ÂNGELA MARIA PEREIRA DO NASCIMENTO

BENEFICIAL MICROORGANISMS IN GROWTH PROMOTION AND STRESS TOLERANCE IN PETUNIA PLUGS

LAVRAS-MG
2017

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/ Fitotecnia, área de concentração em Produção Vegetal, para a obtenção do título de Doutor.

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RESUMO GERAL

A petúnia é uma espécie comumente propagada por plugs, sendo utilizada comercialmente em vasos e canteiros, apresentando variedade de cores e floração abundante. Como forma de aprimorar a produção de plugs, o uso de bactérias promotoras de crescimento de plantas, pode proporcionar melhor crescimento e resistência a elas. Com essa finalidade, se destacam as bactérias do gênero *Pseudomonas* spp., em especial as produtoras de auxinas e ACC deaminase, por promoverem o crescimento e reduzir sintomas de estresse causados pelo etileno. Dessa forma, objetivou-se identificar e avaliar a utilização de estirpes de *Pseudomonas* spp. na promoção de crescimento e resistência a estresse hídrico em plugs de petúnias. Estirpes de *Pseudomonas* spp. foram selecionadas para detecção de atividade de ACC deaminase, identificação da melhor época de aplicação, e efeito da aplicação desses microrganismos na promoção de crescimento e indução de resistência das mudas em condições de estresse hídrico. Maior atividade de ACC deaminase foi observada para *P. putida* UW4+, *P. poae* 29G9 e *P. brassicacearum* Delaware. A pulverização das soluções contendo essas bactérias aplicadas no plantio, e no terceiro dia após semeadura, promoveu maior desenvolvimento da parte aérea e do sistema radicular dos plugs. Dentre as estirpes estudadas, não foi observado efeito de promoção de resistência para plantas submetidas a estresse no estágio de plugs. Porém, o uso das estirpes de *Pseudomonas* contribuiu para o desenvolvimento das plantas no estágio de plugs, acelerando a floração e promovendo a formação de maior número de flores em plantas que não foram submetidas a estresse no estágio de plugs.

Palavras-chave: *Pseudomonas* spp. Propagação. Bactérias Promotoras de Crescimento. Plantas Ornamentais. Estresse hídrico.

GENERAL ABSTRACT

Petunia is a specie usually propagated by plugs, being used commercially in pots and flowerbeds, presenting variety of colors and abundant flowering. To improve plugs production, the use of plant growth promoting bacteria (PGPR) can improve growth and resistance to plants. With this purpose, we highlight the bacteria of genus *Pseudomonas* spp., specially the auxin and ACC deaminase producers for growth promotion and reducing stress symptoms caused by ethylene. Thus, the objective was to identify and evaluate the use of strains of *Pseudomonas* spp. in growth promotion and water stress in petunias plugs. Strains of *Pseudomonas* spp. were selected for the detection of ACC deaminase activity, identification of the best application time and effect of the application of these bacteria in growth promotion and resistance under water stress conditions. Greater activity of ACC deaminase was observed for *P. putida* UW4 +, *P. poae* 29G9 and *P. brassicacearum* Delaware. The spraying of the solutions containing these bacteria applied at sowing and third day after sowing promoted greater development of shoots and root system of the plugs. Among the studied strains, no resistance promoting effect was observed for plants submitted to stress in plugs stage. However, the use of *Pseudomonas* strains contributed to the development of plugs, accelerating flowering and producing more flowers in plants that were not submitted to stress in the plugs stage.

Keywords: *Pseudomonas* spp. Propagation. Plant growth promoting bacteria. Ornamental plants. Water stress.

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PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

As petúnias estão entre as espécies de flores anuais mais comercializadas no Brasil, e são apreciadas devido a grande variedade de cores e floração abundante, sendo muito utilizadas na formação de canteiros maciços, bordaduras, e em vasos.

Uma das formas de propagação de petúnias é por meio de plugs, que apresentam como vantagens a alta qualidade, uniformidade, qualidade fitossanitária e grande porcentagem de sobrevivência pós-transplante. Por outro lado, devido ao pequeno volume de solo, é comum que durante os processos de transporte e armazenamento, os plugs sejam expostos a condições de altas temperaturas e irregularidade nas regas, promovendo rápido secamento do substrato e murcha das plantas (WATERLAND et al., 2010). Essas condições de estresse promovem alterações bioquímicas e fisiológicas que afetam o desenvolvimento das plantas (AHMAD et al., 2014).

Com o intuito de se obter mudas com alto padrão de qualidade, muitos reguladores de crescimento são utilizados, objetivando-se a produção de mudas compactas e com sistema radicular bem desenvolvido, o que contribui para a maior resistência às condições de estresse hídrico. A maioria desses reguladores são produtos químicos, que apresentam alta toxidez e riscos de contaminação ambiental. Dessa forma, o desenvolvimento de técnicas de manejo envolvendo produtos biológicos é de grande importância para esse setor, sendo a utilização de microrganismos benéficos, uma alternativa promissora.

Nesse contexto, as bactérias promotoras de crescimento constituem um grande potencial, sendo um grupo variado com diversas características, como a capacidade de promoção de crescimento das raízes, indução de resistência contra patógenos, além de produção de fitormônios variados (VERBON; LIBERMAN, 2016).

Dentre essas bactérias benéficas, as do gênero *Pseudomonas* se destacam na promoção de crescimento e resistência ao estresse hídrico (MAYAK; TIROSH; GLICK, 2004a presença de metais pesados (HAN et al., 2015) e salino (CHENG; PARK; GLICK, 2007),), podendo ser utilizadas em grandes culturas e espécies olerícolas. No entanto, não há informações sobre o uso desses microrganismos na produção de mudas do tipo plug, nem a utilização em espécies floríferas. Pelos resultados positivos encontrados em outros sistemas de manejo e espécies, essa poderia ser uma inovação na produção de mudas ornamentais.

Dessa forma, objetivou-se identificar estirpes de *Pseudomonas* com potencial para promoção de crescimento, e indução de resistência ao estresse em mudas do tipo plugs de petúnias.

2 REFERENCIAL TEÓRICO

2.1 Produção de mudas de espécies anuais ornamentais

A produção de mudas, sementes e bulbos, se destaca na cadeia produtiva de flores e plantas ornamentais no Brasil. No estado de Minas Gerais, a produção de mudas para jardim corresponde à maior área e volume de plantas dentro do segmento da floricultura (LANDGRAF; PAIVA, 2009), sendo que esse segmento tem crescido e agregado novas tecnologias, cultivares e insumos aos processos produtivos.

Nesse contexto, as flores anuais se destacam, sendo amplamente utilizadas na confecção de jardins, em razão do grande número de flores que produzem, pela variedade de cores, rápido crescimento, e por causa do longo período de florescimento (GROLLI, 2008). Devido ao ciclo rápido, são substituídas todos os anos nos jardins, representando uma demanda constante por novas plantas.

Plantas anuais são aquelas que completam o ciclo de vida em um ano, incluindo a germinação das sementes, crescimento, produção de flores e sementes. Essas plantas são, em sua maioria, herbáceas de pequeno porte, com altura inferior a 50 cm (LORENZI; SOUZA, 2008).

A maioria das espécies anuais é propagada por sementes, destacando-se amor-perfeito, begônia, dália, impatiens, petúnia, sálvia e zinia (GROLLI, 2008).

A produção de mudas de plantas anuais apresenta quatro estágios principais, que exigem procedimentos e insumos específicos: no estágio 1, ocorre o início da germinação e emissão da primeira radícula, exigindo umidade elevada; no estágio 2, ocorre a emergência da radícula, do hipocótilo e dos cotilédones, aumentando a necessidade de oxigênio; no estágio 3, surgem as folhas verdadeiras e, no estágio 4, as mudas estão prontas para transplante (STYER; KORANSKI, 1997). Devido aos diferentes procedimentos, insumos e necessidades das mudas em cada estágio, é comum que os produtores se especializem na formação inicial das mudas (estágios 1 e 2) ou na finalização das mudas para comercialização ou transplante para vasos e jardins (estágios 3 e 4) (GROLLI, 2008).

Outro aspecto importante na produção de espécies anuais é o substrato utilizado. No mercado, existem substratos específicos para a produção de mudas em bandejas, os quais já vêm prontos para uso. Muito produtores, visando um melhor aproveitamento

dos resíduos disponíveis nas propriedades rurais, desenvolvem os próprios substratos, o que pode ser uma alternativa viável e mais sustentável (COSTA; COSTA; PEREIRA, 2014). Nesses casos, são necessários cuidados especiais para se evitar proliferação de patógenos e sementes de plantas daninhas.

Diversos recipientes podem ser utilizados na produção de mudas, sendo mais comuns os saquinhos plásticos e bandejas. Dentre as bandejas, as multicelulares do tipo plug são as mais utilizadas (STYER; KORANSKI, 1997).

2.2 Produção de mudas do tipo plug

Plugs referem-se a mudas em tamanho reduzido que apresentam o sistema radicular totalmente enovelado no torrão (SCHMITT et al., 2012).

Dentre as vantagens da produção de plugs cita-se o menor tempo e trabalho para transplantio, uniformidade e rápido crescimento, redução das perdas após transplantio e melhor aproveitamento das sementes e do espaço (STYER; KORANSKI, 1997), além da maior facilidade de controle e manejo de pragas e doenças (GIMENEZ et al., 2008).

A produção de mudas do tipo plug normalmente é realizada de forma mecanizada, incluindo a semeadura, plantio e transplantes, apresentando grande produtividade por área, sendo amplamente utilizado na produção de flores (STYER; KORANSKI, 1997).

Há grande variedade de tamanhos de bandejas para produção de plugs, sendo que a formação ideal das raízes é variável de acordo com a cultura, tamanho da célula, tipo de substrato, bem como o manejo de uma forma geral, que pode afetar tanto o desenvolvimento da muda quanto o desempenho após transplantio (LATIMER, 1991). As mudas são produzidas em um volume muito pequeno de substrato, o qual pode secar rapidamente em condições de casa de vegetação, com redução da fotossíntese e, em alguns casos, ocasionar a morte das mudas (BURNETT; THOMAS; IERSEL, 2005).

No que diz respeito à produção de plugs, outro aspecto importante são as condições de armazenamento e transporte. Muitas vezes, o produtor necessita armazenar as mudas por um período específico de tempo para atender as demandas de mercado (KUBOTA; SEIYAMA; KOZAI, 2002), ou em função da disponibilidade de equipamentos e mão de obra (SATO et al., 2004).

2.3 Petúnias

Petúnias (*Petunia x hybrida* hort. Vilm.-Andr) são flores nativas da América do Sul, encontradas principalmente na Argentina, Brasil e Uruguai, pertencentes à família Solanácea. Podem ser cultivadas a pleno sol, em vasos, jardineiras ou em canteiros (LORENZI; SOUZA, 2008). Existe uma grande diversidade de variedades de petúnias, sendo as grandifloras as mais comuns, apresentando flores grandes e bom desenvolvimento para cultivo em vasos suspensos (DOLE; WILKINS, 1999).

A propagação de petúnias é realizada por sementes, podendo ser cultivadas o ano todo. Para a produção de plugs de petúnias são utilizadas sementes peletizadas, pois possuem tamanho muito reduzido (STYER; KORANSKI, 1997).

Para a germinação, recomenda-se a temperatura de 20 a 24 ºC e 100 % de umidade até a emergência dos cotilédones. Após, ajusta-se a temperatura para 18-21 ºC e a umidade pode ser reduzida gradualmente até 50%. A fertilização no estágio de plugs deve ser feita com 50 ppm de 15-0-15 quando ocorre a emergência das raízes, após a expansão dos cotilédones, aplica-se 100-150 ppm (DOLE; WILKINS, 1999). Após o plug estar totalmente formado, as mudas devem ser transplantadas para substrato bem drenado e esterilizado, para evitar a contaminação por doenças.

