, stolons, seed tubers, produced tubers and stem tissues (Fig. 6).

However, the multivariate analysis demonstrated the formation of four distinct clusters in relation to the leaves mineral concentration under Cu stress for both the Cambi and Ulti soils (Figs. 7, 8). In the Cambi soils, the axis of the first PC (PC1) explained 39% of the total variance, and K, Ca, Mn, Fe and Zn were positively correlated in this component. The axis of the second PC (PC2) explained 25% of the total variance with no positive correlation with the Cu and Mg concentrations, but a positive correlation with Cu and P. Together, the axes explained 64% of the mineral parameter variation for plants grown in Cambi soils (Fig. 7). This soil type indicated four patterns of response: I) the pattern related to high Cu content in soil/Cu toxicity, which was highly correlated with Cu and P concentrations; II) the pattern related to moderated Cu content in soil and P deficiency, correlated to Mg; III) the pattern related to lower/natural Cu content in soil and fertility, correlated to K and Ca concentrations; and IV) the pattern related to soils with or without high Cu content, correlated with Zn, Fe and Mn concentrations with the previous cluster (Fig. 7).

Group I only clustered the tissues of plants grown in Cambi VN2 and Cambi VN3 treatments. Most of the leaf tissues in this cluster were from the apex and middle parts, with only the SMINIA793101-3 basal leaves and total leaves (Fig. 7). Group II clustered tissues from plants grown in Cambi C and Cambi VN1 and only tissues of the apical and middle leaves (Fig. 7). Group III was the only cluster with just one type of soil, Cambi C+PK, and with tissues of the apex, middle and basal parts (Fig. 7). The last group, IV, clustered soils with and without Cu contamination, as well as with or without PK fertilization, and was mostly represented by the total leaves tissues (Fig. 7).

For the Ulti soils, PC1 explained 47% of the total variance, and Cu, Ca, Zn and Mn were positively correlated in this component. Overall, PC1 separated the tissues from plants grown in soil with natural/low Cu (Ulti) and with intermediate Cu contamination (Ulti VN1), from high Cu contamination (Ulti VN2) (Fig. 8). The PC2 explained 25% of the total variance, where concentrations of Fe and K were positively correlated. Together, PC1 and PC2 explained 72% of the variation in growth parameters (Fig. 8).

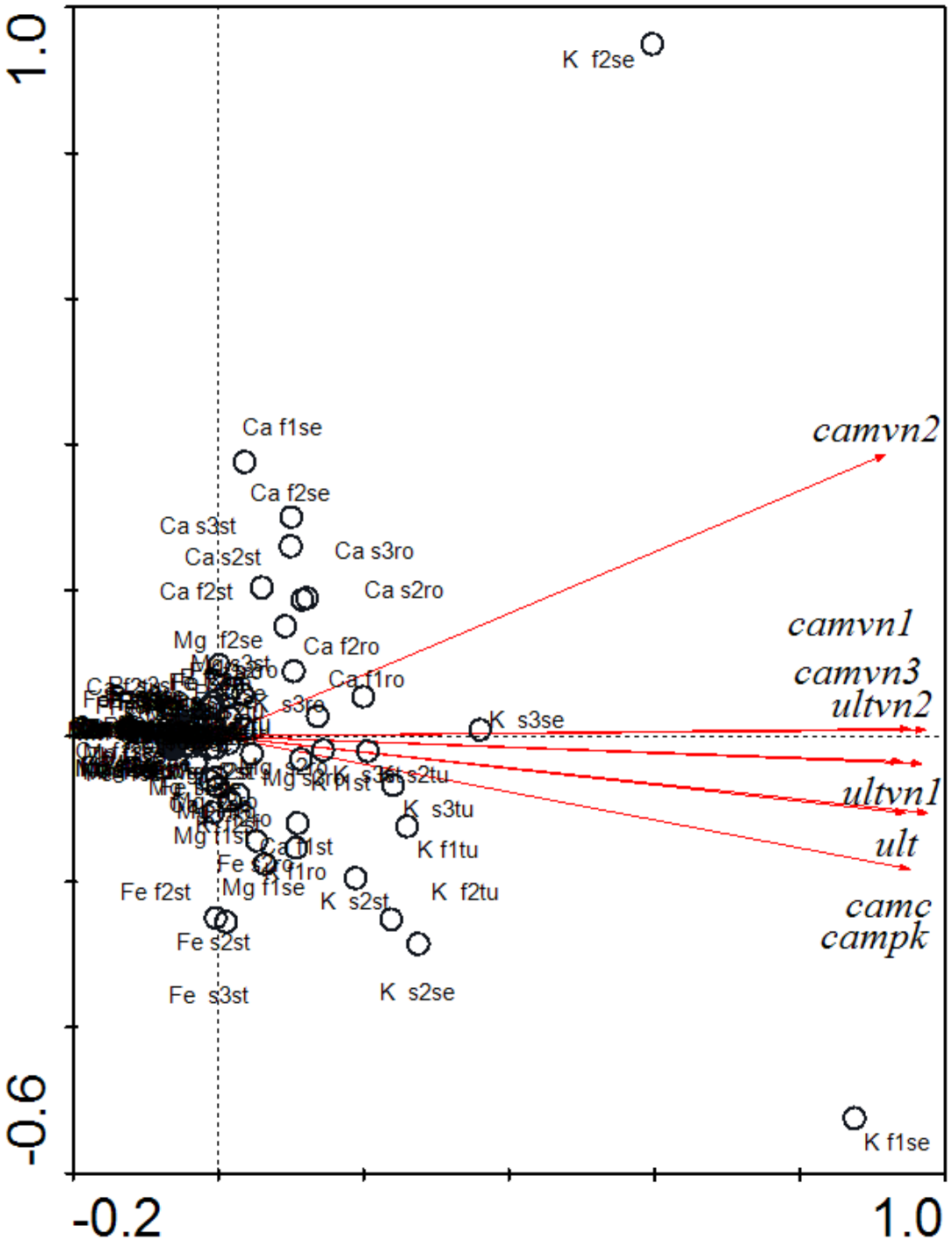

Fig. 6. Biplot graphic of scores and weights (loadings) for the first two principal components (PC1 and PC2) for mineral parameters (Ca, K, Mg,P, Cu, Fe, Mn and Zn) and tested soils at fall (f) and spring (s) growing seasons for SMIE040-6RY (1), SMINIA793101-3 (2) and SMIF 212-3 (3) genotypes. Total stem (ste); apex stem (aste); middle stem (mste); basal stem (bste); produced tubers (tu); seed tubers (se); stolon (st) and root (ro).

