JÉSSICA FIGUEIREDO REZENDE

HERANÇA DA TOLERÂNCIA AO ESTRESSE SALINO EM TOMATEIROS DERIVADOS DE *Solanum galapagense* E *Solanum pennellii*

LAVRAS – MG
2019

JÉSSICA FIGUEIREDO REZENDE

HERANÇA DA TOLERÂNCIA AO ESTRESSE SALINO EM TOMATEIROS DERIVADOS DE *Solanum galapagense* E *Solanum pennellii*

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética e Melhoramento de Plantas, para a obtenção do título de Doutora.

Orientador
PhD Wilson Roberto Maluf

LAVRAS - MG
2019

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Rezende, Jéssica Figueiredo.
Herança da tolerância ao estresse salino em tomateiros derivados de *Solanum galapagense* e *Solanum pennellii* / Jéssica Figueiredo Rezende. - 2019.
86 p. : il.

Orientador(a): Wilson Roberto Maluf.

Tese (doutorado) - Universidade Federal de Lavras, 2019.
Bibliografia.

1. Tolerância à salinidade. 2. Melhoramento genético. 3. *Solanum lycopersicum*. I. Maluf, Wilson Roberto. II. Título.

JÉSSICA FIGUEIREDO REZENDE

HERANÇA DA TOLERÂNCIA AO ESTRESSE SALINO EM TOMATEIROS DERIVADOS DE *Solanum galapagense* E *Solanum pennellii*

INHERITANCE OF SALT STRESS TOLERANCE IN TOMATOES DERIVED FROM *Solanum galapagense* AND *Solanum pennellii*

Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Genética e Melhoramento de Plantas, área de concentração em Genética e Melhoramento de Plantas, para a obtenção do título de Doutora.

APROVADA em 27 de agosto de 2019.

Dr. Carlos Nick Gomes	UFV
Dr(a). Elaine Aparecida de Souza	UFLA
Dr. Júlio Sílvio de Sousa Bueno Filho	UFLA
Dr. Daniel Furtado Ferreira	UFLA

PhD Wilson Roberto Maluf
Orientador

LAVRAS - MG
2019

Aos meus pais, Márcio e Consuelo, que sempre acreditaram em mim, passaram muitas dificuldades para que eu pudesse estudar, e sem eles eu não teria chegado até aqui.
Meus maiores exemplos de garra, superação e dedicação, me ensinaram a nunca desistir de correr atrás dos meus objetivos, independente de todas as adversidades encontradas no caminho. Por serem minha inspiração e motivação para finalizar mais uma etapa,
A vocês, com gratidão e amor,

DEDICO

AGRADECIMENTOS

À Deus, por todas as graças alcançadas.

Aos meus pais, Márcio e Consuelo, a minha irmã Franciene, e meu sobrinho Luiz, pelo amor incondicional, motivação e inspiração e estarem sempre ao meu lado.

Ao meu namorado, Athos, pelo apoio, amor e carinho.

Ao meu cunhado Cleiton pelo apoio e aconselhamentos.

Ao Professor Maluf pela orientação, confiança, incentivo e ensinamentos transmitidos.

À UFLA e ao Programa de Pós-Graduação em Genética e Melhoramento de Plantas, pela oportunidade de poder realizar o doutorado.

À empresa HortiAgro Sementes Ltda., onde todos os experimentos foram conduzidos, pela disponibilidade de recursos e infraestrutura, e aos seus funcionários pelo apoio na condução dos experimentos e pela amizade, em especial Moretto, Vicente, Ná e Naldo.

Aos professores do Programa de Pós-graduação em Genética e Melhoramento de Plantas pelos ensinamentos transmitidos.

Aos professores Izabela, Júlio, Faquin, Evaristo E João Paulo que sempre se mostraram disponíveis e pelo suporte técnico.

Aos colegas de trabalho, pela amizade e boa convivência Luis Felipe, João, Alex, Natália e Régis.

À todos os colegas de pós-graduação, pela amizade e boa convivência.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) e ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pelo auxílio financeiro e pela concessão de bolsas de estudo.

À todos aqueles contribuíram direta ou indiretamente para a realização deste trabalho e que não foram aqui citados, o meu muito obrigada!

RESUMO

O tomate cultivado, *Solanum lycopersicum*, é uma espécie glicófita e é severamente danificado até mesmo sob baixos níveis de salinidade. Como as áreas para cultivo de tomate estão se tornando cada vez mais escassas no mundo devido ao aumento da salinização de áreas agricultáveis, o melhoramento visando ao aumento da tolerância a salinidade é uma questão urgente. Genótipos tolerantes à salinidade poderiam atenuar essas perdas, no entanto, o progresso em direção a esse objetivo tem sido limitado, principalmente devido à falta metodologias de triagem de genótipos tolerantes ao estresse salino que possam ser utilizadas rotineiramente em programas de melhoramento. Neste estudo, uma metodologia de seleção de genótipos tolerantes à salinidade é proposta, bem como um caráter capaz de predizer esta tolerância no estádio de plântula. Foi estudado o controle genético dos sintomas de estresse causados pela salinidade em *Solanum galapagense*. Populações segregantes foram obtidas de *Solanum lycopersicum* 'TOM-684' x *Solanum galapagense* 'LA1401' aos 35 dias após a semeadura, as plantas foram expostas a um estresse salino (NaCl) de 300 mM, e foram avaliadas com base nos sintomas de estresse. A área sob a curva de progresso dos sintomas de estresse foi usada para testar a hipótese de herança monogênica, e modelos genéticos foram testados utilizando funções de máxima verossimilhança para determinar o controle genético deste caráter. Os sintomas de estresse em *S. galapagense* 'LA1401' são controlados por mais de um locus, caracterizando-se como poligênico. As herdabilidades no sentido amplo e restrito foram estimadas em 0,66 e 0,27, respectivamente. *S. lycopersicum* 'BPX-441E-88', um genótipo previamente identificado como tolerante ao estresse hídrico (tolerância obtida de *S. pennellii*), foi identificado como tolerante à salinidade. Populações P_1 (BPX-441E-88), P_2 (TOM760), F_1, F_2, $F_1BC_{1(1)}$ e $F_1BC_{1(2)}$ foram cultivadas sob condições salinas (300 mM de NaCl) em sistema hidropônico, e avaliadas para sintomas de estresse salino, que foram utilizados para estimar a área sob a curva de progresso dos sintomas de estresse ao longo do tempo. Os sintomas de estresse salino em BPX-441E-88 são controlados por um gene de efeito maior mais poligenes modificadores, com efeitos aditivos e não aditivos. As estimativas de herdabilidade nos sentidos amplo e restrito foram 0,26 e 0,10, respectivamente.

Palavras-chave: Tolerância à salinidade. Melhoramento genético de plantas. *Solanum* spp. Recursos Genéticos. Metodologia de seleção. Fenotipagem. Controle genético.

ABSTRACT

The cultivated tomato, *Solanum lycopersicum*, is a glycophyte species and is severely damaged even at low salinity levels. As areas for growing tomatoes are becoming narrower around the world due to the increased salinization of arable lands, breeding for salt-tolerant crops is a pressing issue. Salt tolerant genotypes could attenuate these losses, however, in spite of the substantial efforts, progress towards this goal has been limited, mainly due to the lack of a screening criteria for salt tolerance viable for routine breeding. In this study, a new screening methodology to select salt tolerant genotypes is proposed, as well as a trait that can predict salt tolerance at the seedling stage. Also, to elucidate the genetic control of stress symptoms caused by salinity in *Solanum galapagense*, segregating populations were obtained from *Solanum lycopersicum* 'TOM-684' x *Solanum galapagense* 'LA1401'. 35 days after sowing, the plants were exposed to a salt stress of 300 mM, and were evaluated based on stress symptoms. The area under the stress symptoms progress curve was used to test a hypothesis of monogenic inheritance under different presumed degrees of dominance, and genetic models were tested using maximum likelihood tests of genetic control. Broad-sense heritability was of 0.66 and narrow-sense heritability was of 0.27. Stress symptoms in *S. galapagense* 'LA1401' is controlled by more than one locus, and might be under polygenic control. *S. lycopersicum* 'BPX-441E-88', a genotype previously identified as drought-tolerant (tolerance obtained from *S. pennellii*), was identified as salt-tolerant. In this study, populations P_1 (BPX-441E-88), P_2 (TOM760), F_1, F_2, $F_1BC_{1(1)}$, and $F_1BC_{1(2)}$ were grown under saline conditions (300 mM NaCl) in a hydroponic system, and assessed for stress symptoms, which were converted to area under the stress symptoms progress curve. This trait was found to be controlled by a major gene plus modifier genes, with both additive and non-additive gene effects. The estimates of broad-sense and narrow-sense heritability are 0.26 and 0.10, respectively.

Keywords: Salinity tolerance. Plant breeding. *Solanum* spp. Genetic resources. Screening methodology. Phenotyping. Genetic control.

SUMÁRIO

CAPÍTULO 1

1 INTRODUÇÃO

A salinidade é considerada um dos principais entraves para a produção agrícola mundial. Estima-se que 20% das áreas cultivadas e quase metade das áreas irrigadas no mundo são afetadas pela salinidade (BOTELLA et al., 2005), que tem um impacto negativo na produção agrícola, afetando o crescimento da planta e restringindo o uso da terra (TURAN; CORNISH; KUMAR, 2012). Uma alternativa para contornar este problema é a utilização de genótipos tolerantes à salinidade, que podem ser obtidos por meio do melhoramento convencional (BOTELLA et al., 2005).

A introgressão de genes de interesse por meio do melhoramento convencional é a base de vários programas de melhoramento. Para isto é necessário que haja variabilidade genética para os caracteres relacionados ao aumento da tolerância à salinidade, dentro da espécie a ser melhorada ou em espécies relacionadas. Apesar do tomateiro (*Solanum lycopersicum*), a terceira hortaliça mais cultivada no mundo (GRUBER, 2017), não ser tolerante à salinidade, é possível fazer introgressão de genes presentes no seu *gene pool* por meio do intercruzamento com espécies selvagens, como *S. galapagense* e *S. pennellii*, as quais são capazes de sobreviver em solos altamente salinos (RUSH & EPSTEIN, 1976; ZAMIR & TAL, 1987; CUARTERO & FERNÁNDEZ-MUÑOZ, 1999).