2.4 Bactérias promotoras de crescimento de plantas

Bactérias que estabelecem interações benéficas com as plantas são classificadas em dois grupos: as denominadas simbiontes que induzem à formação de estruturas especializadas e nódulos; e bactérias promotoras de crescimento, que são de vida livre e encontradas na rizosfera das plantas (FROMMEL; NOWAK; LAZAROVITS, 1991).

As bactérias promotoras de crescimento podem atuar de forma direta ou indireta sobre o desenvolvimento das plantas. O efeito indireto é descrito quando reduzem ou previnem efeitos deletérios de organismos fitopatogênicos por um ou mais mecanismos. Também podem atuar diretamente na promoção de crescimento sintetizando ou facilitando a absorção de nutrientes do meio ambiente e sintetizando fitormônios e enzimas que contribuem nos mais variados mecanismos de desenvolvimento das plantas (GLICK, 2014).

Com essas características, destacam-se os gêneros *Azospirillum, Bacillus, Burkholderia* e *Pseudomonas,* que já foram isolados e adicionados a formulações comerciais, como é o caso de bacilos para controle biológico. Esses microrganismos promovem efeitos significativos no aumento da altura de plantas, comprimento de raízes e produção de massa seca em algumas culturas (BHATTACHARYYA; JHA, 2012).

Algumas espécies de bactérias promotoras de crescimento têm a capacidade de aumentar a disponibilidade de nutrientes para as plantas. Estirpes dos gêneros *Enterobacter*, *Pantoea* e *Klebsiella* apresentam potencial de solubilizar fontes inorgânicas de fósforo disponibilizando o fósforo para absorção das plantas, ou atuando na fixação de nitrogênio (SOUZA; AMBROSINI; PASSAGLIA, 2015).

Bactérias promotoras de crescimento de plantas também vêm sendo amplamente estudadas em situações de estresse hídrico, principalmente em regiões áridas em função das mudanças climáticas e escassez de água (GLICK, 2014). Essas bactérias apresentam atividade de ACC deaminase e produção de auxinas que auxiliam no controle da produção de etileno em condições de estresse.

2.4.1 Bactérias com atividade de ACC deaminase e síntese de auxinas

As raízes de diversas plantas liberam exsudatos que contém ACC (1- amino-ciclopropano-1-carboxilato), atraindo as bactérias promotoras de crescimento para a rizosfera e estabelecendo a interação raiz-bactéria (PENROSE; GLICK, 2003). Bactérias contendo ACC deaminase são relativamente comuns no solo, sendo encontradas em diferentes ambientes (GLICK, 2014).

Bactérias que apresentam atividade ACC deaminase hidrolisam o ACC em alfa-ketobutirato e amônia. Assim, bactérias que apresentam atividade de ACC deaminase utilizam o ACC como fonte de nitrogênio, diminuindo, consequentemente, a produção de etileno pelas plantas (PENROSE; GLICK, 2003) .

Em condições de estresse, as plantas produzem altos níveis de etileno, afetando a homeostase e, consequentemente, reduzindo crescimento das raízes e parte aérea. Estudos realizados com bactérias isoladas de regiões áridas (GLICK, 2014), comprovaram que a atividade de ACC deaminase de algumas bactérias reduziu os sintomas de estresse causados pelo etileno possibilitando que a planta recuperasse o seu desenvolvimento (VAN LOON; BAKKER; PIETERSE, 1998).

A síntese de auxinas por bactérias contribui para melhorar o desenvolvimento do sistema radicular, além de auxiliar em alguns processos de defesa das plantas (SOUZA; AMBROSINI; PASSAGLIA, 2015). Algumas bactérias secretam ácido indol acético (AIA), ao utilizar o triptofano exsudato pelas raízes das plantas como precursor para síntese desse fitormônio (DUCA et al., 2014).

Auxinas secretadas pelas bactérias da rizosfera atuam em conjunto com as endógenas das plantas. O efeito pode ser positivo ou negativo, dependendo das concentrações produzidas e secretadas, além da sensibilidade dos tecidos das plantas a esse fitormônio (ALI; SABRI; HASNAIN, 2010).

Auxinas sintetizadas por bactérias podem estimular a proliferação celular e alongamento das células, e também podem induzir a transcrição da enzima ACC sintase que catalisa a formação de ACC. Nesse caso, a auxina contribui para síntese de etileno nas plantas, inibindo seu crescimento (GLICK, 2014). No entanto, a redução de crescimento por níveis de auxinas não é observada, uma vez que o aumento da produção de etileno pela planta submetida a estresse inibe o sinal de transdução de auxinas limitando a interferência da auxina na transcrição de ACC sintase (GLICK; CHENG; CZARNY, 2007).

Ressalta-se que a ACC oxidase possui maior afinidade com ACC do que com a ACC deaminase, dessa forma, a eficiência do tratamento com bactérias sintetizadoras de ACC deaminase é dependente da relação das concentrações dessas duas enzimas (GLICK; CHENG; CZARNY, 2007).

2.4.2 Bactérias e a síntese de etileno

A síntese de etileno pelas plantas é afetada por fatores como temperatura, luz, gravidade, nutrição, fitormônios e condições de estresse (TAIZ; ZEIGER, 2013).

A síntese de etileno é elevada em condições de estresse como seca, inundação, resfriamento, exposição ao ozônio ou danos mecânicos. Nessas situações, o etileno é produzido por uma rota comum, e o aumento da produção pode ser atribuído, em parte, pelo aumento na transcrição do mRNA da ACC sintase. O etileno produzido em situações de estresse induz a abscisão foliar, senescência, geração de lesões e resistência a doenças (TAIZ; ZEIGER, 2013).

A síntese de etileno como resposta a condições de estresse inclui dois picos de produção, primeiramente ocorre o consumo do ACC existente nos tecidos das plantas

(GLICK; CHENG; CZARNY, 2007). No segundo pico, que pode ocorrer alguns dias após o primeiro, a planta já produziu mais ACC em resposta ao estresse, iniciando os processos de senescência, clorose e abscisão foliar. Nesse sentido, qualquer tratamento que possa reduzir a magnitude desse segundo pico de produção de etileno pode reduzir a magnitude dos danos causados por situações de estresse (GLICK, 2014).

2.4.3 Bactérias do gênero *Pseudomonas*

O gênero *Pseudomonas* é constituído por mais de 100 espécies, divididas em linhagens, grupos e subgrupos. Constituem um dos grupos mais estudados pela capacidade de promoção de crescimento de plantas, atuando na ciclagem de matéria orgânica, promoção de resistência a estresses bióticos e abióticos e degradação de compostos xenobióticos (MAYAK; TIROSH; GLICK, 2004b).

As *Pseudomonas* spp. atuam na defesa das plantas contra patógenos, devido a produção de compostos antimicrobianos, competição e indução de mecanismos de defesa. Dentre as *Pseudomonas*, se destacam as fluorescentes que apresentam mais de 50 espécies, e têm recebido destaque devido a sua versatilidade, capacidade de colonizar raízes das plantas, produzir enzimas e outros metabólicos favoráveis ao desenvolvimento de algumas espécies de plantas (MAYAK; TIROSH; GLICK, 2004b).

É importante ressaltar, que existem variações, pois, por exemplo, algumas estirpes de *P. fluorescens* têm atividade de promoção de crescimento, mas outras estirpes, isoladas em outras condições, não apresentam os mesmos efeitos nas plantas (GLICK; CHENG; CZARNY, 2007).

Dentre os efeitos da aplicação de diferentes espécies e estirpes de *Pseudomonas* cita-se a utilização de *Pseudomonas stutzeri* A1501 (HAN et al., 2015), *Pseudomonas putida* UW4 (CHENG; PARK; GLICK, 2007) e *Pseudomonas putida MTCC5279* (TIWARI et al., 2016), na indução de resistência a metais pesados, salinidade e estresse hídrico, respectivamente.

2.4.4 Interação planta bactéria

As bactérias promotoras de crescimento podem colonizar a rizosfera, folhas ou habitar em superfícies internas dos tecidos das plantas estabelecendo relações endofíticas. As bactérias endofíticas normalmente formam nódulos em algumas plantas

hospedeiras específicas, sendo amplamente estudadas (WANG; MARTINEZ-ROMERO, 2000) e encontradas em produtos comerciais.

A efetividade da utilização dessas bactérias na agricultura, é determinada por fatores como espécie estudada, tipo de solo, pH, fertilidade do solo, temperatura, exsudatos produzidos e outros fatores ambientais (SOUZA; AMBROSINI; PASSAGLIA, 2015). Apesar do grande potencial desses microrganismos, informações acerca da identificação, mecanismos de ação, aplicação e formulações ainda são escassas e exploradas por poucos sistemas de produção.

2.5 Estresse hídrico

O estresse hídrico é um fator limitante para o crescimento e produtividade de muitas culturas, principalmente em regiões semiáridas (LIM; KIM, 2013). Devido ao pequeno volume de solo e as dificuldades relacionadas ao transporte e armazenamento de plugs, é comum que esse tipo de muda também seja muito suscetível ao estresse hídrico.

A exposição das plantas a condições de estresse hídrico gera alterações morfológicas mediadas pela ação de auxinas, espécies reativas de oxigênio e etileno. Normalmente, observa-se a inibição de crescimento e ocorre maior ramificação das raízes e parte aérea (TOBERGTE; CURTIS, 2013).

Estresse abióticos como a deficiência hídrica induzem a uma série de respostas nas plantas, como mudança na expressão de genes, metabolismo celular, índice de crescimento e produtividade. A duração e a severidade do estresse a que a planta é exposta determina as variações fisiológicas de resposta (AHMAD et al., 2014).

Uma das principais respostas adaptativas das plantas ao estresse hídrico é a redução da área foliar, para diminuição das perdas de água pela evapotranspiração. Ainda, a redução da parte aérea contribui para o balanço osmótico na planta em função da redistribuição dos solutos nos tecidos (CLAEYS; INZE, 2013). Também, plantas submetidas a estresse hídrico apresentam aumento na área ocupada pelas raízes, como estratégia para melhor absorção de água do solo (TAIZ; ZEIGER, 2013).

O tratamento das plantas com bactérias promotoras de crescimento aumenta o crescimento da parte aérea das plantas, mantendo os níveis de produtividade mesmo em condições de estresse (NGUMBI; KLOEPPER, 2016), como já observado em tomate e

pimenta (MAYAK; TIROSH; GLICK, 2004a), grão-de-bico (TIWARI et al., 2016) e milho (NAVEED et al., 2014).

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 Increased development of petunia in plugs by the inoculation of plant growth-promoting bacteria

RESUMO

A utilização de bactérias promotoras de crescimento apresenta inúmeras potencialidades, destacando-se a promoção de crescimento e resistência a condições de estresses bióticos e abióticos em plantas. Objetivou-se avaliar o efeito de 4 estirpes de *Pseudomonas* na promoção de crescimento de petúnias produzidas em plugs, bem como determinar o melhor estádio para inoculação. Sementes de petúnia híbrida 'Carpet White' foram dispostas em bandejas do tipo plug e pulverizadas com soluções de *Pseudomonas putida* UW4+, *Pseudomonas poae* 29G9 e *Pseudomonas brassicacearum* Delaware e *Pseudomonas fluorescens* 48D1, mais um tratamento controle com pulverização de água destilada. Foram testados 4 períodos de aplicação: somente após a semeadura, após semeadura + terceiro dia, após semeadura + sétimo dia, após semeadura + décimo quarto dia. Vinte e oito dias após plantio, as mudas foram avaliadas quanto ao índice de crescimento, número de folhas, massa fresca e seca da parte aérea e raízes e estudos da morfologia das raízes, por meio da determinação do diâmetro médio e área total ocupada pelas raízes com auxílio do software WhinRhizo®. Foram observados efeitos benéficos da aplicação de bactérias promotoras de crescimento em plugs de petúnia. O maior desenvolvimento da parte aérea e sistema radicular ocorreu com a aplicação das estirpes no plantio e no terceiro dia. As estirpes *Pseudomonas putida* UW4+, *Pseudomonas poae* 29G9 e *Pseudomonas fluorescens* 48D1 contribuíram para o maior desenvolvimento da parte aérea, área total e diâmetro das raízes. *Pseudomonas brassicacearum* Delaware promoveram maior acúmulo de massa nas raízes a parte aérea mais compacta para plugs de petúnia 'Carpet White', características desejáveis para esse tipo de muda.