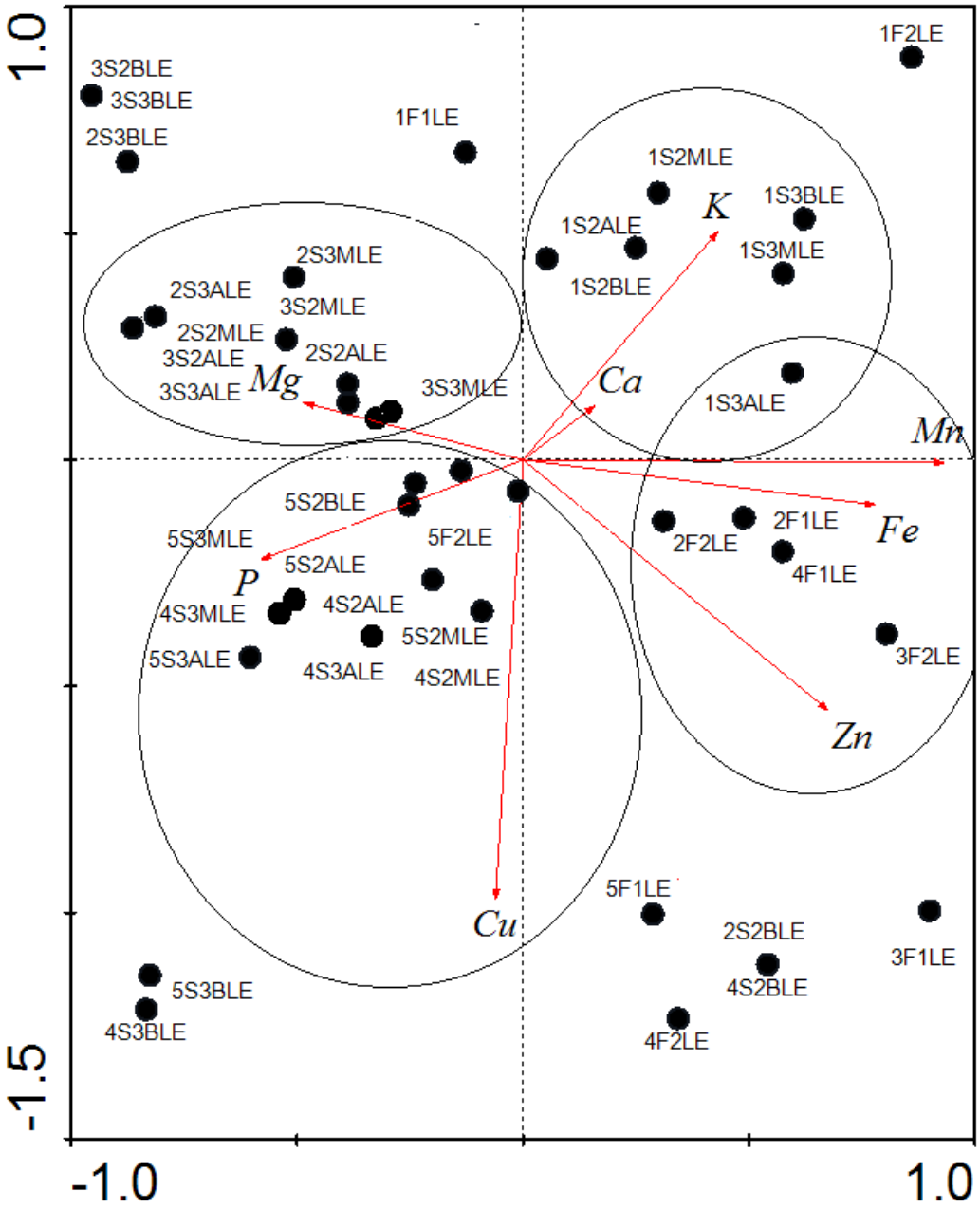

Fig. 7. Biplot graphic of scores and weights (loadings) for the first two principal components (PC1 and PC2) for mineral parameters (Ca, K, Mg,P, Cu, Fe, Mn and Zn) and Cambi soils (identified for the first number), Cambi C (1), Cambi C+PK (2), Cambi VN1 (3), Cambi VN2 (4) and Cambi VN3 (5); at fall (f) and spring (s) growing seasons for SMIE040-6RY (1), SMINIA793101-3 (2) and SMIF 212-3 (3) genotypes. Total leaves (le); apex leaves (ale); middle leaves (mle) and basal leaves (ble)

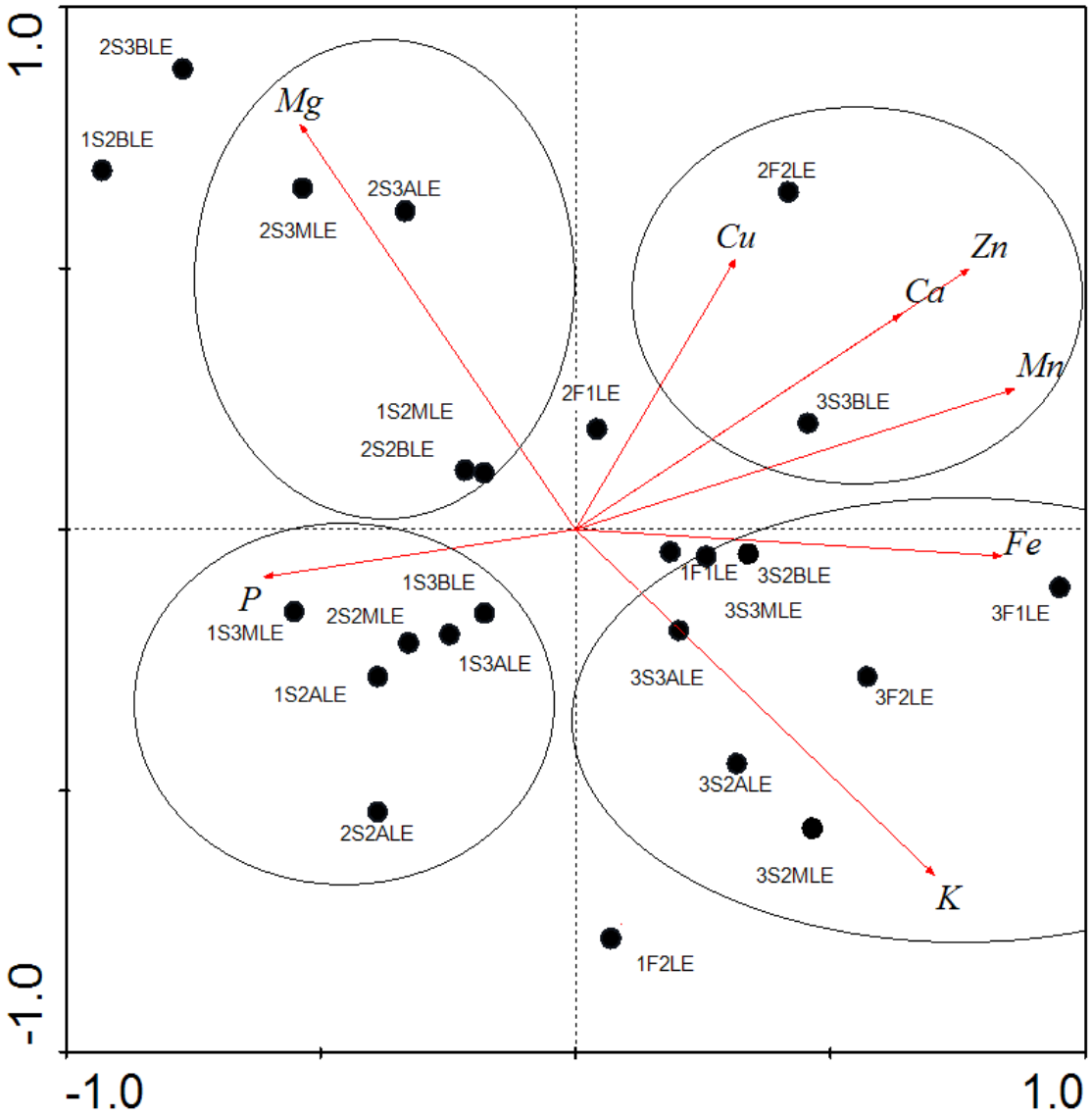

Fig. 8. Biplot graphic of scores and weights (loadings) for the first two principal components (PC1 and PC2) for mineral parameters (Ca, K, Mg,P, Cu, Fe, Mn and Zn) and Ulti soils (identified for the first number), Ulti (1), Ulti VN1 (2) and Ulti VN2 (3); at fall (f) and spring (s) growing seasons for SMIE040-6RY (1), SMINIA793101-3 (2) and SMIF 212-3 (3) genotypes. Total leaves (le); apex leaves (ale); middle leaves (mle) and basal leaves (ble)

4.Discussion

Mineral nutrients are necessary for optimal plant growth, which is only achieved after controlling the level of essential minerals [26]. Mineral uptake by roots is subject to selective properties of the plasma membrane [41]. However, at high levels, some nutrients have direct toxic effects and might drastically interfere on the uptake and homeostasis of other nutrients [24]. Additionally, Cu toxicity has been shown to affect photosynthesis, enzyme activity, pigment synthesis, protein synthesis and cell division [4]. These disturbances can result in

damages, such as the peroxidation of membrane lipids, thus leading to ion leakage [5] and further accumulation of Cu in the plant tissues [6]. However, the mechanisms of nutrient uptake and accumulation associated with growth responses in Cu-treated potato plants are not well characterized.

In the present study, the genotypes pre-classified as efficient in use and not responsive to P (ENR) were able to expand leaves and produce tubers in all of the tested soils; in contrast, the genotype pre-classified as inefficient in use but responsive to P (NER) showed the highest sensitivity to Cu excess in growth terms. The response to Cu toxicity included plants without expanded leaves and plants lacking tuber production (Table 2; Fig. 1). In view of the internal requirement of plant nutrition, efficiency is normally related to the biomass produced per unit of nutrient applied to the soil, and it depends on two main components: efficiency of acquisition and utilization [15]. The first component depends on the absorption efficiencies and root production, including both architecture and exudates [16,17]. The second component depends on the efficiency of translocation and conversion into biomass. Together, these components ensure a good plant nutrition, which usually reflects in a higher biomass production, which may mitigate the excess of Cu through dilution of the nutrient in the tissuel [13].