Acessos da espécie selvagem *Solanum galapagense* (sin. *Lycopersicon cheesmannii* var. *minor*) resistentes à salinidade (RUSH & EPSTEIN, 1976), são capazes de sobreviver em concentrações salinas equivalentes a da água do mar, ou seja, 460 mM de NaCl (RUSH & EPSTEIN, 1976). Por tolerarem potenciais hídricos extremamente baixos, também podem ser explorados potencialmente como fontes de resistência ao estresse hídrico. A espécie *S. pennellii* é naturalmente tolerante à seca (RICK, 1973; HOLTAN; HAKE, 2003; GONG *et al.*, 2010) e há relatos de que alguns acessos são também tolerantes à salinidade (ZAMIR & TAL, 1987; PÉREZ ALFOCEA; ESTAN, 1993; PÉREZ ALFOCEA; ESTAN, 1993;[i] MITTOVA *et al.*, 2004; CUARTERO; FERNÁNDEZ-MUÑOZ, 1999). Portanto, estas espécies podem ser utilizadas como fonte de variabilidade genética de caracteres morfológicos, fisiológicos e bioquímicos que proporcionam a tolerância à salinidade.

Para o tomateiro cultivado poucos avanços foram obtidos para o aumento da tolerância à salinidade até o presente momento. Um dos fatores limitantes para este avanço são as

inconsistências das metodologias de seleção de genótipos superiores e de indução de estresse salino. O objetivo deste trabalho foi padronizar uma metodologia de indução de estresse salino que possibilitasse a identificação e seleção precoce de genótipos de tomateiro tolerantes à salinidade, bem como determinar a herança dos sintomas de estresse em resposta à salinidade em *Solanum galapagense* 'LA-1401' e *Solanum lycopersicum* 'BPX-441E-88'.

2 REVISÃO DE LITERATURA

2.1 O problema da salinização dos solos

A salinização de áreas agricultáveis, é um dos problemas mais graves e persistentes na agricultura e, segundo modelos climáticos preditivos, a tendência é que este tipo de problema aumente num futuro próximo (CABOT, SIBOLE, BARCELÓ, & POSCHENRIEDER, 2014), especialmente em regiões áridas e semi-áridas ou regiões irrigadas e quentes, onde há uma perda excessiva de água por meio da evapotranspiração (TURAN; CORNISH; KUMAR, 2012). Além da perda imediata de produtividade, existem os riscos de degradação e desertificação irreversíveis do solo e contaminação das águas e solos a jusante (CABOT, SIBOLE, BARCELÓ, & POSCHENRIEDER, 2014).

O processo natural de formação de alguns solos pode originar solos salinos, mas um dos principais fatores responsáveis pela salinização dos solos é a atividade humana, como o manejo inadequado da irrigação (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999). Normalmente as regiões sujeitas ao problema de salinização são regiões com clima favorável para a produção de tomate. Portanto, seria interessante não só poder cultivar nestes solos, mas também aumentar a produtividade e aproveitar a água salobra para irrigação, visto que recursos hídricos estão cada vez mais escassos (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999).

O tomate poderia funcionar com uma cultura modelo para recuperação de áreas salinizadas e utilização de água de baixa qualidade, visto que há um grande conhecimento a respeito da fisiologia e genética desta espécie (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999).

2.1.1 Mensuração da salinidade

Salinidade é uma condição do solo caracterizada pela alta concentração de sais solúveis. Os solos são classificados como salinos quando a concentração de íons é tanta que a pressão osmótica produzida pelos íons é equivalente àquela gerada por 40 mM de NaCl i,e. 0.2 MPa (200 KPa) (TURAN; CORNISH; KUMAR, 2012). Já a salinidade da água é medida por meio da condutividade elétrica (CE), que pode ser convertida em sais totais dissolvidos (STD). A CE não identifica quais são os sais solubilizados ou quais os efeitos que eles têm sob os solos e culturas, mas fornece uma medida confiável do nível de salinidade. A tabela 1 foi adaptada de Prince (2016), e mostra a classificação geral de salinidade para a água.

Tabela 1 Classificação geral de salinidade para a água.

CE (dS/m)	CE (mS/m)	STD (ppm)	Classificação
0 – 0.8	0 – 80	0 – 440	Salinidade Baixa
0.8 – 2.5	80 – 250	440 – 1375	Salinidade Média
2.5 – 5	250 – 500	1375 – 2750	Salinidade Alta
> 5	>500	>2750	Salinidade Muito Alta

100 mS/m = 1 dS/m
STD = Sais Totais Dissolvidos
CE = Condutividade Elétrica

2.2 Relação entre estresse salino e seca

Estresse salino e seca possuem um alto grau de similaridade no que diz respeito aos efeitos fisiológicos, bioquímicos, moleculares e genéticos. A seca fisiológica ocorre quando os níveis de sais solúveis, na solução do solo, são suficientemente altos para limitar a absorção de água devido ao baixo potencial osmótico da mesma, induzindo assim o *deficit* hídrico (LEKSUNGNOEN, 2012).

A principal diferença entre ambientes com baixo potencial osmótico devido a salinidade *versus* seca, é a quantidade total de água disponível. Durante a seca, uma quantidade finita de água pode ser obtida do perfil do solo pela planta, causando uma redução do potencial hídrico do solo. Em ambientes salinos, uma grande quantidade de água está disponível, mas sob um baixo potencial osmótico (LEKSUNGNOEN, 2012).

Ambos os estresses levam à desidratação celular, promovendo estresse osmótico e remoção de água do citoplasma para o espaço intracelular, resultando na redução dos volumes citosólico e vacuolar. As primeiras respostas ao *deficit* hídrico e ao estresse salino são praticamente idênticas, exceto pelo componente iônico nas células de plantas sob estresse salino. Estas semelhanças incluem processos metabólicos, por exemplo, uma diminuição da fotossíntese ou aumento na produção de hormônios (ABA). Plantas sob estresse salino tem um problema adicional de elevadas concentrações intracelulares de sódio e cloreto (LEKSUNGNOEN, 2012).

Assim, as plantas podem usar vias comuns em resposta aos estresses, a qual é conhecida como tolerância cruzada, que permite que as plantas se adaptem a diferentes estresses após a exposição a um estresse específico. Portanto, uma espécie tolerante à salinidade também

poderia ser tolerante à seca ou vice-versa, e ter mecanismos semelhantes para lidar com esses estresses (LEKSUNGNOEN, 2012).

2.3 Desvendando o estresse salino

Plantas submetidas ao estresse salino enfrentam pelo menos quatro entraves: *deficit* hídrico, toxicidade iônica, desequilíbrio nutricional (LEKSUNGNOEN, 2012) e estresse oxidativo (MILLER et al., 2010).

2.3.1 *Deficit* hídrico

A redução na disponibilidade de água se dá pelo decréscimo do potencial osmótico do solo, na qual espécies mais sensíveis, inaptas a regular seu potencial hídrico, perdem o turgor celular (CABOT, SIBOLE, BARCELÓ, & POSCHENRIEDER, 2014). De acordo com Bai et al. (2018), o estresse osmótico nas raízes provoca redução do crescimento das folhas e da parte aérea de modo geral.

2.3.2 Toxicidade iônica

Subsequentemente, o estresse salino leva a um desequilíbrio iônico que causa necrose e morte prematura das folhas mais velhas (BAI et al., 2018). O excesso de íons, especialmente Na^+ e Cl^-, é extremamente tóxico para a maior parte das plantas, comprometendo o metabolismo e o crescimento das plantas por afetar negativamente a atividade enzimática, a estabilidade das membranas e aumentar a produção de ROS (*Reactive Oxygen Species*), que levam ao estresse oxidativo (CABOT, SIBOLE, BARCELÓ, & POSCHENRIEDER, 2014).

2.3.3 Estresse Oxidativo

O estresse salino prejudica a fotossíntese e aumenta a fotorrespiração, alterando a homeostase das células e causando um aumento na produção de espécies reativas de oxigênio (ROS) (MILLER et al., 2010), provocando o estresse oxidativo.

O oxigênio é indispensável para as plantas, pois está envolvido no metabolismo, na respiração mitocondrial e na fosforilação oxidativa para produção de energia. No entanto, o oxigênio ativa as espécies reativas de oxigênio (ROS) durante o metabolismo (LIANG et al., 2018). O estresse oxidativo se dá por meio de um desequilíbrio entre os fatores pró-oxidantes e antioxidantes, em favor dos primeiros (BARBOSA et al., 2010). Sob condições ótimas de

crescimento, as ROS são produzidas principalmente em baixo nível em organelas, como cloroplastos, mitocôndrias e peroxissomos. No entanto, durante o estresse, sua taxa de produção é drasticamente elevada (MILLER et al., 2010).

As ROS ($^1O_2$, H_2O_2, $O_2^{\cdot -}$ e HO·) desempenham um papel duplo na resposta das plantas aos estresses abióticos, pois além de funcionarem como moléculas tóxicas capazes de causar danos oxidativos às proteínas, ao DNA e aos lipídios (SAIRAM; RAO; SRI, 2002; MILLER et al., 2010), também funcionam como importantes moléculas de transdução de sinais (MILLER et al., 2010). As ROS geradas devido a desequilíbrios metabólicos durante o estresse também podem ser canalizadas pela planta para servir como um sinal de estresse, ativando mecanismos de aclimatação e defesa que, por sua vez, neutralizam o estresse oxidativo associado ao estresse (MILLER et al., 2010; ABDELGAWAD et al., 2016).

2.3.4 Desequilíbrio nutricional

De acordo com Leksungnoen (2012), o estresse salino pode induzir o desiquilíbrio nutricional, reduzindo a absorção de nutrientes como cálcio, potássio, nitrato, magnésio, manganês e fósforo (Ca^{2+}, K^+, NO_3^-, Mg^{2+}, Mn, e P), e provocando deficiências na planta. A deficiência de cálcio provoca podridão apical nos frutos (YARA, 2019), inviabilizando a comercialização dos mesmos. A deficiência de potássio compromete a abertura e fechamento dos estômatos, dificultando o movimento de água na planta (YARA, 2019). O nitrogênio é essencial para o crescimento e desenvolvimento da planta (YARA, 2019), e é por sua deficiência que plantas sob condições de estresse salino têm seu crescimento inibido. O magnésio é constituinte da molécula de clorofila, e sua deficiência é responsável pela inibição do crescimento das plantas sob condições salinas (YARA, 2019). O manganês está envolvido na síntese de ácido ascórbico (Vitamina C) (YARA, 2019), que por sua vez pode melhorar os danos causados pelo estresse salino ao inibir o aumento da concentração de prolina foliar e o vazamento eletrolítico das células foliares (BASTAM; BANINASAB; GHOBADI, 2016).