Palavras-chave: *Pseudomonas* spp. *Petunia hybrida*. Produção de mudas. Plantas anuais.

ABSTRACT

The use of plant growth-promoting bacteria has several potentialities, highlighting growth promotion and resistance to biotic and abiotic stress conditions in plants. The aim of this study was to evaluate the effect of four strains of *Pseudomonas* in the growth promotion of petunias produced in plugs, as well as to determine the best stage for inoculation. Seeds of 'Carpet White' hybrid petunia were arranged in plug-type trays and sprayed with solutions of *Pseudomonas putida* UW4+, *Pseudomonas poae* 29G9, *Pseudomonas brassicacearum* Delaware and *Pseudomonas fluorescens* 48D1, plus a control treatment with spraying of distilled water. Four application periods were tested: only after sowing, after sowing + third day, after sowing + seventh day, after sowing + fourteenth day. After 28 days of planting, the seedlings were evaluated regarding the growth index, number of leaves, shoot and root fresh and dry weight, and root morphology studies, by determining the average diameter and total area occupied by the roots with the aid of WhinRhizo® software. Beneficial effects on the application of plant growth-promoting bacteria on petunia plugs were observed. The greatest development

of shoot and root system occurred with the application of strains in the planting and on the third day. The strains *Pseudomonas putida* UW4+, *Pseudomonas poae* 29G9 and *Pseudomonas fluorescens* 48D1 contributed for the greater development of shoot, total area and diameter of roots. *Pseudomonas brassicacearum* Delaware promoted greater mass accumulation in the roots with more compact shoot for 'Carpet White' petunia plugs, which are desirable characteristics for this type of seedlings.

Keywords: *Pseudomona* spp. *Petunia hybrida*. Seedling production. Annual plants.

1 INTRODUCTION

Annual flowers represent an important niche in floriculture agribusiness in Brazil, standing out petunias, heartseases, tagetes, geraniums, impatiens, dahlias, gardenias and zinnias. The propagation of these species is a process that includes many inputs, such as seeds, trays, substrates and growth regulators, besides the generation of jobs due to the high need for labor.

The commercialization of seedlings currently is made by plugs, because they are considered of high quality, being compact, with the roots completely tangled in the cell of tray, resistant stem and high root biomass (RANDALL et al., 2014). In order to obtain these characteristics, procedures are performed in order to reduce the shoot of plants and to promote improvements in the morphology and quantity of roots, often through the mediation of chemical regulators. In this context, the use of biological products is promising for growth modulation, mainly by the use of beneficial microorganisms, which are highly efficient and do not damage the environment.

Several microorganisms, mainly fungi and bacteria, interact with plants, promoting growth and resistance to diseases. Among the main mechanisms used by the microorganisms in these interactions are auxin synthesis, ACC deaminase synthesis, and leaching and nutrient supply (GLICK, 2014).

Some bacteria, using L-tryptophan exuded by the plant roots, synthesize indole-3-acetic acid (IAA) (SOUZA; AMBROSINI; PASSAGLIA, 2015), which acts in the development of root system by increasing lateral roots and primary roots that allow better absorption of water and nutrients (DUCA et al., 2014), thus contributing to plant growth.

Another mechanism used by these microorganisms is the synthesis of ACC deaminase that reduces the synthesis of ethylene under stress conditions, allowing developing plants even under adverse conditions (GLICK, 2014).

The genus *Pseudomonas* sp. is one of the largest groups that contains naturally occurring plant growth-promoting bacteria in the soil promoting diverse benefits for plants (SRIVASTAVA et al., 2012). The growth promotion mediated by the inoculation of *Pseudomonas* spp. in plants has already been observed in crops, such as tomato and cucumber (SARAVANAKUMAR; SAMIYAPPAN, 2007), chick-pea (TIWARI et al., 2016), and pepper (MAYAK; TIROSH; GLICK, 2004a).

Besides the identification of these microorganisms, it is essential to establish the best method of inoculation, as well as the appropriate stage for the application of microorganisms to guarantee the success of interaction and growth and resistance promotion. Factors as root exudation, colonization ability and soil characteristics should be considered, since they influence the effectiveness of plant-bacterial interaction (SOUZA; AMBROSINI; PASSAGLIA, 2015).

The aim of this study was to evaluate the effect of plant growth-promoting bacteria on the development of plug-seedlings of petunias, and to determine the most appropriate stage for the application of these microorganisms, aiming the production of compact seedlings with a well-developed root system.

2 MATERIAL AND METHODS

Seeds of 'Carpet White' hybrid petunia were arranged in a 128-cell plug tray filled with commercial Promix PGX® substrate, based on peat moss.

Four periods for application of *Pseudomonas* were tested: only after sowing, after sowing + third day, after sowing + seventh day, after sowing + fourteenth day. The seasons were chosen according to the development stages of petunia plugs, being the third day corresponding to the radicle emission, the seventh to the total expansion of cotyledons, and the fourteenth to the presence of the first fully expanded leaves.

The applied strains were *Pseudomonas putida* UW4+, *Pseudomonas poae* 29G9, and *Pseudomonas brassicacearum* Delaware with ACC deaminase activity and *Pseudomonas fluorescens* 48D1 with potential for auxin synthesis, whereas the control treatment consisted only of distilled water.

For the preparation of bacterial solutions, plates were grown from the stocks stored in a freezer at -80 ºC, using Luria Bertani (LB) medium plus agar (SAMBROOK; FRITSCHI; MANIATIS, 1989). The plates were incubated at 28 °C for 24 h. After this period, a single colony was added to the liquid LB medium and cultured for another 12 h, at 28 °C and 250 rpm. Bacterial growth was measured through the absorbance of culture. The final solution was diluted to the concentration 1 x 10^7 CFU mL^{-1}, and 3 ml of the final solution was applied per cell with the aid of previously calibrated sprays. Spray inoculation was adopted due to the viability and suitability of plug production process, since it is a practice commonly adopted by producers.

After the sprays, all the trays were covered with transparent lids for 24 h. The trays were kept in an air-conditioned oven for 28 days with daytime temperature from 21.1 to 24.4 °C, night temperature from 15.5 to 18.3 °C and photoperiod of 14h of light.

Moreover, they were irrigated daily with distilled water. In the second week, the application of the fertigation started with NPK15-5-15 Ca+- Mg, at the concentration of 75 ppm and 150 ppm in the third and fourth weeks, respectively, always with two weekly applications.

Seedlings were collected and evaluated 28 days after planting in relation to growth index, number of leaves, number of lateral shoots, fresh and dry weight of shoot and roots, and root morphology studies.

The calculation of the growth index (GI) was performed based on measurements of diameters (D1, D2) and height (H) of seedlings, calculated by the formula GI= [(D1+D2/2)+H]/2 (NIU; RODRIGUEZ; STARMAN, 2010).

For root morphology studies, the substrate was carefully removed from the roots, which were washed, scanned and evaluated using the software WhinRhizo®. The average diameter and the total area occupied by the roots were determined.

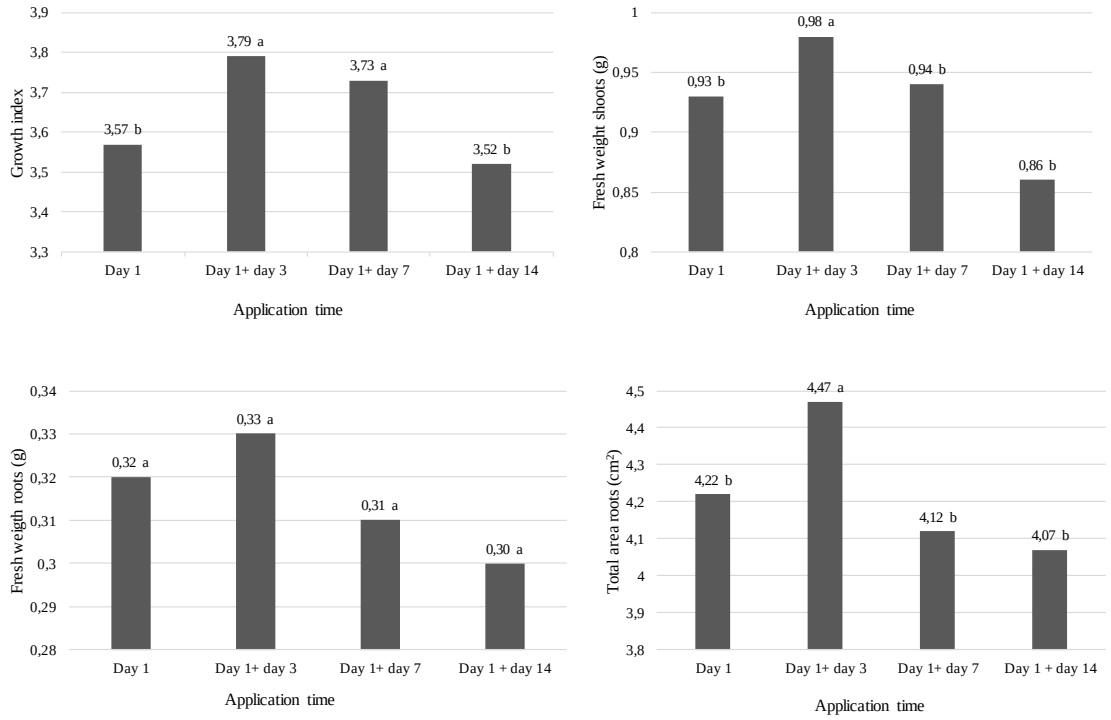
The experiment was conducted in a completely randomized design (CRD), in factorial design 5 x 4 (treatments x application times), being each replicate composed of four plugs.

The data were submitted to analysis of variance and the Scott-Knott test at 5% probability was performed when differences were observed.

3 RESULTS AND DISCUSSION

The inoculation time of different strains from *Pseudomonas* (FIGURE 1) affected the development of shoot and root system of the 'Carpet White' petunia.

Figure 1 - Growth index, fresh weigth (g) of shoot and roots and total area occupied by roots of 'Carpet White' petunia plugs inoculated with *Pseudomonas* at different times. Averages followed by the same letter belong to the same group by Scott-Knott test at 5% probability.