An important difference between the ENR and NER genotypes was the seed tuber dry weight. Seed tubers are an important factor in ensuring a good crop of tubers, by providing the initial plant nutrition [25], which can vary in response to plant requirements [26]. During the fall and spring growing seasons, ENR plants (SMINIA793101-3 and SMIF212-3) had a higher absorption of seed tubers in Cambi VN2, Cambi VN3, Ulti VN1 and Ulti VN2, whereas NER plants (SMIE040-6RY) absorbed more in Cambi C, Cambi VN1, Ulti and Ulti VN1 (Table 1). These data suggest that the seed tubers might mitigate the initial Cu toxicity; the initial mineral supply of tissue reserves for ENR plants does not occur in NER plants.

Although there was a significant difference among genotypes, soils and seasons, in general Cu toxicity inhibited shoot growth with a greater inhibition in Cambi VN2, Cambi VN3 and Ulti VN2 as described by Girotto [27] for *Avena sativa* under the same tested soils. This difference might be due to the inhibition of cell division resulting from the decline of growth [28].

Previous studies have also indicated a remarkable reduction in root weight [10,29]. Contrary to these findings, our study demonstrated that potato cultivation in soils containing high Cu levels did not negatively affect the root dry weight. Furthermore, ENR SMINIA793101-3 increased the root dry weight with Cu increases in both the Cambi and Ulti

soils during the fall season and in Ulti soils during the spring season. This response might be due to the hormetic effect. Growth hormesis represents an overcompensation due to a disruption in homeostasis that has been described in relation to different factors (Calabrese, 1999). In contrast, for NER plants, the stolon dry weight decreased in Ulti VN1 and Ulti VN2 when compared with the Ulti control during the fall season; during the spring, for SMIF212-3 plants grown in Cambi VN2 and Cambi VN3, the stolon dry weight also decreased when compared with the Cambi controls (Table 2).

Interestingly, Cambi C+PK conferred a significant decrease in Cu concentration on the tested tissues when compared with Cambi C. This decrease was followed by a biomass increase regardless of the growing season or genotype tested. Previous studies have shown a reduction of Cu transport to shoots in plants grown in contaminated soils [13] through the formation of less soluble compounds with added phosphate [30]. Other protective effects of P might include metal dilution in plant tissue as a result of greater biomass production and/or chelation by the exuded compounds in rhizospheres [31].

Moreover, in plants grown under Cambi VN1 (which presented high Cu levels and high P and K concentrations), the tuber and shoot production was not negatively affected. In contrast, plants grown in Cambi VN1 had values of dry weight similar to those of Cambi C+PK, and they showed a significant increase compared with Cambi C and Ulti (Table 2; Fig. 1). The element K is the most abundant cation in the cytoplasm, and it contributes to the cell's osmotic potential. Furthermore, many enzymes are either completely dependent on K^+ or stimulated by K^+. Moreover, K is required for protein synthesis and cell extension, which affects many components of photosynthesis [26]. In addition, potato plants are responsive to K fertilization with effects observed in the tuber number and tuber weight per plant [32]. This might explain the mitigation of the Cu toxicity effects on plant grown in Cambi VN1 through metal dilution associated with P complexation (Figs. 2, 3).

Overall, the Ulti control, even with agriculturally suitable concentrations of P and K, and without Cu contamination, had tuber and shoot production values similar to Cambi C, which had a P deficit. Furthermore, the production of tubers and shoot biomass was significantly lower in the Ulti control when compared with Cambi + PK and Cambi VN1 (Table 2). The concentration of Cu found in the Ultisols with greater accumulations of Cu (Ulti VN1 and Ulti VN2) was approximately 6 times less than that found in the Cambisols (Table 1), which can be explained by the natural composition of the soil. The tested Ultisols had lower levels of OM and clay when compared with the Cambisols (Table 1), however, the levels of soluble Cu were higher in Ulti soils [27]. Additionally, Rooney et al. [10] used single

regressions performed between Cu toxicity threshold values and various soil properties to show that exchangeable calcium, soil cation exchange capacity, iron oxide concentration, soil pH, clay, and organic carbon content are the best predictors for Cu toxicity in barley and tomato plants.

In the two growing seasons (fall and spring), the Cu concentration in roots, stolons and tubers (seed tubers and produced tubers) increased with the Cu contamination level (Fig.s 2, 3, 4, 5) and greatly varied among the tested tissues. The higher concentration was found in root tissues, as reported in other studies of distinct species. However, most of the Cu taken up by the plants was accumulated in tubers (Figs. 4, 5). Remarkably, the root and stolon Cu concentrations were greater in Ulti VN2, Cambi VN2 and Cambi VN3 regardless of the tested genotype and growing season, with similar values among these treatments. Much of the variation in the Cu uptake and its consequent accumulation is attributed to the Cu content in the soil. However, an interesting finding in the Faucon et al. [33] study was the interaction among several transition metals, which played a key role in determining population-specific Cu accumulation patterns. In particular, Mn was the most influential soil factor for Cu (positive influence) and Co (negative influence) accumulation. Cu-Mn interactions in plant nutrition are reported to be both synergistic and antagonistic [34]. Our data suggest a pattern of response to Cu excess in Cu accumulation independent of the soil Mn concentrations, where a higher content of Mn in soils did not result in a higher accumulation of Cu in potato plant tissues (Table 1; Figs. 2, 3, 4, 5).

Regardless of the significant difference in Cu content, there was a great reduction in plant growth. This reduction was accompanied by an increase in the Cu concentration in root tissues in Ultisols with Cu contamination even when Cu was below the established threshold of 200 mg kg^{-1} [35]. These findings were similar to the effects observed in Cambisols with Cu toxicity. Additionally, the values of Cu concentration in tested tissues under Cu contamination were also similar between Cambi and Ulti soils (Figs. 2, 3). Moreover, in general Ulti VN3 showed the highest Cu concentration in root and stolon tissues among the tested soils. The behavior of metals is influenced by attributes in the soil solid phase, type of adsorbent (organic matter, silicate minerals, iron oxides, manganese, and phosphate groups) and geochemical conditions of the solution, particularly the proton concentration and ionic strength [36]. In the tested soils of this study, OM and CECs had remarkable lower values, at about 3 fold, in Ultisols as compared to Cambisols, which was determinant for a lower difference between total Cu content and CuEDTA (Table 1), and consequently a high fraction of available Cu.

The acquisition and transport pathways of Cu in plants remain not fully understood. Copper uptake via transporters such as COPT1 (Copper Transporter) has been described in *Arabidopsis thaliana* [37]. Prior to uptake, this transporter requires a reduction step of Cu (II) by a specific Cu reductase [38]. Additionally, it was suggested that ZIP (Zinc/Iron-regulated Proteins) and PS (phytosiderophore) transporters play an important role in the Cu uptake [2]. In the two growing seasons (fall and spring), the Cu concentration in roots, stolons and tubers (seed tubers and produced tubers) increased with the Cu contamination level (Figs. 2, 3, 4, 5), and the highest concentration was found in the tissues of the roots and stolon. This information agreed with previous reports [5], which also founded the higher content of Cu in roots. Remarkable, our data demonstrate that most of the Cu taken up by the potato plants was accumulated in produced tubers (Figs. 4, 5). Interestingly, some plants are able to reduce metal translocation to shoot parts trough an excluding-behavior [39]. This mechanism prevents metal excessive translocation to shoot by metal accumulation in roots. Under Cu contamination, it allows plants to have a satisfactory growth and development under high levels. However, when overpassing a certain concentration threshold, the progressive root decay may result in death due to toxic effect of Cu on cell membranes [5]. In this view, the accumulation of Cu in produced tubers may be an alternative to the potato root system, which is reduced in comparison with other plant species. Furthermore, ENR plants were less affected by Cu toxicity as compared to NER plants and also showed higher Cu content in root, stolon and produced tubers in high contaminated soils (Cambi VN2, Cambi VN3 and Ulti VN2).