Sob alta concentração de cloro (Cl^-), a absorção de nitrato (NO_3^-) é reduzida. Solos com alta concentração de Na^+ trocável e sem quantidades adequadas de Ca^{2+} e Mg^{2+}, têm sua permeabilidade reduzida. O Na^+ também compete pelos sítios de ligação com o K^+, e a alta proporção de Na^+/K^+ leva a inativação de enzimas e inibem proteínas (LEKSUNGNOEN, 2012).

2.4 Efeitos do estresse salino na cultura do tomateiro

2.4.1 Germinação

O estudo dos efeitos da salinidade sobre a germinação é relevante para o caso de semeadura direta, na qual a má germinação e emergência das plântulas comprometeriam a viabilidade econômica do plantio. A salinidade não afeta somente a porcentagem de germinação das sementes, mas também o tempo necessário para a sua germinação. Sementes de tomate demoram 50% a mais do tempo para germinarem sob uma concentração salina de 80 mM e quase 100% de dias a mais para germinar sob uma concentração de 190 mM. No caso de semeadura direta isto pode ser um problema, uma vez que a superfície do solo pode formar uma crosta que dificulta a emergência das plântulas. Ao que tudo indica, a salinidade dificulta a germinação da semente por dificultar a absorção da água do solo pela mesma, na primeira fase da germinação – embebição (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

2.4.2 Desenvolvimento radicular

Quando as raízes são expostas à salinidade, inicia-se o estresse salino que provoca alterações no seu crescimento, morfologia e fisiologia, os quais influenciam na absorção de água e íons, além de alterar a produção de fitohormônios, afetando a planta toda (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

A salinidade afeta negativamente a biomassa da raíz. Acima do limite de tolerância, estimado entre 4 dS/m e 6 dS/m, o peso da raíz começa a decrescer. Um estudo comparou raízes de plantas de tomateiro cultivadas com 135 mM de NaCl e sem NaCl (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999). As raízes das plantas cultivadas com 135 mM de NaCl demoraram uma semana a mais para surgirem, e houve um atraso de 20 dias para que as raízes destas plantas atingissem 80 cm de profundidade do solo e a densidade destas raízes foi reduzida a um quarto quando comparada com as plantas não tratadas com NaCl. Existem várias possíveis razões para o crescimento reduzido das raízes sob condições de estresse salino, como restrição do crescimento celular - devido ao baixo potencial hídrico do meio externo, interferência dos íons na nutrição da planta ou a toxicidade de íons (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

A salinidade pode causar um *déficit* hídrico similar àquele produzido pela seca. Ela não só diminui o crescimento das raízes do tomateiro como também aumenta a extensão de raízes mortas nos genótipos sensíveis ao sal (SNAPP; SHENNAN, 1992).

Apesar dos efeitos negativos provocados pela salinidade do solo, as raízes são menos afetadas que a parte aérea. Estudos apontam que a permeabilidade da raiz (expressa como condutância hidráulica do sistema radicular) decresce significativamente sob condições de estresse salino, que pode ser uma explicação para a redução na absorção de água sob condições de salinidade. Em tomate, existe uma alta correlação negativa entre a condutância hidráulica da raiz e a concentração de NaCl. É difícil saber se a redução no fluxo de água através do sistema radicular é devida às mudanças no gradiente de potencial hídrico na raiz, às mudanças na condutância hidráulica produzidas por modificações na estrutura da raiz, ou devida a ambos (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

2.4.3 Desenvolvimento da parte aérea

A salinidade reduz o crescimento da parte aérea da planta, no estádio de *seedling*. Quanto mais cedo as plântulas forem submetidas ao estresse salino, menor será o crescimento da parte da aérea. Nos estádios de florescimento e frutificação as plantas conseguem sobreviver a concentrações de NaCl que são suficientes para matá-las no estádio de plântula. A capacidade de adaptação à salinidade aumenta conforme a planta se desenvolve (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

Tanto o caule quanto o teor de matéria seca das folhas diminuem sob condições de estresse salino (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999). Também ocorre uma redução do número de folhas e da área foliar (NAJLA et al., 2009). A taxa fotossintética e, consequentemente o crescimento da planta, também são reduzidos sob condições de estresse salino (NAJLA et al., 2009; Rouphael et al., 2018).

As plantas possuem mecanismos para reduzirem a perda de água, tais como aumento da suculência das folhas, redução do número de estômatos, distribuição dos estômatos (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999) e espessura da cutícula na folha (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999; NAJLA et al., 2009), que contribuem para o aumento da tolerância à salinidade (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999).

Em folhas totalmente expandidas, os sais são sequestrados pelo vacúolo até que o limite máximo seja atingido. Quando isto ocorre, a folha continua a transpirar e os sais que forem

absorvidos começam a ser armazenados no citoplasma com a subsequente inativação enzimática e morte celular. Os sais também podem ser armazenados na parede celular com subsequente desidratação e morte celular. Os íons acumulados no vacúolo são osmoticamente regulados no citoplasma por outros solutos não tóxicos para as enzimas. Um modelo de planta tolerante à salinidade seria aquela que mantém baixos teores de Na^+ e Cl^- nas folhas jovens e a concentração de NaCl nas folhas velhas esteja em equilíbrio com a concentração nas raízes (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

2.4.4 Características relacionadas com produtividade

O tomate cultivado é considerado moderadamente sensível à salinidade, o que significa que ele tolera uma condutividade elétrica de aproximadamente 2.5 dS/m (salinidade média; Tabela 1). Quando o tomate é cultivado em hidroponia ou em substrato inerte, a condutividade elétrica da solução normalmente varia entre 2.0 dS/m a 2.5 dS/m, portanto, mesmo sob condições normais de cultivo, a raiz está exposta a uma condutividade elétrica próxima do limite máximo tolerado, acima do qual ocorre uma redução da produtividade. Outros fatores que devem ser levados em conta são a umidade relativa do ar e a temperatura no ambiente, ou seja, quando a planta está exposta ao estresse salino sob alta temperatura e baixa umidade relativa do ar, as perdas na produtividade serão mais pronunciadas. Estas condições induzem o aumento da transpiração e a salinidade reduz o potencial osmótico, consequentemente o fluxo de água para o fruto será reduzido e haverá um decréscimo na taxa de expansão do fruto (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

A redução na produtividade é, em termos econômicos, o caráter mais importante afetado pela salinidade (VILLALTA et al., 2007). A produtividade do tomateiro neste caso é reduzida tanto pelo menor número de frutos por planta, quanto pela redução no peso médio dos frutos. Os frutos obtidos de plantas sob estresse salino apresentam um crescimento normal durante a fase de divisão celular e os efeitos deletérios são observados na fase de expansão do fruto. Portanto, genótipos com frutos menores, como caso de tomate tipo cereja, são uma opção para serem cultivados sob condições de estresse salino (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

No caso de plantas submetidas ao estresse salino, o período de tempo desde a fertilização até o amadurecimento do fruto é menor quando comparado com plantas que não estão sob estresse salino. Obviamente, esta precocidade depende da cultivar e do nível de estresse salino

utilizado. A cultivar “Moneymaker” pode suportar um estresse salino de 150 mM de NaCl – considerado alto - por até 10 dias (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

2.4.5 Qualidade do fruto

O teor total de sólidos solúveis (TSS), medido em graus Brix, é o critério mais importante para a indústria de processamento de polpa de tomate e serve como parâmetro para fixar o preço que será pago para o produtor. Plantas submetidas ao estresse salino apresentam um maior TSS. A vida de prateleira e a firmeza do fruto são reduzidas em plantas submetidas a concentrações salinas acima de 100 mM de NaCl. No caso de genótipos portadores de mutantes de amadurecimento como o gene rin (*rin/+*), a firmeza e tempo de prateleira não são alteradas nessas condições. Frutos oriundos de plantas sob estresse salino necessitam de um maior cuidado durante a pós colheita, pois os frutos apresentam maior taxa de respiração e maior produção de etileno (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999).

Sob condições de salinidade, há também uma maior incidência de podridão apical nos frutos. Os sintomas de podridão apical dos frutos iniciam-se com um leve escurecimento do tecido placentário distal, que progressivamente atinge o pericarpo. Além da necrose atingir o tecido, o fruto também interrompe seu crescimento e começa a maturação precocemente. Salinidade, alta temperatura e baixa umidade relativa do ar favorecem o desenvolvimento de podridão apical: a salinidade reduz a absorção de Ca^{+}, a alta temperatura acelera o crescimento do fruto e a baixa umidade do ar aumenta a transpiração, consequentemente o Ca^{+} se move para as folhas e sua disponibilidade para o fruto é reduzida (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999). O Ca^{+} é um nutriente essencial para o funcionamento adequado da membrana plasmática, em organelas de armazenamento para contrabalancear as cargas iônicas, no citosol para as respostas de sinalização e no apoplasto para formar a estrutura da parede celular (TONETTO DE FREITAS et al., 2014).

Cultivares que produzem frutos menores, que apresentem bom desenvolvimento e distribuição do xilema ao longo da parte distal do fruto, associado a maior eficiência em absorver Ca^{+}, fazem com que o fruto seja menos suscetível à podridão apical (Cuartero & Fernández-Muñoz, 1999).

2.5 Importância do melhoramento visando a tolerância a salinidade

O manejo adequado e o melhoramento genético são as principais ferramentas para aumentar a adaptação das plantas às condições adversas (CHAVES; FLEXAS; PINHEIRO, 2009).

O tomate cultivado é moderadamente sensível ao estresse salino (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999; FRARY et al., 2011) e é por ele afetado negativamente em todos seus estádios de desenvolvimento (CUARTERO & FERNÁNDEZ-MUÑOZ, 1999; FRARY et al., 2011; LEKSUNGNOEN, 2012). Além disso, a salinidade induz a senescência prematura das folhas e reduz a produtividade (LEKSUNGNOEN, 2012).