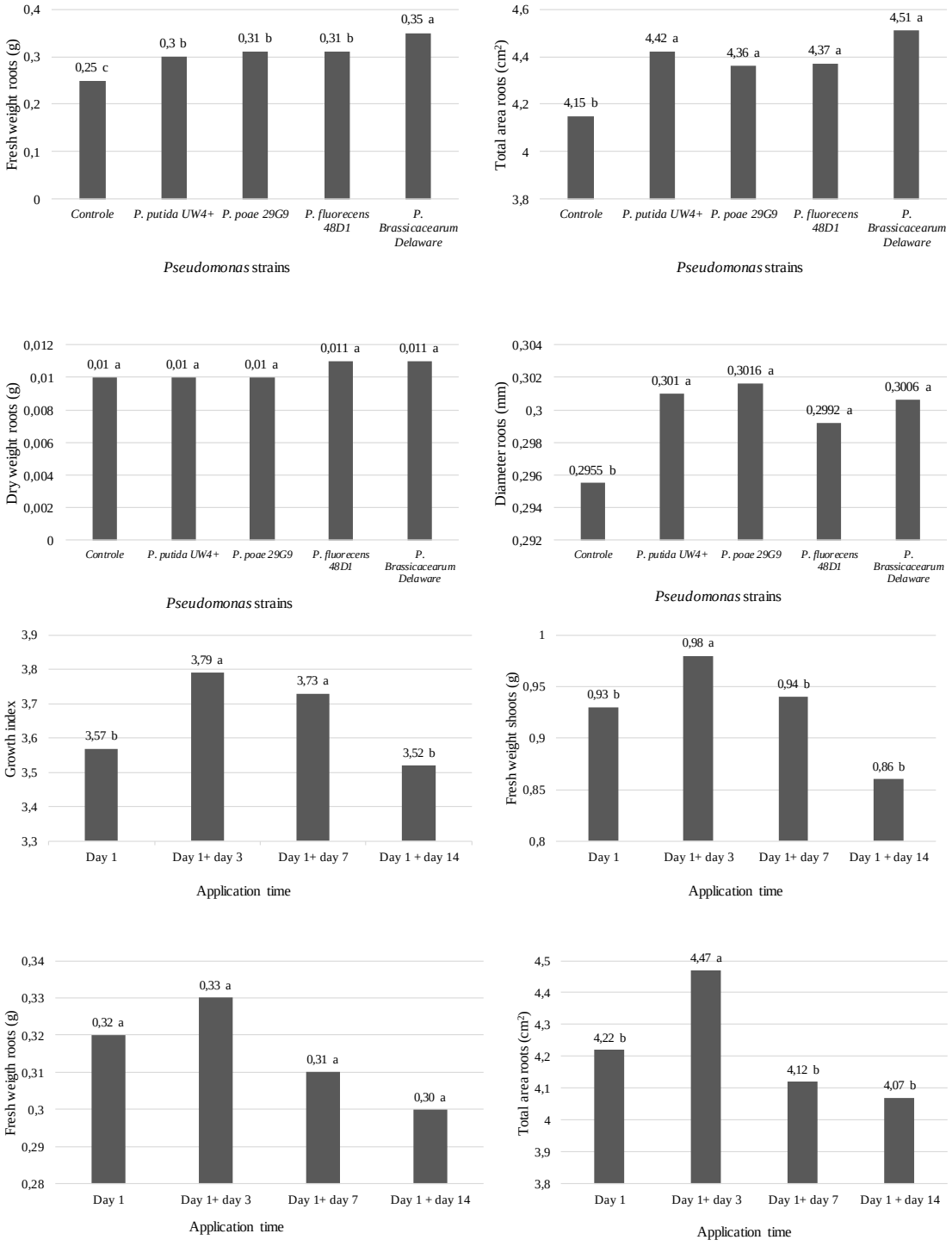


Higher growth index, fresh shoot weight and total area occupied by roots were observed when inoculation was performed on the first and third days after planting. In the production of petunia plugs, in the third day occurs the stage of radicle emergence, so it can be inferred that the greatest development of plants inoculated at that time was due to the interaction between the roots of seedlings and bacteria in the initial stage development of petunia plugs.

Plant hormone production that contribute to plant development, such as auxins, are influenced by the stage of development and availability of substrates exuded by roots (SHOKRI; EMTIAZI, 2010), and when plant growth-promoting bacteria effectively interact with the plant roots, biomass gains are usually observed (COOK, 2002).

Effect of the different *Pseudomonas* strains was observed on the root system development of 'Carpet White' petunia (FIGURE 2).

Figure 2 - Fresh weight (g), total area occupied by roots (cm^2), dry weight (g), and root diameter (mm) of 'Carpet White' petunia plugs inoculated with different strains of *Pseudomonas.* Averages followed by the same belong to the same group by Scott-Knott test at 5% probability.

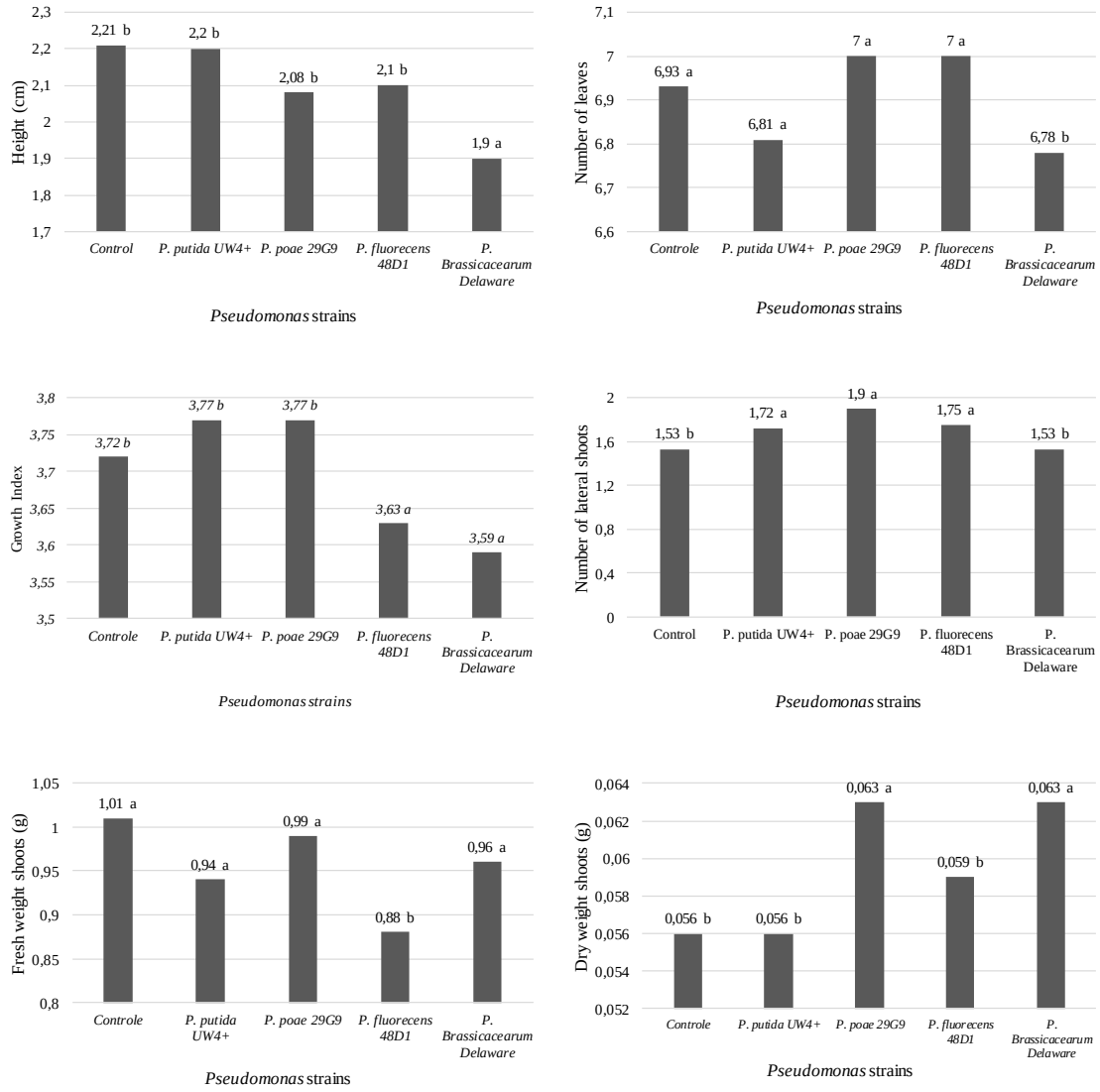


Regarding the accumulation of root fresh weight, the highest values were observed in plants inoculated with the *Pseudomonas brassicacearum* Delaware strain. Considering the root morphology, the plants inoculated with all strains of *Pseudomonas* showed higher values of total area and average root diameter compared to the control treatment. The modifications in the root system induced by these microorganisms are due to the production of auxins, inhibition of the ethylene synthesis or mineralization of nutrients (STEENHOUDT; VANDEREYDEN, 2000).

Plant growth-promoting bacteria affect root development of plants by altering cell division and differentiating primary roots and developing lateral roots (VERBON; LIBERMAN, 2016), resulting in a more branched root system architecture (BHATTACHARYYA; JHA, 2012). This higher branching of lateral roots, observed by the greater area occupied by roots, is extremely advantageous in the production of plugs, since it contributes to the twisted roots in the clod, facilitating the mechanized transplant and survival of seedlings. Different strains of *Pseudomonas* also provided higher development of root system in other species, such as tomato and cucumber (SARAVANAKUMAR; SAMIYAPPAN, 2007).

Inoculation of different strains of *Pseudomonas* also affected the shoot development of the 'Carpet White' petunia plugs (FIGURE 3).

Figure 3 - Height (cm), number of leaves, growth index, number of lateral shoots, fresh (g) and dry weight(g) of shoots of 'Carpet White' petunia plugs inoculated with different strains of *Pseudomonas*. Averages followed by the same letter belong to the same group by Scott-Knott test at 5% probability.



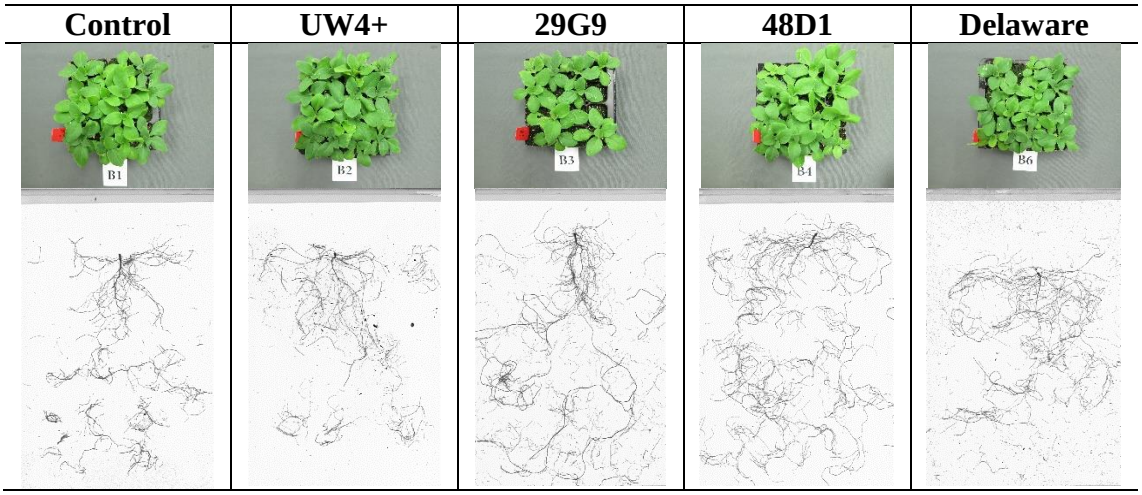
Petunia plugs treated with *Pseudomonas brassicacearum* Delaware showed lower values for height, number of leaves, growth index and lateral shoots, being plants more compact, which is highly desirable with respect to the production of seedlings per plugs. Furthermore, they showed the better characteristics for the root morphology (Figure 2), modulating plant growth in a desirable way with respect to the production of plugs.

Petunia plants inoculated with *P. putida* strain UW4+ showed high growth index, with similar results found on the application of this strain in rape plants (CHENG; PARK; GLICK, 2007).

The inoculation with beneficial bacteria contributes to the development of the root system and hence can increase the shoot of plants due to the higher absorption of water and nutrients (VERBON; LIBERMAN, 2016). On the other hand, it is also possible that the shoot reduction is due to the use of photoassimilates for the root formation. The inoculation with *Pseudomonas brassicacearum* Delaware contributed to the compaction of plants, however, they were also the plugs that showed higher root development. Thereby, the shoot reduction can be attributed to the higher root development.