The concentrations of Fe, Mn and Zn also showed high levels in the root and stolon tissue. The most common general symptom of Cu toxicity is chlorosis of the vegetative tissue, which can be related to a reduction in Fe uptake, even to the point of deficiency, depending on the form of Fe available in the soil [26]. In the shoots, the thylakoid membrane of the chloroplast, especially photosystem II (PSII), is a primary target of Cu toxicity [40]. Remarkably, the Fe and Zn concentrations increased with Cu increases in the root and young leaves tissues (apex and middle), whereas Fe decreases in the basal leaves and stem tissues during the spring season (Tables 7, 8, 9, 10). Conversely, Jouvin et al. [41] reported an increase in the Cu concentration in the roots of tomato plants with a Fe increment, with no difference in shoot parts, and the opposite response in wheat plants under the same conditions, which were correlated to PS transporters competition. This information may elucidate some data in *Avena sativa* plants grown under Cu excess, where was suggested Fe deficiency by Cu excesses resulting in reticular chorosis (data not published). Jouvin et al. [41] also reported a reduction of Cu(II) at the surface of the plasma membrane by an FRO-type reductase (e.g.,

specific Cu-reductase as suggested by Zheng et al. [38] for red clover or FRO3 as suggested for *A. thaliana* by Mukherjee et al. [42] and an uptake of the reduced Cu by the specific carrier protein COPT1, which leads to an enrichment of isotopic light Cu in the root. However, Zn uptake was related to ZIP proteins with strong affinity for Zn^{2+}, thus leading to a fraction toward the uptake of heavier isotopes and complexation by the phytosiderophores (strategy II plants only). Conversely, and contrary to Cu, in Jouvin et al. [41] the Zn uptake was negatively affected by the Fe concentration in both root and shoot tissues in tomato and wheat plants. This data may explain higher values of Zn in root and stolon tissues of potato at Ulti soils as compared to Cambi soils, once Cambi soils showed 10 fold of Fe content in soil in relation to Ulti treatments (Tables 1, 7, 8, 9, 10). In addition, our data showed Zn concentration encompassing similar values in root and stem tissues, and Mn also occurred in high levels in leaves and stem tissues (Tables 7, 9, 10).

In general, the interpretation of data of mineral elements concentrations and growth parameters is performed either using univariate analysis or through the correlation among the parameters. However, these variables can interact leading to misinterpretation. PCA analyses can detect differences between samples or between different measured variables, thereby reducing the number of variables to explain the same amount of variance [43]. Throught the results obtained by PCA based on the correlation matrix of different nutrients in the potato tissue in relation to the tested soils, it was possible to suggest that concentration of nutrients in root, stolon, tubers and stem tissues were not good predictors of Cu toxicity for the experimental system tested, once there was no cluster formation through the tested soils (Fig. 6). Furthermore there was no pattern of response to Cu toxicity among these tested tissues. On the other hand, by using the leaf tissues it was possible to observe the formation of distinct clusters in response to Cu toxicity, as well as to other soil fertility parameters (Figs. 6, 7, 8). In addition, there were remarkable differences among tissues response according to the development stages of leaves and the correlations with macro and micronutrients analyzed. These differences were also noticed with the use of more conventional statistic analyses as univariate analysis through Tukey test (Tables 6, 10).

Our data showed a decrease of the macronutrients Ca, K and Mg in leaf tissues with Cu increment in Cambi soils (Table 6). In addition NER plants showed a higher decrease when compared to the ENR plants. Moreover, the concentration of these elements showed continuous increase from the apex leaves to basal leaves. Plants require Ca, K and Mg in relatively large amounts (> 0.1% of dry weight) and each of these elements is essential for a plant to complete its life cycle. Normally, these minerals are taken up by plant roots from the

soil solution in ionic form with the metals Ca^{2+}, Mg^{2+} and K^{+} present as free cations, but by distinct transporters and/or channels [44]. In plant tissues, Ca^{2+} is relatively immobile and tends to be sequestered in the large vacuole of mature cells, which supports our data of higher Ca concentration in basal leaves (Table 6). The uptake de Ca^{2+} is carried out by members of the CAX H^{+}: Ca^{2+} antiport family and by ATP-driven P-type ATPases (McAinsh and Pittman, 2009). No transporters have been identified that are responsible for Ca^{2+} xylem loading and a proportion of xylem Ca^{2+} may arrive via the apoplast [45], but also in vascular system Ca^{2+} mobility is low.

Differently, a substantial fraction of the K^{+} that is taken up is translocated to the shoot mediated by SKOR type channels that release K^{+} into the xylem [46]. In addition to the delivery K^{+} to green tissue, a large phloem-mediated shoot to root K^{+} flux is maintained. The resulting cycling of K^{+} is believed to be important in K^{+} homeostasis and to provide a constant supply of cations to accompany anions such as NO^{-3} on their way to the shoot [44]. In the present study, the tissue used was collect next to the end of the cycle, when the plants were still producing tubers, and hence tubers were the principal sink. The Cu toxicity may inhibit uptake of nutrients as a result to disturbances in root membrane cell. Furthermore the reduction of K concentration may be attributed to K-leakage indicating membrane damage and loss of selective properties [47].

Rossini Oliva et al. [5] reported decrease of Ca, K and Mg concentrations in leaves of *Erica andevalensis* exposed to Cu toxicity (250 uM). In addition, it was reported an increase in P concentration with Cu increment, as it was noticed in our work (Table 6). Furthermore, in contrast to Ca, K and Mg, P concentrations increased from basal parts to apex.

It is usual the use of third and fourth leaves for investigations of the nutritional status of the potato plants [48]. Conversely, through PCA analysis, our results showed that apex and middle leaves were more correlated to alterations in the concentration of nutrients as compared to either basal or whole leaves. Thus, apex and middle leaves were better predictors to Cu toxicity and its implication to mineral nutrition.

Interestingly, in both Cambi and Ulti soils, the determinant factor for clustering plants were the soil treatments, but with no genotypic difference (Figs. 6, 7, 8). Moreover, in Cambi soils, Cu and P concentrations in tissue were correlated with high Cu contamination (Cambi VN2 and Cambi VN3), and hence might be possible predictors to Cu toxicity. The results obtained through univariate analysis for growth parameters showed a significative reduction of shoot dry weight, which may concentrate P on tested tissues. Remarkable, Mg concentration was correlated with Cambi VN1 and Cambi C. In view of contrasting

production of tubers between these treatments (Table 2), Mg correlation seems to result from two distinct situations: I) the good fertility under moderate Cu level in Cambi VN1, which conferred a high fitomass production; II) P deficiency under normal Cu levels, which reduced fitomass production and concentrate Mg in shoot tissue. Moreover, Ca and K concentrations were good predictors for fertility, with an individual correlation with Cambi C+PK. Overall, in Cambi soils the micronutrients were not good predictors either to Cu toxicity or fertility, with the exception of Cu concentration. Mn, Fe and Zn were correlated with all soils with the exception of Cambi VN3.

In Ulti soils a distinct response compared to Cambi soils was noticed. During the fall growing season, only NER plants showed decrease in Mg concentrations with increasing Cu levels. In addition, it showed a significant increase in Ca, K and P concentrations (Fig. 6). In contrast, ENR plants were not affect in the tissue Ca concentration, but they showed increase in K and decrease in both Mg and P concentrations (Fig. 6). It is important to emphasize the extreme sensibility of NER plants to Cu toxicity in both Cambi and Ulti soils (Cambi VN2, Cambi VN3 and Argi VN2), which resulted in plants not fully developed and with morphological changes (Fig. 1). This fitomass reduction may have contributed to concentrate some nutrients in the tissues. During the spring season, in general, tissue Ca and K concentrations increased with increasing Cu levels, while Mg and P decreased (Fig. 6). Encompassing, throught PCA analysis, it was possible to infer that tissue nutrients concentrations of plants grown in Ulti soils had multiple response to Cu stress, which difficult a determination of toxicity predictor. For this soil, Cu, Ca, Zn, Mn, Fe and K were more correlated to Cu contamination in both high levels (Ulti VN1 and Ulti VN2), while Mg and P were more correlated to control and the first Cu level of contamination (Ulti and Ulti VN1) (Fig. 8).