A tolerância à salinidade é um caráter complexo, controlado por diversos genes e envolve vários componentes fisiológicos, bioquímicos e genéticos. Assim, a dissecação genética da tolerância a salinidade é difícil; no entanto, é necessária para o desenvolvimento de cultivares tolerantes à salinidade (FRARY et al., 2011).

Apesar dos grandes esforços investidos no melhoramento genético para tolerância à salinidade (CUARTERO et al., 2006), o desenvolvimento de variedades tolerantes tem sido lento não somente devido à natureza complexa deste caráter, mas também devido à baixa variabilidade genética existente no tomate cultivado (EGEA et al., 2018).

Algumas espécies selvagens relacionadas ao tomate cultivado são descritas como tolerantes ao estresse salino e podem ser utilizadas como recursos genéticos em programas de melhoramento para obtenção de genótipos tolerantes ao estresse salino (FRARY et al., 2011).

2.5.1 *Solanum galapagense*

Solanum galapagense é uma espécie nativa das Ilhas Galápagos, região vulcânica localizada a aproximadamente 1000 km da costa oeste da América do Sul na República do Equador, encontrada frequentemente no litoral das ilhas ocidentais e sul (DARWIN; KNAPP; PERALTA, 2003). Por cruzar facilmente com o tomate cultivado e produzir descendentes férteis (RICK, 1979), esta espécie tem sido extensivamente investigada como fonte de características de interesse para melhorar o tomate cultivado (DARWIN, 2009).

Além de possuir frutos com altos teores de sólidos solúveis (GARVEY; HEWITT, 1991), folhas com a presença de tricomas do tipo IV (ANDRADE et al., 2018), serem resistentes a mosca-branca (*Bemisia* spp.), mosca minadora (*Liriomyza trifolii*) e ácaros (*Tetranychus urticae*) (VOSMAN et al., 2018), *Solanum galapagense* também é amplamente

descrito como tolerante à salinidade, permitindo potencialmente o desenvolvimento de cultivares mais adaptadas a solos com concentrações salinas elevadas (RUSH & EPSTEIN, 1981).

2.5.2 *Solanum pennellii*

Solanum pennellii é uma espécie de tomate selvagem endêmica das regiões andinas da América do Sul (desertos Chileno e Peruano) (DARWIN; KNAPP; PERALTA, 2003), onde evoluiu para se desenvolver em habitats áridos. Devido à sua adaptação a condições extremas, apresenta tolerância a estresses como seca (YU, 1972; RICK, 1973; KAHN, FENDER, BRAY, & O'CONNELL, 1993; ZSÖGÖN, 2011; EGEA et al., 2018) e salinidade (TAL & SHANNON, 1983; FRARY et al., 2011; BOLGER et al., 2014), sendo um importante germoplasma para utilização no melhoramento genético do tomate cultivado *Solanum lycopersicum* (BOLGER et al., 2014) visando a possibilitar a produção de tomate, em ambientes áridos e semi-áridos, que possuam disponibilidade limitada de água e/ou sofram de problemas de salinização do solo (EGEA et al., 2018).

2.5.2.1 *S. lycopersicum* 'BPX-441E-88'

Um componente monogênico de *S. pennellii* "LA716", foi introduzido na cultivar Micro-Tom, resultando no genótipo WELL, "Water Economy Locus in Lycopersicum" (ZSÖGÖN, 2011). No entanto, como este genótipo, um microtomateiro do tipo cereja, não possui características agronômicas em padrões comerciais, a linhagem avançada BPX-441E-88, com frutos graúdos, foi obtida após cinco autofecundações a partir {TOM-684 x (WELL x M-82)}. A linhagem BPX-441E-88 foi testada sob condições de seca, e apresentou baixa incidência de frutos com podridão apical, um bom parâmetro para selecionar genótipos tolerantes à seca (MORALES et al., 2015). Além disso, o genótipo BPX-441E-88 foi testado sob altas concentrações salinas por Rezende et al. (2019, não publicado) e mostrou-se altamente tolerante quando comparado aos demais genótipos pré-comerciais testados. Como BPX-441E-88 já possui boas características agronômicas, este genótipo é uma interessante fonte de tolerância à salinidade, uma vez que ao contrário dos acessos selvagens, está menos sujeito ao problema de *linkage drag*.

2.6 Metodologias para identificação e seleção de genótipos tolerantes à salinidade

2.6.1 Mecanismos de tolerância à salinidade

O tomate, é uma espécie glicófita, ou seja, altas concentrações salinas (100 a 200 mM de NaCl), mesmo a curto prazo, interferem no seu crescimento e podem provocar a morte da planta (ACOSTA-MOTOS et al., 2017). Plantas halófitas possuem mecanismos de escape e/ou tolerância à salinidade mais eficientes, e por isso podem sobreviver na presença de altas concentrações de NaCl (300-500 mM) por longos períodos (ACOSTA-MOTOS et al., 2017). Algumas glicófitas também são capazes de sobreviver sob altas concentrações salinas, mas são incapazes de compartimentar o sal residual absorvido tão efetivamente quanto as halófitas. A maioria das glicófitas tem uma fraca capacidade de excluir sais, os quais se concentram em níveis tóxicos nas folhas (MUNNS, 2002).

De modo geral, as plantas possuem diversos mecanismos para lidar com o problema de salinidade, e estes mecanismos podem ser basicamente divididos em dois grupos: mecanismos de escape e tolerância. Normalmente estes mecanismos são acompanhados por alterações fisiológicas, metabólicas e morfológicas (ACOSTA-MOTOS et al., 2017).

Os mecanismos de escape, comuns em halófitas (GUPTA; GOYAL; SINGH, 2018), são aqueles que minimizam a entrada de sais na planta (LIANG et al., 2018), através de sua exclusão ou excreção (GUPTA; GOYAL; SINGH, 2018).

Os mecanismos de tolerância tem por objetivo minimizar a concentração de sais no citoplasma (LIANG et al., 2018), e são eles ajuste osmótico (LIANG et al., 2018), diluição de sais (GUPTA; GOYAL; SINGH, 2018), compartimentação iônica (GUPTA; GOYAL; SINGH, 2018), e indução de enzimas antioxidantes (MARTINEZ et al., 2018).

2.6.2 Sintomas foliares em resposta ao estresse salino

Nas folhas, altas concentrações salinas causam o fechamento dos estômatos, além de comprometerem o transporte de elétrons e o aparato fotossintético, levando à redução da fotossíntese (ABDELGAWAD et al., 2016).

Fotossíntese e crescimento celular são os primeiros processos a serem afetados pelo estresse salino e pelo *déficit* hídrico (CHAVES; FLEXAS; PINHEIRO, 2009). Outros autores também demonstraram um impacto negativo do estresse salino na atividade fotossintética da planta (CUARTERO; FERNÁNDEZ-MUÑOZ, 1999; SAIRAM; RAO; SRI, 2002; GUPTA;

HUANG, 2014; TONETTO DE FREITAS et al., 2014; ZHAI; YANG; HOU, 2015; ZHAI; YANG; WU, 2016; HURA; SZEWCZYK-TARANEK, 2017; ALVES et al., 2018; MORTON et al., 2019). A redução da taxa de fotossíntese pode ser devida à redução da disponibilidade de CO_2 devido às limitações de difusão por meio dos estômatos e do mesofilo (CHAVES; FLEXAS; PINHEIRO, 2009), redução da permeabilidade da membrana ao CO_2 (KUMAR et al., 2018), redução da absorção de nitrogênio do solo (LEKSUNGNOEN, 2012), fechamento estomático (MORADI; ISMAIL, 2007; ROY; NEGRÃO; TESTER, 2014; ACOSTA-MOTOS et al., 2017; ROUPHAEL et al., 2018; MORTON et al., 2019) ou diminuição da atividade enzimática (HURA; SZEWCZYK-TARANEK, 2017; MARTINEZ et al., 2018).

Muitos estudos têm analisado os efeitos da salinidade em diferentes funções na escala foliar. Além da redução na fotossíntese, na transpiração e no status hídrico da planta, a salinidade também tem um efeito pronunciado sobre a estrutura da planta, sendo observada uma redução na elongação e no espessamento do caule, na área foliar, na taxa de crescimento das folhas, e aumento da espessura da folha (NAJLA et al., 2009). Estes efeitos variam de acordo com a intensidade e duração do estresse, bem como com a idade da folha: as folhas mais velhas são mais afetadas e acumulam maiores quantidades de sal (CHAVES; FLEXAS; PINHEIRO, 2009). Altas concentrações de NaCl induzem clorose, necrose e seca das folhas mais intensamente (HURA; SZEWCZYK-TARANEK, 2017).

Em um trabalho realizado por Asins et al. (1993), 206 progénies derivadas do cruzamento interespecífico, S. *lycopersicum* (=*L. esculentum*) x *S. pimpinellifolium* (=*L. pimpinellifolium*), foram testadas quanto à tolerância ao estresse salino sob uma concentração de 171.1 mM de NaCl. Sintomas de estresse (SS) foram avaliados, e a característica apresentou maior herdabilidade no sentido amplo (91,67%). Os "sintomas de estresse" (SS) foram avaliados na 6ª semana de tratamento e classificados em cinco classes de acordo com a clorose das folhas e do meristema apical e o grau de desfolhação, etc. Quanto pior o aspecto da planta, devido à severidade dos sintomas, maior a índice atribuído à ela (1 – 5).

Além dos sintomas de amarelecimento, desfolha e nanismo, plantas submetidas ao estresse salino podem apresentar sinais de murcha, mesmo que o solo esteja adequadamente úmido (PRINCE, 2016). Estes sintomas de murcha também foram relatados em genótipos de tomateiro cultivados em sistema hidropônico sob altas concentrações salinas (300 mM) por Shalata; Neumann (2001).

O estresse salino também provoca sintomas foliares de deficiência de alguns nutrientes como Ca^{2+}, K^{+}, NO_3^{-}, Mg^{2+}, Mn e P, uma vez que a alta concentração de sódio (Na^{+}) inibe a absorção destes nutrientes (LEKSUNGNOEN, 2012).

Portanto, os sintomas visuais do estresse salino na planta são variáveis e dependem da concentração salina (HURA; SZEWCZYK-TARANEK, 2017), e apesar do estresse salino alterar o fenótipo da planta, provocando sintomas visíveis, nenhum artigo retrata detalhadamente quais são exatamente os sintomas visuais decorrentes do estresse salino na cultura do tomateiro. Além disso, a alta herdabilidade do caráter "sintomas de estresse" encontrada por Asins et al. (1993), que inclui sobretudo sintomas foliares, mostra que estes têm um grande potencial para serem utilizados como parâmetro na seleção indireta de genótipos tolerantes à salinidade, por ser um caráter de mais fácil mensuração.