The application of *Pseudomonas* strains contributed to higher development of root system and shoot reduction of the 'Carpet White' petunia plugs compared to the control treatment (FIGURE 4).

Figure 4 - Shoot and root system of 'Carpet White' petunia plugs treated with *Pseudomonas* strains at sowing + third day.



In the plug production segment, plants with well-developed roots and twisted in the clod are desired, since contribute mainly to the survival of plants after transplant (STYER; KORANSKI, 1997). Moreover, seedlings should be compact, since larger plants are more difficult to handle, making transportation more expensive and less appreciated by consumers (BURNETT; THOMAS; IERSEL, 2005). The inoculation of the *Pseudomonas brassicacearum* Delaware strain was used to modulate the size of 'Carpet White' petunia plugs, promoting a good development of the root system and

plugs with compact shoot. In this way, it is a potential microorganism for use in formulations of biological products that, besides efficient, are not toxic to plants, do not contaminate the environment and is safe for use.

4 CONCLUSIONS

The application of solutions with bacteria from the genus *Pseudomonas* promotes greater development of shoot and root system of 'Carpet White' petunia plugs when performed at planting and on the third day after sowing.

Plants treated with *Pseudomonas brassicacearum* Delaware have desirable characteristics for the production of compact plugs and with a well-developed root system.

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ARTIGO 2 - ACC deaminase-synthesizing bacteria in the control of abiotic stress in petunia plugs

RESUMO

Bactérias que apresentam atividade de ACC deaminase, apresentam potencial de promoção de crescimento e atenuação de sintomas de estresse em plantas, uma vez que diminuem as concentrações de etileno, principalmente em condições de estresse. Dessa forma, objetivou-se selecionar bactérias do gênero *Pseudomonas* com atividade de ACC deaminase e avaliar o efeito da aplicação dessas bactérias em plugs de petúnia submetidos a simulações de estresse hídrico, bem como do desenvolvimento inicial das plantas em vasos. Dentre as estirpes estudadas, *P. putida* UW4+, *P. poae* 29G9 e *P. brassicacearum* Delaware apresentaram atividade de ACC deaminase, sendo selecionadas para aplicação nas mudas, juntamente com os produtos comerciais Actinovate ® e Environoc ®, além do tratamento controle, pulverizado apenas com água destilada. As plantas foram submetidas à simulação de transporte pelo armazenamento em câmara fria, simulação de armazenamento em casa de vegetação sem irrigação e um controle, sem aplicação de estresse. Após aplicação de estresse, as plantas foram avaliadas quanto à massa fresca e seca da parte aérea e conteúdo relativo de água. As mudas foram transplantadas para avaliar desenvolvimento inicial das plantas em vaso, sendo avaliados quanto ao índice de crescimento, massa fresca e floração. Mudas e plantas submetidas a condições de estresse apresentaram menores valores de crescimento. Plugs submetidos à simulação de transporte apresentaram ganhos de massa fresca quando inoculados com *P. putida* UW4+, *P. poae* 29G9 e *P. brassicacearum* Delaware e produtos comerciais avaliados. Plugs não submetidos a estresse apresentaram maior número de flores e florescimento mais rápido quando tratados com *P. putida* UW4+ ou *P. poae* 29G9.

Palavras-chave: *Petunia hybrida*. Bactérias promotoras de crescimento de planta. Propagação. Plantas anuais.

ABSTRACT

Bacteria that show ACC deaminase activity have potential for promoting growth and attenuation of stress symptoms in plants, since they decrease ethylene concentrations, especially under stress conditions. Thus, the aim was to select bacteria from the genus *Pseudomonas* with ACC deaminase activity and to evaluate the application effect of these bacteria on petunia plugs subjected to water stress simulations, as well as the initial development of the potted plants. Among the studied strains, *P. putida* UW4+, *P. poae* 29G9 and *P. brassicacearum* Delaware showed ACC deaminase activity, being selected for application in the seedlings, together with the commercial products Actinovate ® and Environoc®, besides the control treatment, spread only with distilled water. Plants were submitted to simulation of transport by cold chamber storage, simulation of storage in a greenhouse without irrigation, and a control without stress application. After applied the stress, the plants were evaluated in relation to shoot fresh and dry weight and relative water content. The seedlings were transplanted in order to

evaluate the initial development of potted plants, being evaluated in relation to growth index, fresh weight and flowering. Seedlings and plants subjected to stress conditions showed lower growth values. Plugs subjected to the transport simulation showed fresh weight gains when inoculated with *P. putida* UW4+, *P. poae* 29G9 and *P. brassicacearum* Delaware and evaluated commercial products. Non-stressed plugs showed higher number of flowers and faster flowering when treated with *P. putida* UW4+ or *P. poae* 29G9.

Keywords: *Petunia hybrida*. Plant growth-promoting bacteria. Propagation. Annual plants.

1 INTRODUCTION

Plant growth-promoting bacteria are microorganisms that can protect against pathogens, provide nutrients, and produce plant hormones and enzymes (LUCY; REED; GLICK, 2004). These bacteria are found in the rhizosphere and show varied action mechanisms and interactions with plants.

Many of these bacteria have already had their action mechanisms elucidated and are found in commercial products, such as the genera *Azospirillum, Bacillus* and *Burkholderia*. Some bacteria from the genus *Pseudomonas* have also been identified with potential for growth promotion, but there are still few studies and records of their commercial use in commercial formulations.

One of the mechanisms used by plant growth-promoting bacteria is the reduction of ethylene levels produced under stress situations by plants. These bacteria synthesize the enzyme ACC deaminase that hydrolyzes 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene, to alpha-ketobutyric acid and ammonia (GLICK, 2014), besides using these nitrogen compounds to synthesize proteins, thus reducing the ethylene synthesis by plants and stimulating resistance to stress (PENROSE; GLICK, 2003).

In the production processes, the plants are subjected usually to stress conditions, especially water. In the case of seedling production, the producer often needs to store the seedlings for a specific period to meet the market demands (KUBOTA; SEIYAMA; KOZAI, 2002) or due to the availability of equipment and labor (SATO et al., 2004). Moreover, during transport and storage in retail, the irrigation of plants is not adequate (WATERLAND, et al., 2010), with losses occurring due to water stress conditions.

Among the commercialized seedlings, the plug type stand out because they are miniaturized, showing the root system very well-developed and totally twisted in the

clod. Among the advantages are the shorter planting time and work, uniformity, rapid growth, reduced losses after transplanting, better use of seeds and space (STYER; KORANSKI, 1997), besides favoring storage and transport conditions.

In the floriculture segment, petunia is one of the main species propagated by plugs. It is an annual plant widely used due to the diversity of colors and abundant flowering, which makes it important for the Brazilian market of ornamental plants (FERRAZ M. V.; CEREDA M.P., 2010).

The aim of this study was to select *Pseudomonas* species of ACC deaminase, the application effect of these bacteria on petunia plugs subjected to water stress conditions and the effect of these microorganisms on the initial development of plants and flowering.

2 MATERIAL AND METHODS

2.1 Selection of strains containing ACC deaminase

A total of 15 *Pseudomonas* strains were selected from the collection of microorganisms from the Department of Plant Pathology of the Ohio Agricultural Research and Development Center (OARDC) in the United States. The studied strains were *P. putida* UW4+, *P. putida* UW4-, *P. poae* 29G9, *P. poae* 36C8, *P. brassicacearum* Wood 3, *P. brassicacearum* 36B7, *P. brassicacearum* 36D4, *P. brassicacearum* 93F8, *P. brassicacearum* Wood 1, *P. brassicacearum* Delaware, *P. frederiksbergensis* 94G2, *P. frederiksbergensis* 36C6, *P. lini* 48C10, *P. protogens* 15G2, and *P. fluorescens* 36G2.

The bacteria were previously cultured in plates containing Luria Bertani (LB) medium (SAMBROOK et al., 1989) and agar, incubated at 28 °C for 18 h. A single colony was added to tubes containing 3 mL of TSB medium (Tryptic Soy Broth) and cultured at 28 °C for further 48 h. After growth in liquid medium, the pellet was suspended and washed in minimal DF medium (DWORKIN; FOSTER, 1958) and centrifuged again. The pellet was resuspended in minimal DF medium plus 3 mM of ACC, incubated for further 24 h at 200 rpm for 24 h. The cells were then washed and suspended in Tris-HCl plus toluene and incubated in water bath for 15 min at 30 °C. After incubation, the absorbance was measured at 600 nm (PENROSE; GLICK, 2003).

ACC deaminase activity is measured through the amount of alpha-ketobutyric acid produced when the enzyme cleaves ACC. By comparing the absorbance at 600 nm of a previously generated standard curve with concentrations from 0.1 to 1.0 μmol of alpha-ketobutyric acid, it is possible to determine the value of this substance, allowing estimating ACC deaminase activity.

2.2 Growth curves

After selection of strains with higher activity of ACC deaminase, growth curves were made to determine the culture time. New plates were initiated from glycerol stocks maintained at -80 °C by inoculation in plates containing LB medium. The plates were incubated at 28 °C for 18 h.

A single colony was isolated from the solid medium and added to 50 ml of liquid LB medium, homogenized for 1 min on a stirrer table at 250 rpm to determine the initial concentration of bacteria. From the initial solution, 1.0 mL was removed to determine the absorbance at 600 nm, and then serial dilutions and culture in plates for counting of colony-forming units (CFU) were performed using the microdroplet technique (ROMEIRO, 2001).

The flasks containing liquid medium were returned to incubator and were kept under stirring at 250 rpm and 28 ºC. Every 3 h, 1.0 mL of each solution was withdrawn and the absorbance and CFUs were measured in order to generate the growth curves. Nine samples were collected, corresponding to the 24-h period.

2.3 Treatment and preparation of solutions

Strains were grown on plates containing LB culture medium to grow and placed in air-conditioned oven at 28 °C for 24 h. After this period, an isolated colony was added to 10 mL of liquid LB medium and subjected to stirring at 250 rpm for 10 h. The bacterial growth was measured by reading the absorbance at 600 nm and the application solutions were diluted in distilled water and sprayed immediately.

The solutions were diluted to the concentration of 1 x 10^7 CFU/ml and the commercial products were diluted according to the manufacturers' recommendations, using the concentrations 1 x 10^7 CFU/ml for Actinovate SP ® and 1 x 10^9 CFU/mL for Environoc ®.

Treatments consisted of spraying the strains *P. putida UW4+*, *P. poae 29G9*, and *P. brassicacearum* Delaware. In addition, the commercial products Actinovate SP ® (biofungicide based on *Streptomyces lydicus*, WYEC108) and EnviroNoc ® (biostimulant containing 23 microorganisms) were also evaluated, whereas the control treatment consisted of spraying with distilled water.

Two applications were performed: the first on the sowing time and the second was three days after sowing. According to previous studies, these stages correspond to the best time for inoculation by spraying, since it occurs on the radicle emission, favoring the colonization of roots by the bacteria.

The spray application was adopted because it is the most viable alternative for plug production, since the processes are mostly automated. The sprayers were previously calibrated and adjusted to apply 3 ml of solution per cell. After application of treatments, the trays were covered for 24 h with transparent plastic caps to create a wet chamber.

2.4 Greenhouse assays

Pelleted seeds of 'Carpet White' hybrid petunia were arranged in a 128-cell plug tray containing commercial Promix Flex® substrate, based on peat moss. The trays were covered with transparent plastic caps until the emergence and kept in greenhouses with daytime temperature from 21.1 to 24.4 ºC and night temperature from 15.5 to 18.3 ºC and 14 h-photoperiod. After 28 days, when the plants reached the commercialization stage of plugs, they were subjected to water stress simulations.

According to the development stage, the seedlings were fertigated with NPK15-5-15 water-soluble fertilizer added with calcium and magnesium, in two weekly applications, starting in the second week, using a concentration of 75 ppm, whereas the concentration of 150 ppm was used in the third and fourth weeks.