The opposite response of tissue P concentration in Ulti soils in relation to Cambi soils may be elucidated by Cu and OM contents in soil, as well as the physics attributes (Table 1). In Cambi soils, as previously reported, Cu increase resulted in increase of the P concentration, that is contrary to the Ulti soils response. Overall, the tested Cambi soils had 3 fold OM in relation to Ulti soils. Copper biogeochemistry is largely controlled by its bonding to natural organic matter for reasons not well understood. Manceau and Matynia [49] studied this affinity and proposed that the most stable Cu–organic matter chelates at acidic pH are formed with closely-spaced carboxyl groups and hydroxyl donors, oxalate-type ring chelates are not observed. Further, Cu(II) bonds the four equatorial oxygens to the heuristic distance of 1.94 ± 0.01 Å , compared to 1.97 Å in water. This shortening increases the ligand field strength, and

hence the covalency of the Cu–Oxigen bond and stability of the chelate, and steric hindrances in organic matter were the main reason for the absence of Cu–Cu interactions, which otherwise are common in carboxylate coordination complexes. The formation of this complexs, the ligand field strength and the high affinity to Cu, probably reduced the P-Cu complex in Cambi soils. On the other hand, Ulti soils had low OM content, so it is possible to infer that a higher complexation of P-Cu may occurred, and turned P less available to plants and, consequently, resulted in decrease of the concentration of P in the tissues.

5.Conclusion

Vineyard soils with a long history of cupric fungicide application showed toxic Cu levels to potato plants. Additionally, at higher concentrations of Cu, tissue nutrient concentrations were remarkably affected. Mineral parameters greatly varied among the genotypes, with the ENR genotypes being less sensitive to Cu excess. Additionally, there were significant differences between the two ENR genotypes studied, suggesting that there is difference between efficiency of mineral acquisition.

Both soil types tested had distinct responses for Cu excess in mineral nutrition of potato. In Cambisols, tissue P and Cu were the main nutrients related to high Cu exposure, while in Ultisols, Fe and K were more related.

Furthermore, our data suggests the use of middle and apex leaf tissues to investigate Cu toxicity in potato plants. This study also provides evidence of non-competitive uptake of Cu and Fe by potato plants. However, further studies at the molecular level are needed to clarify the mechanisms involved in the interaction between mineral nutrition and Cu uptake.

Acknowledgments

The authors would like to thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação e Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa de Estado do Rio Grande do Sul (FAPERGS) for financial support.

References

[1]S.R. Smith, A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge, Environ. Int. 35 (2009) 142-156.
[2]V. Chaignon, I. Sanchez-Neira, P. Herrmann, B. Jaillard, P. Hinsinger, Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area, Environ. Pollut. 123 (2003) 229-238.
[3]N. Mirlean, A. Roisenberg, J.O. Chies, Metal contamination of vineyard soils in wet subtropics (Southern Brazil), Environ. Pollut. 149 (2007) 10–17.
[4]M.P. Mourato, L.L. Martins, M.P. Campos-Andrada, Physiological responses of *Lupinus luteus* to different copper concentrations, Biol. Plant 53 (2009) 105–111.
[5]S. Rossini Oliva, M.D. Mingorance, B. Vladés, E.O. Leidi, Uptake, localisation and physiological changes in response to copper excess in *Erica andevalensis*, Plant Soil 328: (2010) 411-420.
[6]Z.H. Cao, Z.Y. Hu, Copper contamination in paddy soils irrigated with wastewater, Chemosphere 41(2000) 3–6.
[7]FAO, FAOSTAT, 2008. Disponível em: <http://faostat.fao.org/faostat>. Access: December 12, 2011.
[8]H.-Y. Lai, K.-W. Juang, B.-C. Chen, Copper concentrations in grapevines and vineyard soils in central Taiwan, Soil Sci. Plant Nutr, 56 (2010) 601–606.
[9]X.Y. Guo, Y.B. Zuo, B.R. Wang, J.M. Li, Y.B. Ma, Toxicity and accumulation of copper and nickel in maize plants cropped on calcareous and acidic field soils, Plant Soil 333 (2010) 365–373.
[10]C.P. Rooney, F-J. Zhao, S.P. Mcgrath, Soil factors controlling the expression of copper toxicity to plants in a wide range of European soils, Environ. Toxicol. Chem. 25 (2006) 726-732.
[11]G. Weng, L. Wu, Z. Wang, Y. Luo, P. Christie, Copper uptake by four *Elsholtzia* ecotypes supplied with varying levels of copper in solution culture, Environ. Int. 31 (2005) 880-884
[12]R.X. Cao, L.Q. Lena, M. Chen, S.P. Singh, W.G. Harris, Phosphateinduced metal immobilization in a contaminated site, Environ. Pollut. 122 (2003) 19-28.
[13]Y.-J. Lee, E. George, Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply, Plant Soil 278 (2005) 361-370.
[14]L.C. Prezotti, C.A.S. Carmo, A.P.M. Andrade Neto, Nutrição mineral da batata, EMCAPA, Vitória, 1986.
[15]L. Bailian, S.E. Mckeand, H.L. Allen, Genetic variation in nitrogen use efficiency of lobeolly pine seedlings, Forest Science 37 (1991) 613-626.
[16]J.P. Lynch, K.M. Brown, Topsoil foraging – an architectural adaptation of plants to low phosphorus availability, Plant Soil 237 (2001) 225-237.
[17]G. Potters, T.P. Pasternak, Y. Guisez, K.J. Palme, M.A.K. Jansen, Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci. 12 (2007) 98-105.
[18]K.-W. Juang, H.-Y. Lai, B.-C. Chen, Coupling bioaccumulation and phytotoxicity to predict copper removal by switchgrass grown hydroponically, Ecotoxicology 20 (2011) 827–835.
[19]CQFSRS/SC- Comissão de Química e Fertilidade do Solo, Manual de adubação e calagem para os Estados do Rio Grande do Sul e de Santa Catarina, tenth ed., UFRGS, Porto Alegre, 2004.
[20]EMBRAPA- Empresa Brasileira de Pesquisa Agropecuária, Manual de métodos de análise de solo, second ed., Rio de Janeiro,1997.

[21]M.J. Tedesco, C. Gianello, C. Bissani, H. Bohnen, S.J. Volkweiss, Análise de solo, plantas e outros materiais, second ed., Universidade Federal do Rio Grande do Sul, Porto Alegre, 1995.
[22]US EPA. Method 1640: Determination of trace metal elements in ambient waters by on-line chelation preconcentration and inductive coupled plasma-mass spectrometry. U.S. Environmental Protection Agency, Office of Water, Engineering and Analysis Division, Washington, D.C. 1996.
[23]J. Murphy, J.P. Riley, A Modified single solution method for determination of phosphate in natural waters, Anal. Chem. Acta 27 (1962) 31-36.
[24]J. L. Burkhead, K. A. Gogolin Reynolds, S. E. Abdel-Ghany,C. M. Cohu, M. Pilon, Tansley review Copper homeostasis, New Phytologist 182 (2009) 799–816.
[25]Badoni, J.S. Chauhan importance of potato micro tuber seed material for farmers of Uttarakhand Hills, Int. J. Sustainable Agriculture 2 (2010) 01-09.
[26]H. Marschner, Mineral Nutrition of High Plants, second ed., Academic Press, London, 2003.
[27]E. Girotto, Alterações fisiológicas e bioquímicas em plantas cultivadas em solos com acúmulo de cobre e zinco, Tese Curso de Doutorado do Programa de Pós-Graduação em Ciência do Solo, UFSM, 2010.
[28]J.C. Fernandes, F.S. Henriques, Biochemical, physiological, and structural effects of excess copper in plants, Bot. Rev. 57 (1991) 246-273.
[29]H. Lequeux, C. Hermans, S. Lutts, N. Verbruggen, Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile, Plant Physiol. Biochem. 48 (2010) 673-82.
[30]S.L. Brown, R.L. Chaney, J.S. Angle, A.J.M. Baker, Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution, Soil. Sci. Soc. Am. J. 59 (1995) 125-133.
[31]M. Kaldorf, A.J. Kuhn, W.R. Schröder, U. Hildebrandt, H. Bothe, Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus, J. Plant Physiol. 154 (1999) 718-728.
[32]B.H. Adhikary, K.B. Karki, Effect of potassium on potato tuber production in acid soils of Malepatan, Pokhara, Nepal Agric. Res. J. 7 (2006) 42-48.
[33]M-P. Faucon, G. Colinet, G. Mahy, M.N. Luhembwe, N. Verbruggen, P. Meerts, Soil influence on Cu and Co uptake and plant size in the cuprophytes *Crepidorhopalon perennis* and *C. tenuis* (Scrophulariaceae) in SC Africa, Plant Soil 317 (2009) 201–212.
[34]A. Kabata-Pendias, H. Pendias, Trace elements in soils and plants. 3.ed. Boca Raton,CRC Press, 2001. 413p.
[35]CONAMA – Conselho Nacional do Meio Ambiente. Resolução nº 420, de 28 de dezembro e 2009. http://www.mma.gov.br/port/conama/legiabre.cfm?codlegi=620/. Access january 20, 2010.
[36]B.J. Alloway, Heavy metals in soils, Blackie Academic & Professional, London, 1995
[37]V. Sancenón, S. Puig, I. Mateu-Andrés, E. Dorcey, D.J. Thiele, L. Peñarrubia, The *Arabidopsis* copper transporter COPT1 functions in root elongation and pollen development, J. Biol. Chem. 279 (2004) 15348-15355.
[38]S.J. Zheng, Y.F. He, Y. Arakawa, Y. Masaoka, C. Tang, A copper-deficiency-induced root reductase is different from the iron-deficiency-induced one in red clover (*Trifolium pretense* L.), Plant Soil 273 (2005) 69-76.
[39]D.Liu, I. Kottke, Subcellular localization of cadmium in the root cells of Allium cepa by electron energy loss spectroscopy and cytochemistry, J. Biosci. 29 (2004) 329-35.
[40]Yruela, Copper in plants, Braz. J. Plant Physiol. 17 (2005) 145-156.
[41]D. Jouvin, D.J. Weiss, T.F.M. Mason, M.N. Bravin, P. Louvat, F. Zhao, F. Ferec, P. Hinsinger, M.F. Benedetti, Stable isotopes of Cu and Zn in higher plants: evidence for Cu