REFERÊNCIAS BIBLIOGRÁFICAS

ABDELGAWAD, H.; ZINTA, G.; HEGAB, M. M.; PANDEY, R. High Salinity Induces Different Oxidative Stress and Antioxidant Responses in Maize Seedlings Organs. **Frontiers in Plant Science**, v. 7, n. March, p. 1–11, 2016.

ACOSTA-MOTOS, J. R.; ORTUÑO, M. F.; BERNAL-VICENTE, A.; DIAZ-VIVANCOS, P.; SANCHEZ-BLANCO, M. J.; HERNANDEZ, J. A. Plant Responses to Salt Stress: Adaptive Mechanisms. **Agronomy**, v. 7, n. 18, p. 1–38, 2017.

ANDRADE, M. C.; DA SILVA, A. A.; CARVALHO, R. D. C.; DE ANDRADE SANTIAGO, J.; DE OLIVEIRA, A. M. S.; FRANCIS, D. M.; MALUF, W. R. Quantitative trait loci associated with trichomes in the Solanum galapagense accession LA1401. **Genetic Resources and Crop Evolution**, n. May, p. 1–15, 2018. Disponível em: <https://doi.org/10.1007/s10722-018-0644-3>.

ASINS, M. J.; BRETÓ, M. P.; CARBONELL, E. A. Salt tolerance in Lycopersicon species. II. Genetic effects and a search for associated traits. **Theoretical and Applied Genetics**, v. 86, n. 6, p. 769–774, 1993.

BAI, Y.; KISSOUDIS, C.; YAN, Z.; VISSER, R. G. F.; VAN DER LINDEN, G. Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. **Plant Journal**, v. 93, n. 4, p. 781–793, 2018.

BARBOSA, K. B. F.; COSTA, N. M. B.; ALFENAS, R. de C. G.; PAULA, S. O. DE; MINIM, V. P. R.; BRESSAN, J. Estresse oxidativo : conceito , implicações e fatores modulatórios Oxidative stress : concept , implications. **Revista de Nutrição**, v. 23, n. 4, p. 629–643, 2010.

BASTAM, N.; BANINASAB, B.; GHOBADI, C. Interactive effects of ascorbic acid and salinity stress on the growth and photosynthetic capacity of pistachio (Pistacia vera L.) seedlings. **The Journal of Horticultural Science and Biotechnology**, v. 0316, n. May, p. 8, 2016.

BOLGER, A.; SCOSSA, F.; BOLGER, M. E.; LANZ, C.; MAUMUS, F.; TOHGE, T.; QUESNEVILLE, H.; ALSEEKH, S.; SØRENSEN, I.; LICHTENSTEIN, G.; FICH, E. a; CONTE, M.; KELLER, H.; SCHNEEBERGER, K.; SCHWACKE, R.; OFNER, I.; VREBALOV, J.; XU, Y.; OSORIO, S.; AFLITOS, S. A.; SCHIJLEN, E.; JIMÉNEZ-GOMÉZ, J. M.; RYNGAJLLO, M.; KIMURA, S.; KUMAR, R.; KOENIG, D.; HEADLAND, L. R.; MALOOF, J. N.; SINHA, N.; VAN HAM, R. C. H. J.; LANKHORST, R. K.; MAO, L.; VOGEL, A.; ARSOVA, B.; PANSTRUGA, R.; FEI, Z.; ROSE, J. K. C.; ZAMIR, D.; CARRARI, F.; GIOVANNONI, J. J.; WEIGEL, D.; USADEL, B.; FERNIE, A. R. The genome of the stress-tolerant wild tomato species Solanum pennellii. **Nature Genetics**, v. 46, n. 9, p. 1034–1038, 2014. Disponível em: <http://dx.doi.org/10.1038/ng.3046>.

BOTELLA, M. A.; ROSADO, A.; BRESSAN, R. A.; HASEGAWA, P. M. Plant adaptive responses to salinity stress. In: **Plant Abiotic Stress**. [s.l: s.n.]p. 209.

CABOT, C.; SIBOLE, J. V.; BARCELÓ, J.; POSCHENRIEDER, C. Lessons from crop plants struggling with salinity. **Plant Science**, v. 226, p. 2–13, 2014a. Disponível em: <http://dx.doi.org/10.1016/j.plantsci.2014.04.013>.

CABOT, C.; SIBOLE, J. V.; BARCELÓ, J.; POSCHENRIEDER, C. Lessons from crop plants struggling with salinity. **Plant Science**, v. 226, p. 2–13, 2014b.

CHAVES, M. M.; FLEXAS, J.; PINHEIRO, C. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. **Annals of Botany**, v. 103, n. 4, p. 551–560, 2009.

CUARTERO, J.; FERNÁNDEZ-MUÑOZ, R. Tomato and salinity. **Scientia Horticulturae**, v. 78, p.

83–125, 1999.

CUARTERO, J.; M. C. BOLARÍN; ASÍNS, M. J.; MORENO, V. Increasing salt tolerance in the tomato. **Journal of Experimental Botany**, v. 57, n. 5, p. 1045–1058, 2006.

DARWIN, S. C. The systematics and genetics of tomatoes on the Galápagos Islands (Solanum , Solanaceae). n. August, p. 1–261, 2009. Disponível em: <http://eprints.ucl.ac.uk/18994/1/18994.pdf>.

DARWIN, S. C.; KNAPP, S.; PERALTA, I. E. Taxonomy of tomatoes in the Galápagos Islands: Native and introduced species of Solanum section Lycopersicon (Solanaceae). **Systematics and Biodiversity**, v. 1, n. 1, p. 29–53, maio 2003. Disponível em: <http://www.tandfonline.com/doi/abs/10.1017/S1477200003001026>. Acesso em: 24 maio. 2014.

EGEA, I.; ALBALADEJO, I.; MECO, V.; MORALES, B.; SEVILLA, A.; BOLARIN, M. C.; FLORES, F. B. The drought-tolerant Solanum pennellii regulates leaf water loss and induces genes involved in amino acid and ethylene/jasmonate metabolism under dehydration. **Scientific Reports**, v. 8, n. 1, p. 1–14, 2018.

FRARY, A.; KELEŞ, D.; PINAR, H.; GÖL, D.; DOĞANLAR, S. NaCl tolerance in Lycopersicon pennellii introgression lines: QTL related to physiological responses. **Biologia Plantarum**, v. 55, n. 3, p. 461–468, 2011.

GARVEY, T. C.; HEWITT, J. D. Starch and Sugar Accumulation in Two Accessions of Lycopersicon cheesmanii. **Journal of the American Society for Horticultural Science**, v. 116, n. 1, p. 77–79, 1991.

GONG, P.; ZHANG, J.; LI, H.; YANG, C.; ZHANG, C.; ZHANG, X.; KHURRAM, Z.; ZHANG, Y.; WANG, T.; FEI, Z.; YE, Z. Transcriptional profiles of drought-responsive genes in modulating transcription signal transduction, and biochemical pathways in tomato. **Journal of Experimental Botany**, v. 61, n. 13, p. 3563–3575, 2010.

GRUBER, K. Agrobiodiversity: The living library. **Nature**, v. 544, n. 7651, p. S8–S10, 2017.

GUPTA, B.; HUANG, B. Mechanism of Salinity Tolerance in Plants : Physiological , Biochemical , and Molecular Characterization. **International Journal of Genomics**, 2014.

GUPTA, S. K.; GOYAL, M. R.; SINGH, A. **Engineering Practices for Management of Soil Salinity: Agricultural, Physiological, and Adaptive Approaches**. 1. ed. [s.l.] Apple Academic Press, 2018.

HURA, T.; SZEWCZYK-TARANEK, B. Physiological Responses of Rosa rubiginosa to Saline Environment. **Water Air Soil Pollut**, v. 228, n. 81, p. 1–11, 2017.

KAHN, T. L.; FENDER, S. E.; BRAY, E. a.; O'CONNELL, M. a. Characterization of Expression of Drought- and Abscisic Acid-Regulated Tomato Genes in the Drought-Resistant Species Lycopersicon pennellii. **Plant physiology**, v. 103, n. 2, p. 597–605, 1993.

KUMAR, V.; WANI, S. H.; SUPRASANNA, P.; TRAN, L.-S. P. **Salinity Responses and Tolerance in Plants, Volume 1: Targeting Sensory, Transport and Signaling Mechanisms**. [s.l.] Springer, 2018.

LEKSUNGNOEN, N. **The Relationship Between Salinity and Drought Tolerance in Turfgrasses and Woody Species**. 2012. Utah State University, 2012.

LIANG, W.; MA, X.; WAN, P.; LIU, L. Plant salt-tolerance mechanism: A review. **Biochemical and Biophysical Research Communications**, v. 495, n. 1, p. 286–291, 2018. Disponível em:

<https://doi.org/10.1016/j.bbrc.2017.11.043>.

MARTINEZ, V.; NIEVES-CORDONES, M.; LOPEZ-DELACALLE, M.; RODENAS, R.; MESTRE, T. C.; GARCIA-SANCHEZ, F.; RUBIO, F.; NORTES, P. A.; MITTLER, R.; RIVERO, R. M. Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. **Molecules**, v. 23, n. 3, p. 1–20, 2018.

MILLER, G. A. D.; SUZUKI, N.; CIFTCI-YILMAZ, S.; MITTLER, R. O. N. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. **Plant, Cell and Environment**, v. 33, p. 453–467, 2010.

MITTOVA, V.; GUY, M.; TAL, M.; VOLOKITA, M. Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii.* **Journal of Experimental Botany**, v. 55, n. 399, p. 1105–1113, 2004.

MORADI, F.; ISMAIL, A. M. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. **Annals of Botany**, v. 99, n. 6, p. 1161–1173, 2007.

MORTON, M. J. L.; AWLIA, M.; AL-TAMIMI, N.; SAADE, S.; PAILLES, Y.; TESTER, M. Salt stress under the scalpel – dissecting the genetics of salt tolerance. p. 148–163, 2019.