2.5 Simulations of water stress

When they reached the commercial stage, 28 days after sowing, the plugs were subjected to transport simulations and storage in greenhouse without irrigation for water stress induction, whereas the control treatment kept receiving irrigation.

Simulation of transport conditions was performed at growth chamber. The trays were covered with transparent plastic caps used in the commercialization of seedlings, accommodated in transport boxes and kept in growth chambers at 15 ºC for three days, without irrigation.

For induction of water stress and simulation of retail conditions, the plants were kept in greenhouse under 50% shading and maintained without irrigation for three days.

After evaluations, all the seedlings were irrigated again to evaluate the recovery of plugs after stress.

Two plants per treatment per replicate were transplanted into vessels after water stress recovery. We used 0.5 L pots containing commercial Promix BX ® substrate, based on vermiculite, perlite and peat moss. The plants were kept in the same environmental conditions as the plugs and fertigated weekly with NPK 15-5-15 added with calcium and magnesium (Jacks fertilizer ®) at the concentration of 150 ppm.

2.6 Reviews

After stress induction, the plants were submitted to biomass analysis by determination of shoot fresh and dry weight and relative water content (RWC).

To determine the RWC, destructive samples of three plants were collected per replicate, being collected a pair of leaves from each of them. Immediately after harvest, the leaves were weighed to determine the fresh weight on a precision scale. They were then placed in a Petri dish containing distilled water for a period of 24 h. After this period, the excess water was removed with sheets of paper towel and weighed again (turgid weight). The samples were conditioned in Kraft paper bags and taken to a convection oven at 60 ºC for 24 h, being weighed again to obtain the dry weight.

The RWC calculation was determined by the equation RWC= [(FW-PS)/(TW-DW)]x100, where FW = fresh weight, DW = dry weight, and TW = turgid weight (KRAMER, 1983), the results are expressed as a percentage.

After transplanting, an evaluation was performed when all plants emitted at least five flowers, which corresponds to a commercial reference used for petunias in the initial potting stage. Growth index, shoot fresh weight, shoot dry weight and chlorophyll content measured and determined with the aid of chlorophyll meter (SPAD Model 503, Minolta). The flowers were evaluated in relation to number, diameter, fresh and dry weight, besides the date of the first flowering.

The growth index was calculated based on the measurements of shoot diameter (D1, D2) and height (H) in the plants. The index was determined by the formula GI= [(D1+D2/2)+H]/2 (NIU; RODRIGUEZ; STARMAN, 2010).

2.7 Experimental design and statistical analysis

Trays of 128-cell plug were cut in eight parts, where each experimental unit consisted of one part with 32 cells, from which four plants were selected for growth analyses, three plants for destructive analyses and two for transplantation and evaluation of the initial growth stage in pots.

The plug experiment consisted of a factorial design 6 x 3 (treatments x storage conditions), in which six treatments and three stress conditions were evaluated, with four replicates. The post-transplant experiment followed the same design using two pots with one plant per replicate.

The data were submitted to analysis of variance and Scott-Knott test at 5% probability.

3 RESULTS AND DISCUSSION

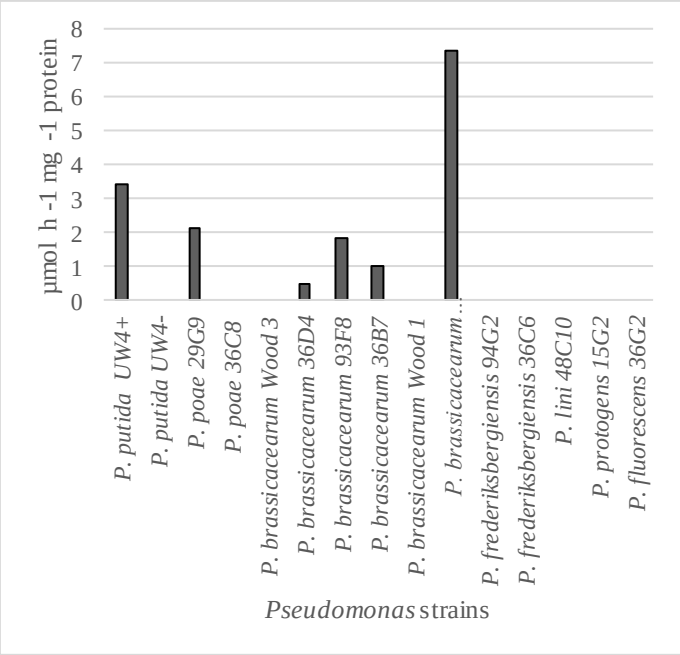
Among the studied *Pseudomonas* strains, bacteria with ACC deaminase activity that influenced the development of the petunia plants were identified at the plug stage and initial flowering. The different forms of storage also affected the plant development.

3.1 Identification of strains and growth curves

Among the 15 tested strains, *P. putida* UW4 +, *P. Poae* 29G9, *P. brassicacearum* 36D4, *P. brassicacearum* 93F8, *P. brassicacearum* 36B7 and *P. brassicacearum* Delaware synthesized proteins in the presence of ACC (Figure 1).

The highest values were observed for strains *P. putida* UW4+, *P. poae* 29G9 and *P. brassicacearum* Delaware, which were selected for application in plugs. These strains also showed more uniform growth as a function of time.

Figure 1 - Protein synthesis by bacterial strains from the genus *Pseudomonas* grown in minimal DF medium added with ACC.

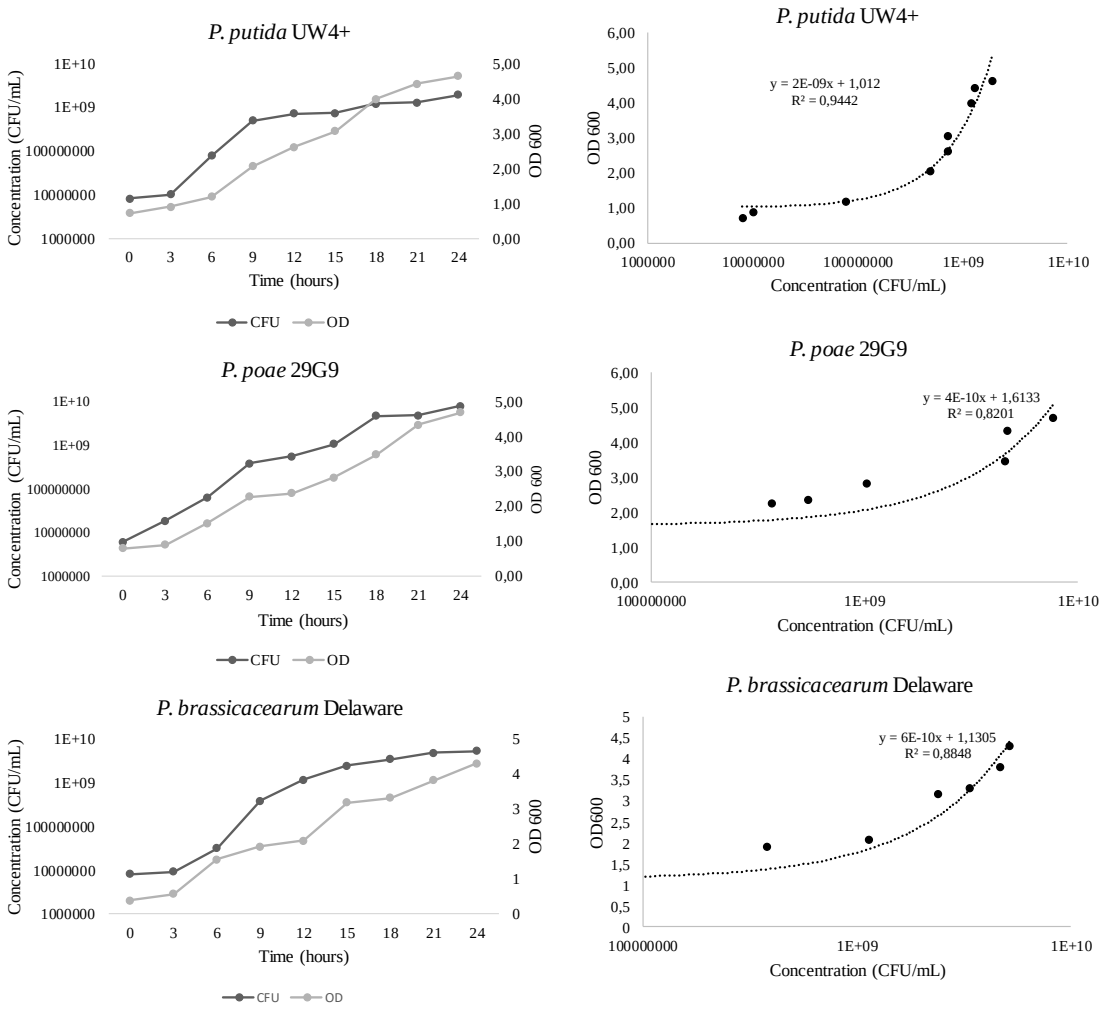


All bacteria were cultured in medium having ACC as the only nitrogen source. Thus, protein synthesis was performed only by bacterial strains with ACC deaminase activity, which generate alpha-ketobutyric acid and ammonia (PENROSE; GLICK, 2003) used in protein synthesis through deamination of ACC.

The strains *P. putida* UW4+, *P. poae* 29G9 and *P. brassicacearum* Delaware (Figure 2) reached the exponential growth stage from 9 h after inoculation, where the average bacterial concentration was 1 x 10^9 CFU/ml.

From the absorbance data and CFU, equations were generated to estimate the bacterial concentration for use as reference in subsequent cultures (FIGURE 2).

Figure 2 - Growth curves of strains *P. putida* UW4+, *P. poae* 29G9 and *P. brassicacearum* Delaware, with concentration as a function of culture time and absorbance as a function of concentration.

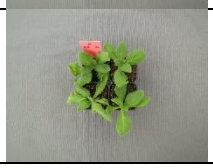


The exponential phase corresponds to the period of high cell division and growth. During this period, cell reproduction is very active, corresponding to the cell cycle of greatest metabolic activity (TORTORA; FUNKE; CASE, 2005). Thus, in the case of *Pseudomonas* strains, the bacterial culture must be inoculated in the plants after 9 h of culture, being the maximum point of cell growth.

3.2 Assays of greenhouse with plugs

Different storage conditions promoted differences between the 'Carpet White' petunia plugs treated with different strains of *Pseudomonas* (Table 1).

Table 1 - General aspect of 'Carpet White' petunia plugs treated with *Pseudomonas* strains and commercial products subjected to different storage conditions.