reduction at the root surface and two conceptual models for isotopic fractionation, Environ. Sci. Technol. 46 (2012) 2652-2660
[42]I.Mukherjee, N.H. Campbell, J.S. Ash , E.L. Connolly, Expression profiling of the Arabidopsis ferric chelate reductase (FRO) gene family reveals differential regulation by iron and copper, Planta, 23 (2006) 1178-90.
[43]M. L. Wu, Y. S. Wang, Using chemometrics to evaluate anthropogenic effects in Daya Bay, China, Estuarine, Coastal and Shelf Science, 72 (2007) 732–742.
[44]F.J.M. Maathuis, Physiological functions of mineral macronutrients, Curr. Opin. Plant Biol. 12 (2009) 250–258.
[45]P.J. White, The pathways of calcium movement to the xylem, J. Exp. Bot. 52 (2001) 891-899.
[46]F. Gaymard, G. Pilot, B. Lacombe, D. Bouchez, D. Bruneau, J. Boucherez, M. Michaux-Ferriere, J. Thibaud, H.Sentenac, Identification and disruption of a plant Shaker-like outward channel involved in K+ release into the xylem sap, Cell 94 (1998) 647-655.
[47]J.L.Hall, Cellular mechanisms for havy metal detoxification and tolerance, J. Exp. Bot. 53(2002) 1-11.
[48]P.T. Gil, P.C.R. Fontes, P.R. Cecon, F.A. Ferreira, Índice SPAD para o diagnóstico do estado de nitrogênio e para o prognóstico da produtividade da batata, Hortic. Bras. 20 (2002) 611-615.
[49]Manceau, A. Matynia, The nature of Cu bonding to natural organic matter, Geochimica et Cosmochimica Acta 74 (2010) 2556–2580.

DISCUSSÃO

O presente estudo visou a caracterização bioquímico-fisiológica de genótipos de batata submetidos à toxidez de Cu. Para tal, foram conduzidos experimentos no período de safra (de setembro a novembro) e safrinha (de março a maio) em cambisolos e argisolos oriundos de vinhedos com histórico de aplicação de fungicidas cúpricos.

A maior exposição ao Cu, embora com diferenças significativas entre genótipos, solos e estações de cultivo, em geral inibiu o crescimento da parte aérea bem como a produção de tubérculos, com maior inibição nos tratamentos Cambi VN2, Cambi VN3 e Ulti VN2, o que pode ser devido à inibição da divisão celular (FERNANDES e HENRIQUES, 1991), além de danos em nível de mebrana resultantes de peroxidação lipídica, e consequente à perda de íons (Van ZWIETEN et al., 2004; ROSINI OLIVA et al., 2010). Resultados semelhantes foram relatados em *Avena sativa* cultivada sob os mesmos solos testados por Girotto (2010).

Os genótipos pré-classificados como eficientes e não responsivos ao P (ENR) SMINIA793101-3 e SMIF212-3 foram capazes de expandir folhas e produzir tubérculos em todos os solos testados. Entretando, o genótipo não eficiente no uso porém responsivo ao P (NER) SMIE040-6RY teve sensibilidade acentuada ao excesso de Cu em parâmetros de crescimento. Neste genótipo, os efeitos à toxidez de Cu incluíram plantas sem folhas expandidas e sem produção de tubérculos. O crescimento e desenvolvimento vegetal dependem da eficiência de aquisição e utilização de nutrientes (BAILIAN et al., 1991). Fageria e Baligar (1993) consideraram a eficiência de utilização de P como sendo a produtividade sobre baixo nível e a eficiência de resposta o incremento na produção à aplicação de P. A classificação utilizada nos genótipos apresentados neste estudo seguiu esta metodologia, sendo então possível sugerir diferentes mecanismos de aquisição entre os genótipos testados, conferindo ou não eficiência de utilização e também de absorção do P. Através dos dados obtidos, parecem ocorrer interações entre o grau de eficiência (genótipos eficientes ou não) e toxidez por Cu. Interessantemente embora com diferenças entre os genótipos testados, a avaliação da resposta de batata à toxidez de Cu em termos nutricionais, em geral apresentou um padrão comum. Desta forma ao contrário do observado em parâmetros de crescimento, sistema oxidante e anti-oxidante, a análise multivariada da concentração de nutrientes em tecidos vegetais gerou grupos distintos de acordo com o solo testado, porém sem separar os genótipos. Esta informação reforça diferenças genotípicas não só de absorção e utilização de nutrientes, mas também do requerimento nutricional para um

crescimento ótimo das plantas.

Interessantemente, em geral não se observou efeito negativo da toxidez por Cu na massa seca radicular, ocorrendo ainda incremento da mesma no genótipo SMINIA793101-3 no período de safra nos solos Cambi, e safrinha nos solos Ulti. Esta resposta pode ser devida ao efeito hormético, que resulta em um crescimento compensatório em resposta a uma perturbação da homeostase celular (CALABRESE et al., 2003).

Outro ponto importante a ser destacado é a diferença marcante entre Argisolos e Cambisolos em termos físico-químicos, resultando, porém em uma resposta semelhante nos parâmetros de crescimento avaliados. Os Argisolos contaminados com Cu (Ulti VN1 e Ulti VN2) apresentaram concentrações de Cu aproximadamente seis vezes menores do que as encontradas em Cambisolos, o que pode ser explicado pela composição natural do solo. Os Argisolos testados apresentaram níveis mais baixos de matéria orgânica e argila, em comparação aos Cambisolos, e esta diferença pode ter sido determinante para os menores níveis de Cu acumulado. Estudos demonstraram que o teor de matéria orgânica desempenha um papel fundamental no controle de adsorção de metais pesados (LEE et al., 1998), e Cu é o metal preferencialente associado à fração orgânica (DRAGOVIC et al., 2008). Além disso, Rooney et al. (2006) relataram que baixas concentrações de argila e a matéria orgânica juntamente com acidez do solo estariam entre os melhores preditores para toxicidade de Cu em plantas de cevada e tomate.