MUNNS, R. Comparative physiologyy of salt and water stress. **Plant, Cell and Environment**, v. 25, p. 239–250, 2002.

NAJLA, S.; VERCAMBRE, G.; PAGÈS, L.; GRASSELLY, D.; GAUTIER, H.; GÉNARD, M. Tomato plant architecture as affected by salinity: Descriptive analysis and integration in a 3-D simulation model. **Botany**, v. 87, n. 10, p. 893–904, 2009. Disponível em: <http://www.nrcresearchpress.com/doi/10.1139/B09-061>.

PÉREZ ALFOCEA, F.; ESTAN, M. Osmotic adjustment in Lycopersicon esculentum and L. Pennellii under NaCl and polyethylene glycol 6000 iso–osmotic stresses. **Physiologia Plantarum**, p. 493–498, 1993. Disponível em: <http://onlinelibrary.wiley.com/doi/10.1111/j.1399-3054.1993.tb02498.x/full>.

PRINCE, R. **Water salinity and plant irrigation**. Disponível em: <https://www.agric.wa.gov.au/fruit/water-salinity-and-plant-irrigation>. Acesso em: 18 set. 2018.

RICK, C. Potential Genetic Resources in Tomato Species : Clues from Observations in Native Habitats. **Genes, Enzymes, and Populations**, p. 255–269, 1973a.

RICK, C. M. Potential Genetic Resources in Tomato Species: Clues from Observations in Native Habitats. In: SRB, A. M. (Ed.). **Genes, Enzymes, and Populations**. [s.l: s.n.]p. 255–269.

RICK, C. M. Biosystematic studies in Lycopersicon and closely related species of Solanum. In: HAWKES, J. G.; LESTER, R. N.; SKELDING, A. D. (Ed.). **Taxonomy of the Solanaceae**. London: The Biology and Academic Press, 1979. p. 667–678.

ROUPHAEL, Y.; RAIMONDI, G.; LUCINI, L.; CARILLO, P.; KYRIACOU, M. C.; COLLA, G.; CIRILLO, V.; PANNICO, A.; EL-NAKHEL, C.; DE PASCALE, S. Physiological and Metabolic Responses Triggered by Omeprazole Improve Tomato Plant Tolerance to NaCl Stress. **Frontiers in Plant Science**, v. 9, n. February, p. 1–18, 2018. Disponível em: <http://journal.frontiersin.org/article/10.3389/fpls.2018.00249/full>.

ROY, S. J.; NEGRÃO, S.; TESTER, M. Salt resistant crop plants. **Current Opinion in Biotechnology**, v. 26, p. 115–124, 2014.

RUSH, D. W.; EPSTEIN, E. Genotypic Responses to Salinity. **Plant physiology**, v. 57, p. 162–166, 1976.

RUSH, D. W.; EPSTEIN, E. Comparative studies on the sodium, potassium, and chloride relations of a wild halophytic and a domestic salt-sensitive tomato species. **Plant physiology**, v. 68, n. 6, p. 1308–1313, 1981.

SAIRAM, R. K.; RAO, K. V.; SRI, G. C. Differential response of wheat genotypes to long term salinity stress in relation to oxidati v e stress , antioxidant acti v ity and osmolyte concentration. **Plant Science**, v. 163, p. 1037–1046, 2002.

SHALATA, A.; NEUMANN, P. M. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. **Journal of Experimental Botany**, v. 52, n. 364, p. 2207–2211, 2001.

SNAPP, S. S.; SHENNAN, C. Effects of salinity on root growth and death dynamics of tomato , Lycopersicon esculentum Mill. **New Phytologist**, v. 121, p. 71–79, 1992.

TAL, M.; SHANNON, M. Salt Tolerance in the Wild Relatives of the Cultivated Tomato: Responses of Lycopersicon esculentum, L. cheesmanii, L. peruvianum, Solanum pennellii and F 1 Hybrids to High Salinity. **Functional Plant BiologY**, v. 10, n. 1, p. 109–117, 1983. Disponível em: <http://www.publish.csiro.au/?paper=PP9830109>.

TONETTO DE FREITAS, S.; MCELRONE, A. J.; SHACKEL, K. A.; MITCHAM, E. J. Calcium partitioning and allocation and blossom-end rot development in tomato plants in response to whole-plant and fruit-specific abscisic acid treatments. **Journal of Experimental Botany**, v. 65, n. 1, p. 235–247, 2014.

TURAN, S.; CORNISH, K.; KUMAR, S. Review article Salinity tolerance in plants : Breeding and genetic engineering. **Austraslian Journal of Crop Science**, v. 6, n. 9, p. 1337–1348, 2012.

VILLALTA, I.; BERNET, G. P.; CARBONELL, E. A.; ASINS, M. J. Comparative QTL analysis of salinity tolerance in terms of fruit yield using two solanum populations of F7 lines. **Theoretical and Applied Genetics**, v. 114, n. 6, p. 1001–1017, 2007.

VOSMAN, B.; VAN, W. P. C.; BETTY, W.; HENRIE, H.; VOS, R. C. H. De; VOORRIPS, R. E. Broad spectrum insect resistance and metabolites in close relatives of the cultivated tomato. **Euphytica**, v. 214, p. 14, 2018.

YARA. **Tomate**. Disponível em: <https://www.yarabrasil.com.br/nutricao-de-plantas/tomate/>.

YU, A. T. **The genetics and physiology of water usage in Solanum pennellii Com. and its hybrids with Lycopersicon esculentum Mill**. 1972. Davis California, 1972.

ZAMIR, D.; TAL, M. Genetic analysis of sodium, potassium and chloride ion content in Lycopersicon. **Euphytica**, v. 36, n. 1, p. 187–191, 1987.

ZHAI, Y.; YANG, Q.; HOU, M. The effects of saline water drip irrigation on tomato yield, quality, and blossom-end rot incidence - A 3a case study in the South of China. **PLoS ONE**, v. 10, n. 11, p. 1–17, 2015.

ZHAI, Y.; YANG, Q.; WU, Y. Soil salt distribution and tomato response to saline water irrigation under straw mulching. **PLoS ONE**, v. 11, n. 11, p. 1–17, 2016.

ZSÖGÖN, A. **Identification and characterization of a tomato introgression line with reduced wilting under drought**. 2011. 2011. Disponível em:

<https://digitalcollections.anu.edu.au/handle/1885/11216>.

CAPÍTULO 2

Screening for salt tolerance of tomato genotypes in a hydroponic system

SUMMARY

The cultivated tomato, *Solanum lycopersicum*, is a glycophyte species that is severely damaged even at low salinity levels. Because areas for optimal tomato growing conditions are becoming scarce around the world due to increased salinization of arable lands, breeding for salt-tolerant cultivars is a pressing issue. Salt tolerant tomato genotypes could attenuate these losses, however, in spite of the substantial efforts, progress towards this goal has been limited, mainly due to the lack of screening criteria that would be viable for routine breeding. In this study, a screening methodology to select salt tolerant genotypes at the seedling stage is proposed.

1 INTRODUCTION

Salinity has been an important target of investigations carried out with agricultural plants, due to the economic losses that it causes every year worldwide (IPCC, 2014; Martinez et al., 2018). In addition to short term losses in crop yield, salinization of croplands may lead to irreversible soil degradation and desertification and long-lasting contamination of downstream waters and soils (Cabot et al., 2014a). It is expected to become more severe in large areas of the planet (Rivero et al., 2018), and this progressive reduction of agricultural yields is a concern due to the escalating calls for more food, contributing to poverty and food insecurity (Ismail and Horie, 2017).

Salt stress impairs the plant growth, development, and yield (Munns and Tester, 2008; Amjad et al., 2019) by causing physiological drought, ionic stress, nutrient imbalance, oxidative stress (Chaves et al., 2009; Amjad et al., 2019), changes in metabolic processes, membrane inefficiency, and decrease of cell division and development (Amjad et al., 2019). Osmotic effects of salinity can be detected immediately after application of salt, and may last for the period of exposure, causing a disturbance in cell development and cell division, as well as the stomatal closure (Munns, 2002, 2005; Amjad et al., 2019). Plant exposure to long-term salinity results in the decrease in the photosynthetic area due to senescence in adult leaves caused by ionic stress (Murphy et al., 2003; Amjad et al., 2015, 2019).

Salinity levels ranging from 3 dS m^{-1} to 5.5 dS m^{-1}, increase tomato organoleptic parameters such as soluble solids, fructose, glucose, titratable acid, and amino acid contents, however, the same salinity levels may lead to deleterious effects such as reduction on yield (-~22.2%), leaf area and chlorophyll content (Zhai et al., 2015). Furthermore, it increases

blossom-end rot (BER) incidence (Cuartero and Fernández-Muñoz, 1999; Zhai et al., 2015; Zhai et al., 2016), which is a major physiological disorder in tomato and may lead to losses of up to 50%. To avoid these deleterious effects, salinity of irrigation water and/or soil should be under 4 dS m^{-1}, and the lower limit of soil matric potential should be greater than -20 kPa (Zhai et al., 2015).

Development of salt-tolerant genotypes would help to provide stability of yield (Flowers and Yeo, 1995), maintaining high yield in areas currently under irrigation, and also for planting new areas with tomato (Cuartero and Fernández-Muñoz, 1999; Zhai et al., 2015). Salt tolerance has already been described in several wild accessions such as *S. galapagense* 'LA1401' (Rush and Epstein, 1981) and *S. pennellii* 'LA716' (Tal and Shannon, 1983; Frary et al., 2011; Bolger et al., 2014).

Despite intensive studies conducted on the mechanisms by which plants cope with saline conditions, no success was obtained to date in developing salt-tolerant tomato cultivars (Cuartero et al., 2006). This fact can be accounted for by (1) that research has consistently shown salt tolerance as a complex trait, precluding direct selection, and making necessary to set up target traits that can predict salt tolerance through indirect selection (Gizaw et al., 2016; Saade *et al.*, 2016); and (2) the difficulties inherent to the evaluation of plants under saline conditions, due to the lack of efficient screening methodologies. Even though many screening criteria have been suggested to distinguish between genotypes for their salt tolerance under controlled environmental conditions (Tavakkoli et al., 2012), the use of those criteria in routine breeding is limited (Chen et al., 2013).

Several studies reported that salinity can be assessed by simple measurements of visual stress related parameters especially when using large number of germplasm, as proposed by Asins et al. (1993), Shalata and Neumann (2001) in tomato, and by Chen et al. (2013) in cucumber.