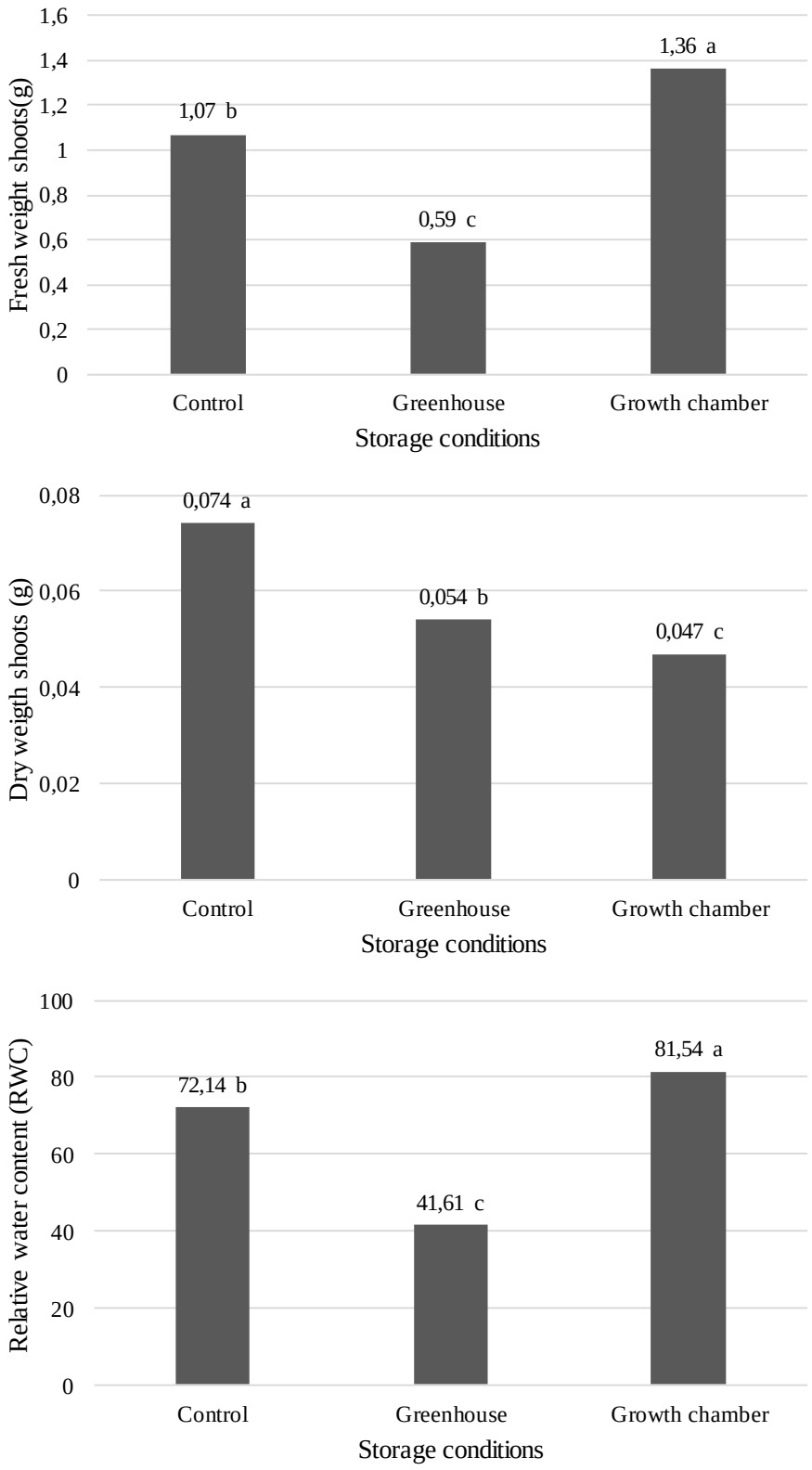
Strain/place	Control	Growth chamber dark	Greenhouse	Greenhouse after irrigation
Control				
UW4+				
29G9				
Delaware				
Actinovate®				
Environoc®				

Plants kept in cold chambers showed yellowish staining after three days in the dark, which can be attributed to light deficiency that degrades chlorophylls in the dark (TAIZ; ZEIGER, 2013), since plants were kept in boxes totally closed during this period. This change promotes a depreciation in the plant value for commercialization, generating losses for the producers.

No visual differences were observed for non-stressed plants. On the other hand, all the plants kept in a greenhouse without irrigation showed visual symptoms of water stress, characterized by the significant turgor loss. All plants were recovered 24 h after irrigation, and no visual differences were observed due to treatment with bacteria in the plug phase.

Differences were observed regarding the development of 'Carpet White' petunia plugs subjected to different stress conditions (FIGURE 3).

Figure 3 - Fresh weight, dry weight and relative water content (RWC) of 'Carpet White' petunia plugs inoculated with different strains of *Pseudomonas* and subjected to different water stress conditions. Averages followed by the same letter belong to the same group by Scott-Knott test at 5% probability.

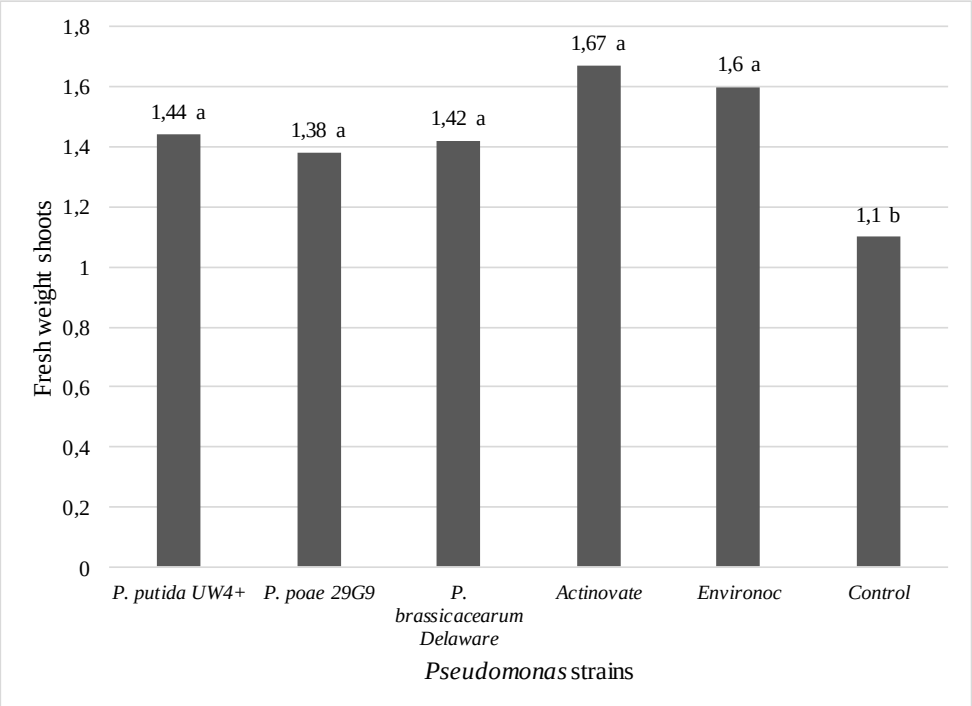


Plants kept in the cold chamber showed higher value of fresh weight and RWC, however, they showed the lower values after determination of dry weight. Plants stored in a cold chamber for transport simulation were not affected by water stress in the plug stage, possibly because fully enclosed boxes contributed to the moisture maintenance in the trays, thus ensuring greater fresh weight and RWC in the plugs. On the other hand, it was observed that the dry weight was lower than to plants subjected to other storage conditions, possibly as a function of the reduction of rate of photosynthesis due to the dark (TAIZ; ZEIGER, 2013).

Plants kept in a greenhouse without irrigation sowed lower values for all evaluated parameters, especially for RWC values, demonstrating the damages generated by stress. The application of different *Pseudomonas* strains was not beneficial to reduce the water stress symptoms.

For the plants subjected to the transport simulation, the effect for different *Pseudomonas* strains was observed (Figure 4), increasing the shoot fresh weight of plants.

Figure 4 - Fresh weight of 'Carpet White' petunia plugs treated with *Pseudomonas* strains and stressed during transport. Averages followed by the same letter belong to the same group by Scott-Knott test at 5% probability.

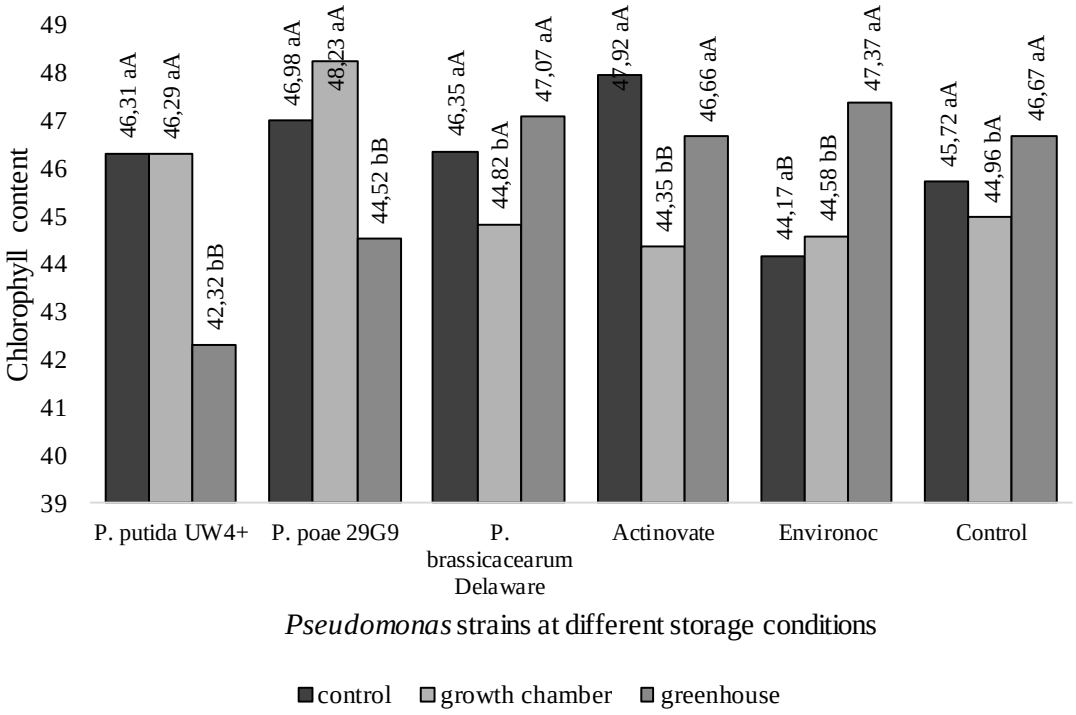


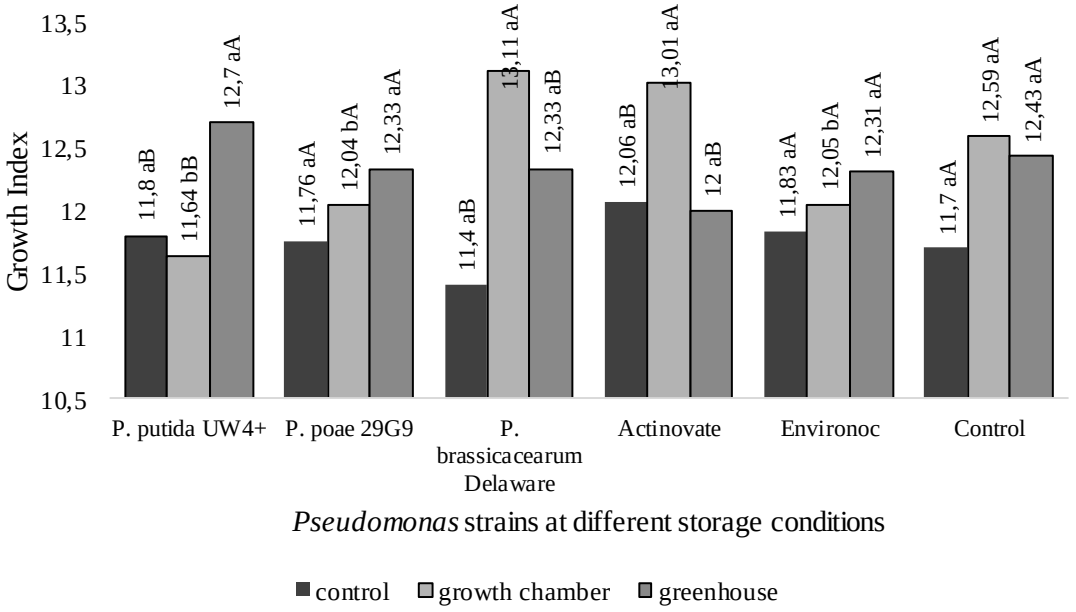
The fresh weight of plants is one of the most affected parameters in water stress conditions (JALEEL et al., 2009). Plants treated with *P. putida* UW4+, *P. poae* 29G9 and *P. brassicacearum* Delaware and commercial products showed higher fresh weight values in relation to the control treatment, and no difference was observed as a function of the different concentrations of ACC deaminase produced by bacteria (Figure 1). During abiotic stress, there is an increase in ethylene levels, consequently inhibiting plant growth (ARSHAD; SHAHAROONA; MAHMOOD, 2008). In this sense, bacteria containing ACC deaminase may contribute to plant growth by attenuating the deleterious effects of ethylene stress (GONTIA-MISHRA; SASIDHARAN; TIWARI, 2014). Increase in shoot weight as a function of the application of plant growth-promoting bacteria subjected to stress conditions were also observed for chick-peas (TIWARI et al., 2016), tomato and pepper (MAYAK; TIROSH; GLICK, 2004a). The use of *P. putida* strain UW4+, besides promoting growth, also contributes to the reduction of stress symptoms in canola plants subjected to salt stress (CHENG; PARK; GLICK, 2007).