É importante ressaltar que mesmo existindo uma considerável amplitude de variação do conteúdo de Cu entre Cambisolos e Argisolos, os efeitos em termos de crescimento, estresse oxidativo e concentração de Cu no tecido vegetal foram muito similares. O comportamento de metais é influenciado por atributos da fase sólida do solo, do tipo de adsorvente (matéria orgânica, silicatados, óxidos de ferro, os grupos de manganês e fosfato) e condições geoquímicas da solução, concentração de prótons e da força iônica (McBRIDE, 1994; ALLOWAY, 1995). Nos Argisolos testados neste estudo, matéria orgânica e CTC tinham valores notavelmente mais baixos, aproximalmente três vezes menor, em relação aos Cambisolos, o que foi determinante para uma menor diferença entre conteúdo total de Cu e Cu extraído por EDTA dos argisolos, devido a grande fração de Cu disponível, tendo assim uma grande capacidade de gerar dano. Adicionalmente, Girotto (2010) descreveu diferenças marcantes no teor de Cu solúvel sobre os mesmo solos testados, sendo o teor significamente maior em argisolos.

A relação de alta afinidade entre Cu e matéria orgânica provavelmente influenciou a disponibilidade de Cu na solução. Parte do P presente em solos com altos níveis de Cu

encontra-se precipitado com o mesmo, entretanto a afinidade do Cu é maior com a matéria orgânica em relação ao P. Como os cambisolos testados possuem uma grande quantidade de matéria orgânica, a formação do complexo P-Cu é reduzida, em relação a solos com pouca matéria orgânica como os Argisolos testados. Este fenômeno tem efeito direto na nutrição vegetal, o que explica a menor produção de tubérculos em Argisolos mesmo sobre doses baixas de Cu, além da redução na concentração de P no tecido. Nos Cambisolos observou-se uma resposta inversa, ocorrendo aumento na concentração de P com incremento de Cu. Através da análise de componentes principais, concentrações de P e Cu no tecido foram os principais nutrientes correlacionados à alta exposição de Cu, enquanto que em Argisolos, Fe e K foram mais correlacionados. Adicionalmente, mesmo sem estar agrupada com a concentração de Fe no tecido, a contaminação de Cu em Cambisolos, em geral aumentou a concentração de Fe no tecido, sugerindo uma absorção não-competitiva de Cu e Fe por plantas de batata.

Através do presente estudo, observou-se que o sistema anti-oxidante bem como os danos resultantes do estresse oxidativo estão diretamente ligados ao status nutricional da planta. Neste sentido, tanto a toxidez por Cu quanto a deficiência de P foram gatilho para ativação enzimática antioxidante em resposta a peroxidação lipídica e concentração de H_2O_2, conforme relatado por trabalhos anteriores (TEWARI, 2007; GIROTO, 2010). Além disso, a utilização de análise multivariada confirmou que o uso de folhas apicais e medianas é um bom preditor para toxidez de Cu, o que justifica a utilização da terceira e quarta folha para análises bioquímicas.

CONCLUSÃO

Em conclusão, Cambisolos de vinhedos da Serra Gaúcha e Argisolos da região da Campanha, com longo histórico de aplicação de fungicidas cúpricos, apresentam teores de Cu tóxicos para as plantas de batata. Além disso, nas concentrações mais elevadas de Cu, ocorreu dano oxidativo, evidenciado pelo aumento da peroxidação lipídica e conteúdo de H_2O_2 elevado, resultando na inibição do crescimento de plantas de batata. Esta resposta bioquímica, bem como a sensibilidade ao Cu dos parâmetros de crescimento variaram amplamente entre os genótipos, sendo os genótipos eficientes no uso de P menos sensíveis à toxidez de Cu. Embora com diferenças marcantes fisico-químicas, observou-se uma resposta semelhante em cambisolos e argisolos contaminados em termos de crescimento e estresse oxidativo. Além disso, os dados sugerem uma resposta semelhante à deficiência de P e toxicidade de Cu em termos de estresse oxidativo. Este estudo também fornece evidência de que os antioxidantes não são suficientes para evitar danos biológicos resultantes de EROS produzidos em concentrações mais elevadas de Cu, resultando em efeitos deletérios.

Nos parâmetros minerais, embora também tenha sido observada ampla variação entre os genótipos, sendo os genótipos eficientes no uso de P menos sensíveis ao excesso de Cu; através de análise multivariada os três genótipos apresentaram o mesmo padrão de resposta à toxidez, sendo agrupados independente da estação de cultivo testada. Em Cambisolos, as concentrações de P e Cu no tecido foram os principais nutrientes correlacionados à alta exposição de Cu, enquanto que em Argisolos, Fe e K foram mais correlacionados. Além disso, os nossos dados apresentam evidências de absorção não-competitiva de Cu e Fe por plantas de batata. Este estudo sugere a utilização da terceira e quarta folha expandidas para avaliação nutricional de plantas de batata, sendo considerados parâmetros indicativos das toxidez de Cu: em nível mineral, as concentrações de Fe, K, P e Cu; em nível bioquímico, as concentraçãos de H_2O_2 e atividade das enzimas SOD e CAT; bem como a produção de tubérculos em peso fresco ou seco para parâmetros de crescimento. No entanto, mais estudos a nível molecular, bioquímico e de campo são necessários para esclarecer os mecanismos envolvidos entre a nutrição mineral da batata e a toxidez de Cu.

REFERENCIAS BIBLIOGRÁFICAS

ABDEL-GHANY, S.E. Contribution of plastocyanin isoforms to photosynthesis and copper homeostasis in Arabidopsis thaliana grown at different copper regimes. **Planta.** v. 229, p. 767–779, 2009.

ABREU, C. A., et al. Total and available copper in some soil profile samples from the state of São Paulo. **Communications in Soil Science and Plant Analysis**, 2012.

ALLOWAY, B.J. **Heavy metals in soils**. Blackie Academic & Professional, London, 1995

ARIAS, M.; et al. Copper distribution and dynamics in acid vineyard soils treated with copper-based fungicides. **Soil Science**, v. 169, p. 796-805, 2004.

ARNON, D.I. Copper enzymes in isolated chloroplasts. Polyphenoloxidse in Beta vulgaris. **Plant Physiology**. v. 24, p. 1–15, 1949.

BAILIAN, L.; McKEAND, S.E.; ALLEN, H.L. Genetic variation in nitrogen use efficiency of lobeolly pine seedlings. **Forest Science**. v. 37, n. 2, p. 613-626, 1991.

BOWLER, C.; VAN MONTAGU, M.; INZE, D. Superoxide dismutase and stress tolerance. **Annual Review of Plant Physiology and Plant Molecular Biology.** v. 43, p. 83–116, 1992.

BROWN, S.L.; et al.. Zinc and cadmium uptake by hyperaccumulator Thlaspi caerulescens grown in nutrient solution. **Soil Science**. v. 59, p. 125–133, 1995.

BRUN, L.A.; et al. Relationships between extractable copper, soil properties sand copper uptake by wild plants in vineyard soils. **Environmental Pollution**. v.10,p.151-161, 1998.

CALABRESE, E.J. Evidence that hormesis represents an ‘‘overcompensation’’ response to a disruption in homeostasis, **Ecotoxicology and Environmental Safety**. v.42, p. 135–137, 1999.

CAO, R.X.; et al. Phosphateinduced metal immobilization in a contaminated site. **Environmental Pollution**. v. 122, p.19–28, 2003.

CARR, H.S.; WINGE, D.R. Assembly of cytochrome c oxidase within the mitochondrion. **Accounts of Chemical Research**. v.36, p. 309–316, 2003.

CASALI, et al . Formas e dessorção de cobre em solos cultivados com videira na Serra Gaúcha do Rio Grande do Sul. **Revista Brasileira de Ciência do Solo**. v. 32, n. 4, 2008 .

CASTRO, Y.G. Eficiência de absorção e utilização de fósforo em batata cultivada in vitro. 2009. 52f. Dissertação (Mestrado em Agronomia) – Programa de Pós-Graduação em Agronomia, Universidade Federal de Santa Maria.

COMISSÃO DE QUÍMICA E FERTILIDADE DO SOLO - CQFSRS/SC. **Manual de adubação e calagem para os Estados do Rio Grande do Sul e de Santa Catarina**. 10.ed. Porto Alegre, Sociedade Brasileira de Ciência do Solo - Núcleo Regional Sul/UFRGS, 2004. 400p.

DONG, J.; KIM, S.T.; LORD, E.M. Plantacyanin plays a role in reproduction in Arabidopsis. **Plant Physiology**. v.138, p. 778–789, 2005.

DRAGOVIC, S., MIHAILOVIC, N. and GAJIC, B. Heavy metals in soils: Distribution, relationship with soil characteristics and radionuclides and multivariate assessment of contamination sources. **Chemosphere**, v. 72, p. 491-495, 2008.