As a consequence, based on previous studies (Rush and Epstein, 1976; Asins et al., 1993; Shalata and Neumann, 2001), the aim of this study was to set up an inexpensive, quick, and reliable methodology to screen salt tolerance in tomato seedlings.

2 MATERIAL AND METHODS

2.1 Plant material

Ten tomato genotypes with different responses to salt stress (highly susceptible to highly resistant) were chosen: (1) *S. galapagense* "LA1401", a strongly salt tolerant accession (Rush and Epstein, 1976; Spooner; Peralta; Knapp., 2014; (2) *S. pennellii* "LA716", accession reported as salt (Frary et al., 2011) and drought tolerant (Easlon; Richards, 2009); (3) Santa Clara, a standard tomato of *S. lycopersicum*, used as a salt sensitive check; (4) Ibiza F1, a commercial hybrid of *S. lycopersicum*, used as a salt sensitive check; (5) TOM760, an inbred line of *S. lycopersicum*, a salt sensitive check; (6) TOM684, an inbred line of *S. lycopersicum*, also used as a salt susceptible check; (7) BPX-441E-55, an advanced inbred line from the interspecific cross between *S. pennellii* "LA716" and *S. lycopersicum* (Morales et al., 2015); (8) BPX-441E-88, a drought tolerant advanced progeny from the interspecific cross between *S. pennellii* "LA716" and *S. lycopersicum* (Morales et al., 2015); (9) F1(BPX-441E-55 x TOM760); and (10) F1(BPX-441E-88 x TOM760).

These genotypes are shown in Table 1. All genotypes were tested under different NaCl concentrations.

Table 1 Tomato genotypes used in the preliminary assays to establish a reliable screening methodology to identify salt-tolerant genotypes.

Genotypes	Salt-tolerant	Drought-tolerant
S. galapagense "LA-1401"	Yes [(1)]	?
S. pennellii "LA-716"	Yes [(2)]	Yes [(3)]
Santa Clara	No [(4)]	?
Ibiza F1	No [(4)]	?
Tom-760	No [(4)]	?
Tom-684	No [(4)]	No [(5)]
BPX-441E-55*	NT	Yes [(5)]
BPX-441E-88*	NT	Yes [(5)]
F1(BPX-441E-55 x Tom-760)	NT	?
F1(BPX-441E-88 x Tom-760)	NT	?

[(1)] According to Rush and Epstein (1976); Spooner et al. (2014); Andrade et al. (2017); [(2)] According to Frary et al., (2011) and drought tolerant (Easlon and Richards, 2009); [(3)] According to Easlon and Richards, (2009); [(4)] Standard cultivars with no salt tolerance; [(5)]According to Morales et al., (2015), where T5 = BPX-441E-55 and T9 = BPX-441E-88. *Inbred lines developed from {TOM-684 x (WELL x M-82)}. WELL was obtained from *S. pennellii* "LA-716"; NT = not tested previously.

2.2 Hydroponic system setup and standardization of the nutrient solution with low osmotic potential

To characterize the differences in salt tolerance among the genotypes, plantlets of all genotypes, with two fully expanded mature leaves, were treated hydroponically according to

Sun *et al.* (2010), with different concentrations of NaCl (90, 200, 300 and 400 mM) to reduce the osmotic potential of the nutrient solution (Rush and Epstein, 1976; Cuartero et al., 2006).

The hydroponic system chosen was an adaptation of the floating raft culture (Furlani et al., 1999), which allowed the assessment of a large number of genotypes in a relatively small area. In all trials the seeds were sown in vermiculite, an inert substratum, in 110cm^3 deepots using 54-cell trays as a support. Each hydroponic pool (length = 3.0 meters, width = 0.6 meters, height = 0.2 meters) was able to fit seven trays of 54-cells, totaling 378 cells per pool. The total volume of nutrient solution used in each pool was approximately 180 liters (L), and the reservoir capacity was 1000 L. A single phase electric motor of 1/3 HP and 3500 rpm, WEG®, was used to pump water.

The basic hydroponic nutrient solution (with NaCl excluded) was developed by Faquin (2008), adapted from leafy vegetables to tomato crop in the following proportions: Macronutrients (MAXSOL F21 - Jaraguá®: N-8%; P-11%; K-38%; Mg-1.6%; S-2.9%; Fe-0.2%; Zn-0.02%; Mn-0.04%; Cu-0.004%; B-0.02%; Mo-0.004%) = 0.725 Kg / 1000 L + Calcium Nitrate (Jaraguá®: N-15.5%; Ca-19%) = 0.540 Kg / 1000 L + 0.035 Kg / 1000 L Micronutrients (ConMicros Standard - Conplant®: B- 1,82%; Cu-1,82% ; Fe-7,26%; Mn-1,82%; Mo-0,36%; Ni-0,335%; Zn-0,73%).

Water used in the hydroponic system came from an artesian well, with an electrical conductivity (EC) varying from 0.257 to 0.328 mS/cm and pH varying from 6.06 to 6.53. In all trials, EC and pH were measured before and after the nutrient solution addition, and after the NaCl addition. Generally, water plus nutrient solution had an EC varying from 1.9 to 2.06 mS/cm and the pH varying from 5.93 to 4.93 (for nutrient solution parameter reading = HI98311 Portable EC/TDS tester, Hanna Instruments®).

When the plants had two fully expanded mature leaves, approximately 30 days after sowing and before applying NaCl, the EC of the nutrient solution was measured again and if necessary the nutrients were replenished with a stock solution (MAXSOL F1 = 12 Kg / 100 L + Calcium Nitrate = 8 Kg / 100 L, Faquin (2008)). After the NaCl addition, the EC and pH were measured again with a Bench Top Water Quality Meter - pH/ORP/Cond./TDS/Salinity – AZ Instrument®.

The amount of NaCl applied was calculated based on the osmotic potential of the nutrient solution and the saline solution according to Silva *et al.* (2013). The electrical

conductivity (dS m^{-1}) is directly proportional to the molar concentration (mM) and inversely proportional to the osmotic potential (kPa) according to Silva *et al.* (2013) as follows:

OP $y = -4.2074x + 15.545$
EC $y = 0.0763x + 0.8779$

Being, OP the Osmotic Potencial (kPa), EC the Electrical Conductivity (dS m^{-1}), and *x* the molar concentration (mM).

2.3 Rating scale based on stress symptoms due to salt stres

After the initial phase (up to five days after NaCl addition), scores were recorded for each plant based on the following symptom scale:

1 = no apparent stress symptoms on leaves, but with stunted growth;

2 = plants with stunted growth + wrinkles on leaves;

3 = plants with stunted growth + leaf wrinkles + leaf curling; light chlorosis of the apical meristem; possibly malformed leaves;

4 = plants with generalized yellowing + wilting and defoliation of the leaves; browning of previously yellow leaves;

5 = generalized leaf yellowing and irreversible leaf wilting; or plant death.

Right after salt stress induction, in the initial phase, some plants showed a reversible leaf wilting, especially during the hottest hours of the day, and were classified as score 1.

2.4 Trials

Four different experiments were carried out in a hydroponic system in a greenhouse of the Research Station of HortiAgro Sementes Ltda. (lat 21º 10' 12" S and long 44º 55' 31" W), Ijaci, MG, Brazil. All the trials were independent and tested the same genotypes (Table 1), differing only on the way of salt stress induction, i.e., in their NaCl concentration (mM) and in the number of doses applied (splitting or not the total amount of NaCl) (Table 2).

Table 2 Trials and NaCl quantity applied on each trial.

Trial	Month/Year (start)	NaCl (mM) (Total amount)	Number of doses	Doses in mM	EC (dS m^{-1})
1	06/2016	400	4	100 + 50 + 50 + 200	30.0
2	09/2016	400	1	400	30.0
3	11/2016	300	2	200 + 100	26.0
4	03/2017	300	2	150 + 150	26.0

Previously, two trials with NaCl concentrations of 90 mM (Two doses of 45 mM - December, 2015) and 200 mM (two doses of 100 mM - March, 2016) were performed, applying NaCl approximately one month after sowing. In these trials the stress symptoms were inconclusive and therefore other experiments were performed with higher NaCl concentrations (Table 2).

Trial 1 (06/2016): 400 mM NaCl, split into four doses (100 mM + 50 mM + 50 mM + 200 mM)

The first trial, performed in July 2016 (sowing date: 06/29), was conducted in a randomized complete blocks design, with 40 plots, with four plots per genotype and five plants per plot. 400 mM NaCl was used to induce salt stress, split into four applications over a 9-day span in 08/01 (100 mM), 08/03 (50 mM), 08/05 (50 mM) and 08/09 (200 mM) (Table 2).

Trial 2 (09/2016): 400 mM NaCl, one dose (400 mM)

The second trial started in September 2016 (sowing date: 09/13), and was conducted in a randomized complete blocks design, with 40 plots, with four plots per genotype and five plants per plot. A NaCl concentration of 400mM was applied as a single dose to the nutrient solution 30 days after sowing (10/12).

Trial 3 (11/2016): 300 mM NaCl, two doses (200 + 100)

The third trial, performed in November 2016 (sowing date: 10/24), was conducted in a randomized complete blocks design, with 40 plots, with four plots per genotype and five plants per plot. A total NaCl concentration of 300 mM was applied. The amount of NaCl was split into two doses, 200 mM of NaCl, applied 27 days after sowing (11/21) and 100 mM of NaCl, applied three days later (11/24).

Trial 4 (03/2017): 300 mM NaCl, two doses (150 + 150)

The fourth and last assay started in March 2017 (sowing date: 03/06). The genotypes were tested in a completely randomized design and the experimental plot consisted of one plant. The number of replications varied for each genotype. The genotypes TOM-684 and Ibiza had 37 plants (plots) and the other genotypes had 38 plants each. The differences in the number of plants per genotype was necessary to fit all of them into one pool (maximum capacity of 378 plants). In this trial, a NaCl concentration of 300 mM was applied when the plants showed two expanded leaves. The total amount of NaCl was split into two equal parts of 150 mM, in a two-day interval (150 mM on 03/31, and 150 mM on 04/03).