3.3 Assays of greenhouse potted plants

Interaction between the different storage conditions and treatments applied in the vegetative growth analyses were observed (FIGURES 5 and 6).

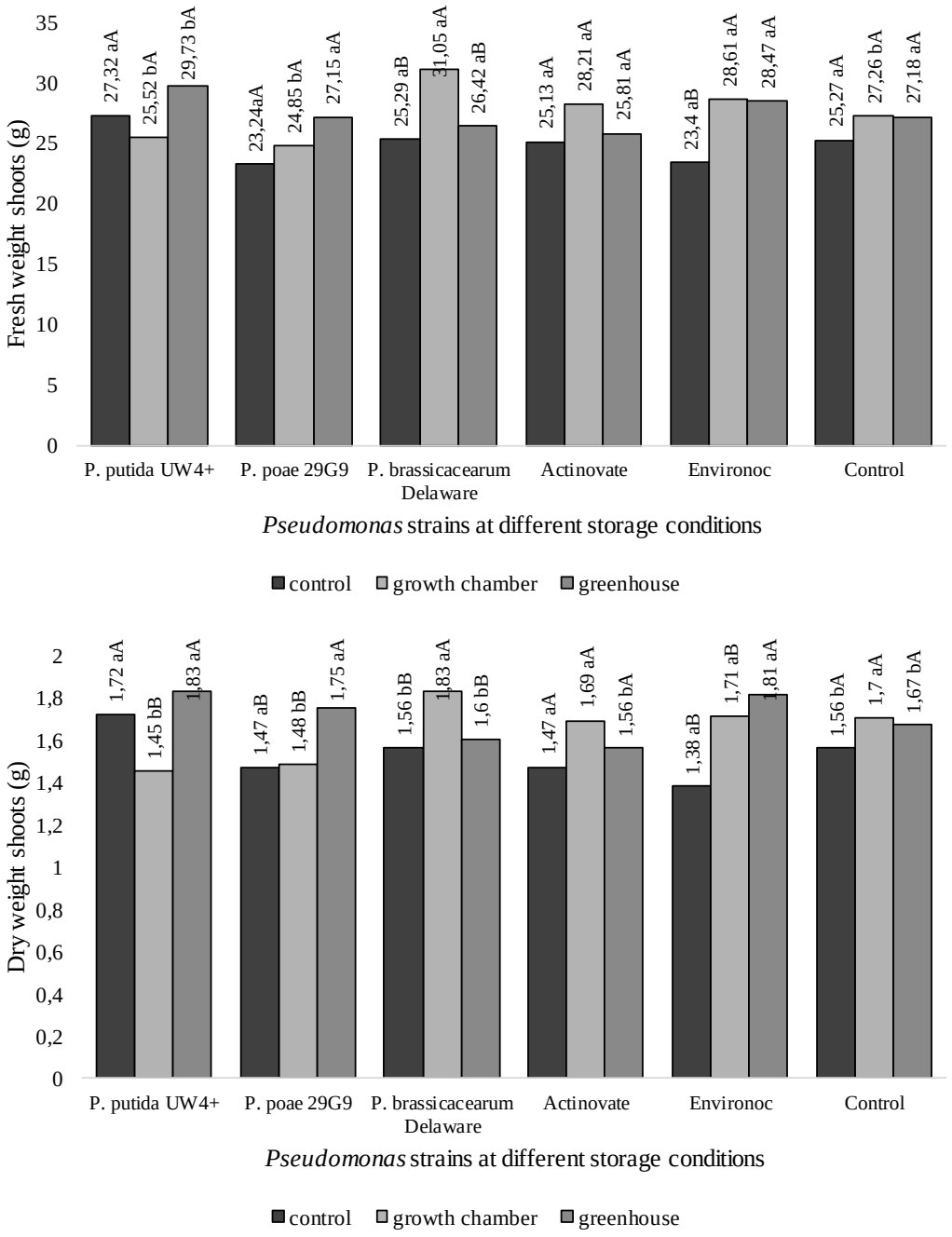
Figure 5 - Chlorophyll content and shoot growth index of 'Carpet White' petunia plugs treated with different strains of *Pseudomonas*, subjected to different storage conditions. Averages followed by the same lowercase letter (for treatments) and same capital letter (for storage conditions) belong to the same group by Scott-Knott test at 5% probability.





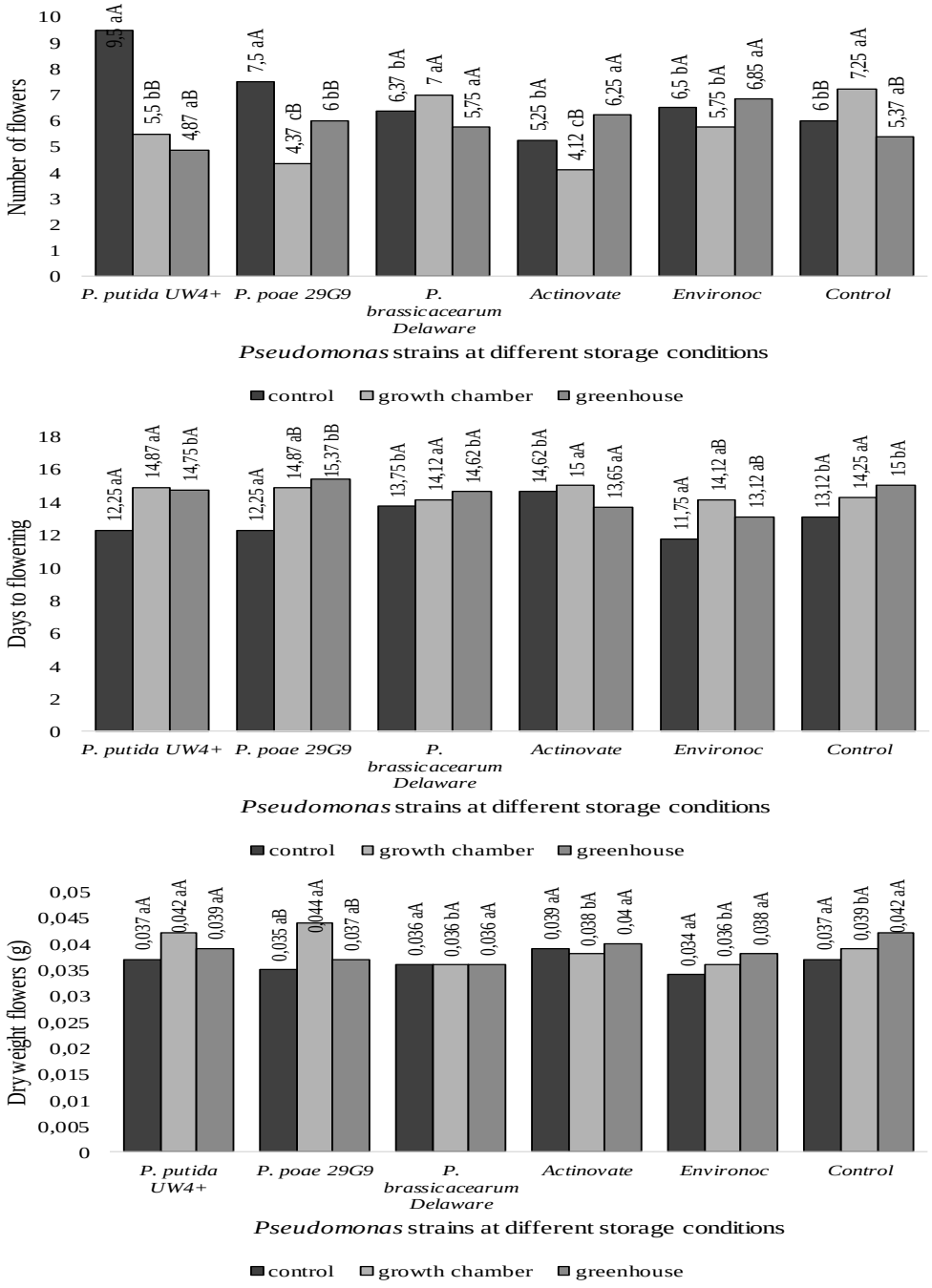
Differences were observed in relation to the accumulation of fresh and dry weight of plants treated with different microorganisms and subjected to different storage conditions.

Figure 6 - Shoot fresh weight (g) and dry weight (g) of 'Carpet White' petunia plugs, treated with different *Pseudomonas* strains and subjected to different storage conditions. Averages followed by the same lowercase letter (for treatments) and same capital letter (for storage conditions) belong to the same group by Scott-Knott test at 5% probability.



Differences were also observed for number of flowers, flowering date and dry weight of ‘Carpet White’ petunia flowers treated with *Pseudomonas* strains and subjected to different storage conditions (FIGURE 7).

Figure 7 - Number of flowers, days for emergence of the first flower and dry weight of flowers of 'Carpet White' petunia plugs treated with different *Pseudomonas* strains and subjected to different storage conditions. Averages followed by the same lowercase letter (for treatments) and same capital letter (for storage conditions) belong to the same group by Scott-Knott test at 5% probability.



In general, the non-stressed plants showed better vegetative growth with earlier flowering and higher number of flowers in relation to stressed plants. Plants reduce leaf growth under water stress conditions, since the reduction of leaf area reduces transpiration rates, contributing to longer soil water maintenance (TAIZ; ZEIGER, 2013). Plant development is negatively affected by abiotic stress conditions, mainly due to the increase of ethylene levels (TIWARI et al., 2016), which changes the growth of roots, stems, flowers and fruits (GLICK, 2014).

Among the non-stressed plants, the treatment with *P. putida* UW4+, *P. poae* 29G9 and Environoc ® favored flowering in comparison to the others, corroborating with the results found by GONTIA-MISHRA; SASIDHARAN; TIWARI (2014), which demonstrate that ACC deaminase synthesizing microorganisms contribute to plant growth even under normal conditions.

In the condition of transport simulation, plants treated with *P. putida* UW4+ and *P. poae* 29G9 showed lower values of growth index, fresh and dry weight of shoots and hence higher accumulation of chlorophyll in the tissues. The highest valuesobserved for flower weight were observed for plants treated with *P. putida* UW4+ and *P. poae* 29G9, possibly due to the accumulation of photoassimilates, since they emitted the least number of flowers. These results contradict several studies that demonstrate the beneficial effect of microorganisms under stress conditions (MAYAK; TIROSH; GLICK, 2004a; TIWARI et al., 2016).

4 CONCLUSIONS

The highest activity values of ACC deaminase are observed for *P. putida* UW4+, *P. poae* 29G9, and *P. brassicacearum* Delaware.

Application of the commercial products Actinovate ® and Environoc ® and of the strains *P. putida* UW4+, *P. poae* 29G9 and *P. brassicacearum* Delaware promote higher fresh weight accumulation of plugs subjected to transport simulation.

Treatment with *P. putida* UW4+ and *P. poae* 29G9 speeds up flowering and promotes a greater number of flowers for non-stressed plants at plug stage.

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