FAGERIA, N.D.; BALIGAR, V.C. Screening crop genotypes for mineral stresses. In: INTSORMIL. Publication n. 94-2. **Proccedings of the workshop on adaptation of plants to soil stresses**. Lincoln: University of Nebraska, 1993.

FERNANDES, J.C.; HENRIQUES, F.S. Biochemical, physiological, and structural effects of excess copper in plants. **The Botanical Review.** v.57, p. 246-273, 1991.

FOX, R.H. Selection for phosphorus efficiency in corn. **Communications in Soil Science and Plant Analysis**, v.9, p. 13-37, 1978.

GIROTTO E. **Alterações fisiológicas e bioquímicas em plantas cultivadas em solos com acúmulo de cobre e zinco**. Tese Curso de Doutorado do Programa de Pós-Graduação em Ciência do Solo. UFSM, 2010.

GUO, W.J.; MEETAM, M.; GOLDSBROUGH, P.B. Examining the specific contributions of individual Arabidopsis metallothioneins to copper distribution and metal tolerance. **Plant Physiology**. v. 146, p. 1697–1706, 2008.

KALDORF, M.; et al. Selective element deposits in maize colonized by a heavy metal tolerance conferring arbuscular mycorrhizal fungus. **Journal of Plant Physiology**. v. 154, p. 718–728, 1999.

KIM, S.; et al. Chemocyanin, a small basic protein from the lily stigma, induces pollen tube chemotropism. **Proceedings of the National Academy of Sciences**. v.100, p. 16125–16130, 2003

KLIEBENSTEIN, D.J.; MONDE, R.A.; LAST, R.L. Superoxide dismutase in Arabidopsis: an eclectic enzyme family with disparate regulation and protein localization. **Plant Physiology**. v. 118, p. 637–650, 1998.

KOCHIAN, L.V. Cellular mechanisms of aluminum toxicity and resistance in plants. Annual **Review of Plant Physiology and Molecular Biology**, v. 46, p.237-260, 1995.

LARGE, E. C. **The Advance of The Fungi**. New York: Holt, 1940, 488p.

LEE SZ, CHANG L, YANG HH, CHEN CM, LIU MC. Adsorption characteristics of lead onto soils. **Journal of Hazard Materials**. v. 63, p. 37–49, 1998.

LEE, Y-J.; GEORGE, E. Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply. **Plant Soil**, v.278, p. 361–370, 2005.

LINDER, M.C.; GOODE, C.A. **Biochemistry of copper**. New York, USA: Plenum Press, 1991

LIPPARD, S.J.; BERG, J.M. **Principles of bioinorganic chemistry**. New York, USA: University Science Books, 1994.

MARINA, M.; et al. Apoplastic polyamine oxidation plays different roles in local responses of tobacco to infection by the necrotrophic fungus Sclerotinia sclerotiorum and the biotrophic bacterium Pseudomonas viridiflava. **Plant Physiology**. v. 147, p. 2164–2178, 2008.

MARSCHNER H. **Mineral nutrition of higher plants**. London, UK: Academic Press, 1995.

MAYER, A.M. Polyphenol oxidases in plants and fungi: going places? **A review. Phytochemistry**. v. 67, p. 2318. 2006

McBRIDE, M.B. **Environmental chemistry of soils**. New York, Oxford University Press, 1994. 406p.

MENCH, M., MOREL, J.L., GUCKERT, A. Action des métaux [Cd(II), Cu(II), Pb(II), Zn(II)] sur la production d'exsudats racinaires solubles chez le maïs (*Zea mays* L.). **Agronomie,** v. 8, p. 237–241,1987.

MENCH, M. Transfert des oligo-éléments du sol à la racine et absorption. **Comptes Rendus de l'Académie d'Agriculture de France**, v.76, n. 2, p. 17–30, 1990.

OLIVEIRA, T. S. **Metais pesados como indicadores de materiais de origem de solos**. Viçosa: Universidade Federal de Viçosa. 1996.

PANOU-FILOTHEOU, H.; BOSABALIDIS, A.M.; KARATAGLIS, S. Effects of copper toxicity on leaves of oregano (Origanum vulgare subsp. hirtum). **Annals of Botany**. v. 88, p. 207-214, 2001.

PARAT, C.; et al. The relationship between copper accumulated in vineyard calcareous soils and soil organic matter and iron. **European Journal of Soil Science**. v. 53, p. 663-669, 2002.

PESARESI, P.; et al. Mutants, overexpressors, and interactors of Arabidopsis plastocyanin isoforms: revised roles of plastocyanin in photosynthetic electron flow and thylakoid redox state. **Molecular Plant**. v. 2, p. 236–248, 2009.

PIETRZAK, U.; MCPHAIL, D. C. Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. **Geoderma**, v. 122, p. 151–66, 2004.

PIGNOCCHI, C.; et al. The function of ascorbate oxidase in tobacco. **Plant Physiology**. v. 132, p.1631–1641, 2003.

RAGHOTHAMA, K.G. Phosphate acquisition. **Annual Review of Plant Physiology and Plant Molecular Biology**, v. 50, p. 665-693, 1999.

RODRIGUEZ, F.I.; et al. A copper cofactor for the ethylene receptor ETR1 from Arabidopsis. **Science**. v. 283, p. 996–998, 1999.

ROONEY, .P.; ZHAO, F-J.; MCGRATH, S.P. Soil factors controlling the expression of copper toxicity to plants in a wide range of european soils. **Environmental Toxicology and Chemistry.** v. 25, p. 726–732, 2006;

ROSSINI OLIVA, S.; et al..Uptake, localisation and physiological changes in response to copper excess in *Erica andevalensis.* **Plant Soil.** v. 328, p. 411-420, 2010.

SCHUBERT, M.; et al. Proteome map of the chloroplast lumen of Arabidopsis thaliana. **Journal of Biological Chemistry**. v. 277, p. 8354–8365, 2002.

SEIDEL, E. P.; COSTA, A. C. S. da; LANA, M. C. Fitodisponibilidade de cobre e produção de matéria seca por plantas de milho em resposta à aplicação de dejetos de suínos. **Revista Brasileira Ciência do Solo**, v. 33, n. 6, p.1871-1878, 2009

SIDDIQI, M.Y.; GLASS, A.D.M. Utilization index: a modified approach to the estimation and comparison of nutrient utilization efficiency in plants. **Journal of Plant Nutrition**, v.4, n.3, p. 289-302, 1981.

TABALDI, L.A.; et al. Micronutrient concentration in potato clones with distinct physiological sensitivity to Al stress. **Ciência Rural**, v. 39, p. 379-385, 2009.

TEWARI R.; et al. Macronutrient deficiences and differential antioxidant responses: influence on the activity and expression of superoxide dismutase in maize. **Plant Science.** v. 166, p. 687-694, 2004;

THIPYAPONG, P.; JOEL, D.M.; STEFFENS, J.C. Differential expression and turnover of the tomato polyphenol oxidase gene family during vegetative and reproductive development. **Plant Physiology**. v. 113, p. 707–718, 1997.

TILLER, K. G. Heavy metals in soil and their environmental significance. **Advances in Soil Science.** v. 9, p. 113–142, 1989.

Van ZWIETEN, L et al. Influence of copper fungicide residues on occurrence of earthworms in avocado orchard soils. **The Science of the Total Environment.** v. 329, p. 29-41, 2004.

WEIGEL, M.; et al. Plastocyanin is indispensable for photosynthetic electron flow in Arabidopsis thaliana. **Journal of Biological Chemistry**. v. 278, p. 31286–31289, 2003.

WELCHEN, E.; CHAN, R.L.; GONZALEZ, D.H.. The promoter of the Arabidopsis nuclear gene COX5b-1, encoding subunit 5b of the mitochondrial cytochrome c oxidase, directs tissue-specific expression by a combination of positive and negative regulatory elements. **Journal of Experimental Botany**. v. 55, p.1997–2004, 2004.

YADAV, R.S.; TARAFDAR, J.C. Phytase and phosphatase producing fungi in arid and semi-arid soils and their efficiency in hydrolyzing different organic P compounds. **Soil Biology and Biochemistry**, v. 35, p. 745-751, 2003.

YRUELA, I. Copper in plants. **Brazilian Journal of Plant Physiology**. v. 17, n. 2, p. 145-156, 2005.