2.5 Statistical analysis

As the scores were collected over time, Cumulative Probit Models (AGRESTI, 2013) with both random and fixed effects were fitted using the Laplace approximation. The model is

$$Y_{ij} = \Phi^{-1}\left[P\left(y_{ij} \leq k\right)\right] = \tau_k - \beta_1 t - \beta_2 t^2 + g_i + p_{ij}$$

Where Y_{ij} is the response for the j^{th} experimental unit in the i^{th} genotype; $\Phi^{-1}(.)$ is the inverse of the standard normal cumulative distribution; τ_k is the k^{th} threshold on a latent continuous variable with standard gamma distribution; t is the standardized time of assessment; β_1 and β_2 are coefficients for the predicted model for time of assessment; g_i is the random effect for genotype i; and p_{ij} is the random effect for the j^{th} plot of the i^{th} genotype. Note that the term p_{ij} is dropped in the 4^{th} trial, as there is only one observation per plot.

All trials were analyzed independently and for each one, the genetic variance (σ_G^2), phenotypic variance (σ_P^2), broad-sense heritability (h^2), BLUPs and prediction error variances (σ_{BLUP}^2) were estimated from the scores.

In trials 1, 2 and 3, the phenotypic variances (σ_P^2) were estimated as follows:

$$\sigma_P^2 = \sigma_G^2 + \sigma_w^2 + 1$$

being σ_w^2 the error within plots, and 1 is the residual of the model.

For the last trial (Trial 4), because there was only one plant per plot, the phenotypic variances (σ_P^2) was estimated by:

$$\sigma_P^2 = \sigma_G^2 + 1$$

being 1 the residual of the model.

In all trials the heritability was estimated by:

$$h^2 = \frac{\sigma_G^2}{\sigma_P^2}$$

Those estimates were considered to evaluate the efficiency of the trials on distinguishing the salt tolerance levels for the different genotypes.

The analyses were performed on R Statistical Software (R Core Team, 2017) through the ordinal package (Christensen, 2018).

3 RESULTS

Stress symptoms

The rating scale was developed during the first trial, under 400 mM NaCl, split into four doses (100 mM + 50 mM + 50 mM + 200 mM), because there were no details of how salt stress visually affected tomato plants.

The first symptoms observed immediately after salt stress induction were wilting and growth inhibition. The growth inhibition (stunting) remained a constant during the entire experiment.

The assessments ceased when most of the plants of the salt-sensitive checks reached score 5. Figure 1 shows the final symptoms scores recorded for the check treatments (left to right: *S. galapagense* 'LA1401', BPX-441E-88, TOM760 and *S. pennellii* 'LA716').

Fig. 1 Plants after a long-term exposure (45 days after salt-stress induction) under high saline concentration (400 mM).

*Photo from Trial 1: 400 mM (100 mM + 50 mM + 50 mM + 200 mM).
Salt-tolerant checks: LA1401; LA716.
Salt-sensitive checks: TOM-760; Santa Clara.
Not tested previously: BPX-441E-88.

Trials

Saline concentrations equal or higher than 300mM, triggered stress symptoms a few days after the salt stress induction, and in all trials, it was possible to visually discriminate the genotypes according to their salt tolerance level. The range of symptoms described in the descriptive scale was observed in all trials.

In the first trial (400 mM NaCl, split in four doses: 100 mM + 50 mM + 50 mM + 200 mM), symptoms assessments started on 08/11, two days after the full amount of NaCl was applied. Twelve unequally spaced evaluations for salt stress symptoms were made. The last one was made 40 days after the total amount of NaCl was applied, or 83 days after sowing, when most of the plants of the salt-sensitive checks had shown severe stress symptoms (scores 4 and 5).

The second trial (400 mM NaCl, one single dose: 400 mM) had the first symptom evaluation, two days after the NaCl addition; the last evaluation was made six days later because the stress symptoms evolved rapidly.

In the third trial (300 mM NaCl, split into two doses: 200 mM + 100 mM) the first assessment started in the same day that the second amount of NaCl (100 mM) was applied (11/24), and the last one was performed 33 days later (12/26). There were a total of eight evaluations based on the stress symptoms scale (Table 2).

In the fourth and last trial (300 mM NaCl, split into two doses: 150 mM + 150 mM), score assessments started two days after the full amount of NaCl was applied and, one month after sowing (04/05). The last evaluation was performed two months after sowing (05/13). Seventeen assessments were made over time (Table 2).

The 4th trial allowed the greatest number of assessments (17 assessments), followed by trials 1 (12 assessments), 3 (8 assessments), and 2 (3 assessments).

In all trials, the longer was the exposure to salt stress, the more severe the stress symptoms became. These increase in the stress symptoms severity was observed even in the wild accessions *S. galapagense* 'LA1401' and *S. pennellii* 'LA716', which are described as salt tolerant.

Heritabilities (h^2), genetic (σ_G^2) and phenotypic variances (σ_P^2), variance within plot (σ_w^2) and the prediction error variances (σ_{BLUP}^2) of each trial are shown in Table 3. The genetic and phenotypic variances and heritability were estimated in the scale of the linear predictor (Table 3).

Table 3 Predicted parameters with the Cumulative Link Mixed Models with random effects via the Laplace approximation of each trial.

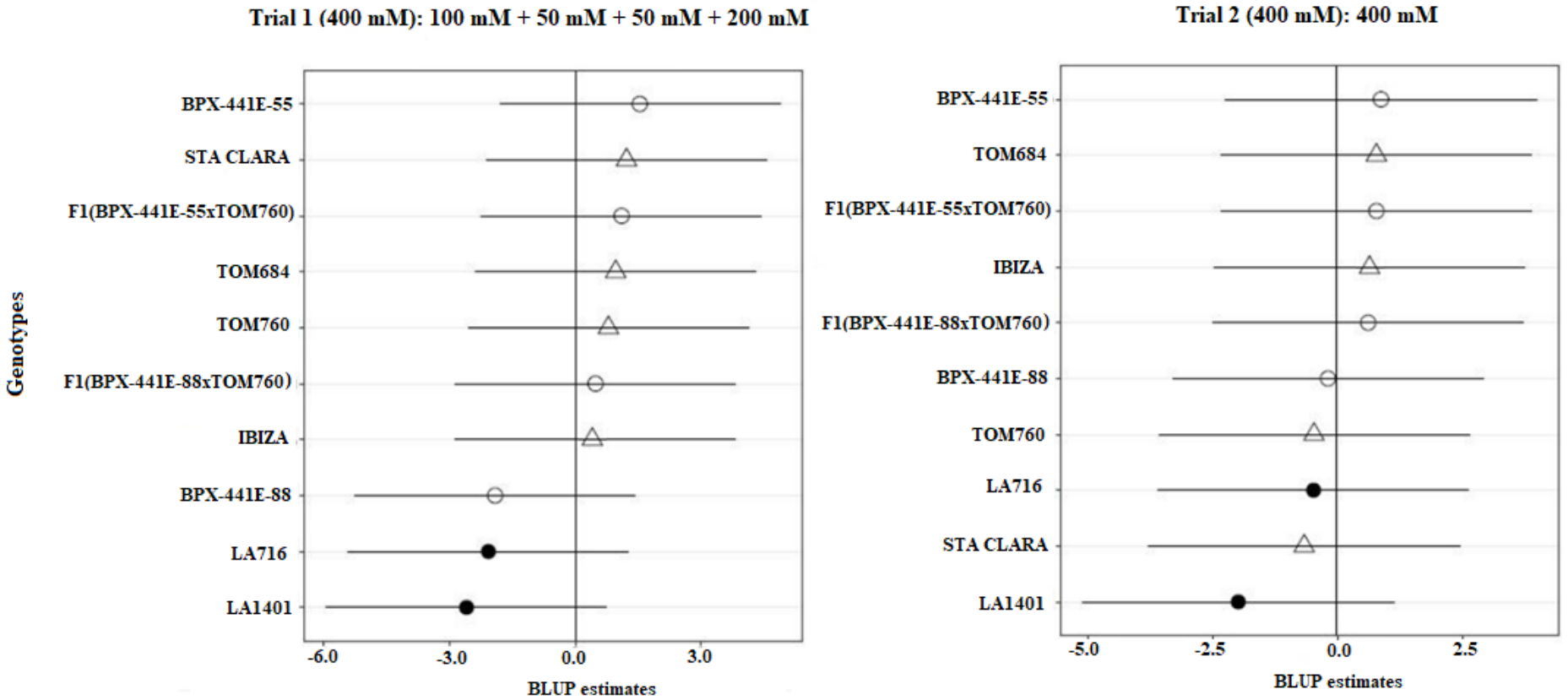
Parameter	Trial 1*	Trial 2*	Trial 3*	Trial 4**
σ_G^2	2.2250	0.8797	0.9388	1.478
σ_w^2	0.1637	0.3215	0.1313	-
σ_P^2	3.3887	2.2012	2.071	2.478
h^2	0.6565	0.3996	0.4533	0.5964
$VAR(BLUP)$	0.2150	0.3302	0.2000	0.0464

* In the fourth trial each plant constitutes an experimental plot. σ_G^2: genetic variance; σ_w^2 variance within plot; σ_P^2: phenotypic variance; $*$ $\sigma_P^2 = \sigma_G^2 + \sigma_w^2 + 1$; ** $\sigma_P^2 = \sigma_G^2 + 1$; h^2: broad-sense heritability; $VAR(BLUP)$: prediction error variances, representing the sum of the squares of the deviations between the observed genetic values and the predicted genetic values.

For all trials, the treatments were ranked according to their salt tolerance estimated by BLUP's (Fig. 3). Considering the salt-tolerant (LA1401 and LA716) and the salt-sensitive (Ibiza, Santa Clara, TOM-684, and TOM-760) checks, Trials 1, 3 and 4 yielded essentially the same ranks (Fig. 3), i.e., LA1401/LA716 were indeed found to be salt-tolerant, whereas Ibiza/TOM684/TOM760/Santa Clara were confirmed as salt-sensitive. Furthermore, in these three trials BPX-441E-88, a known drought-tolerant line, was also considered salt-tolerant. All other lines and hybrids were considered salt-sensitive (Fig. 3). Anomalous results were found for the second Trial (Trial 2), where Santa Clara (salt-sensitive check) was ranked as salt-tolerant. Because of these anomalous results, and because Trial number 2 was short-lived due to too rapid stress symptoms evolution, Trial 2 was disregarded for being an unreliable method of assessing salt stress.

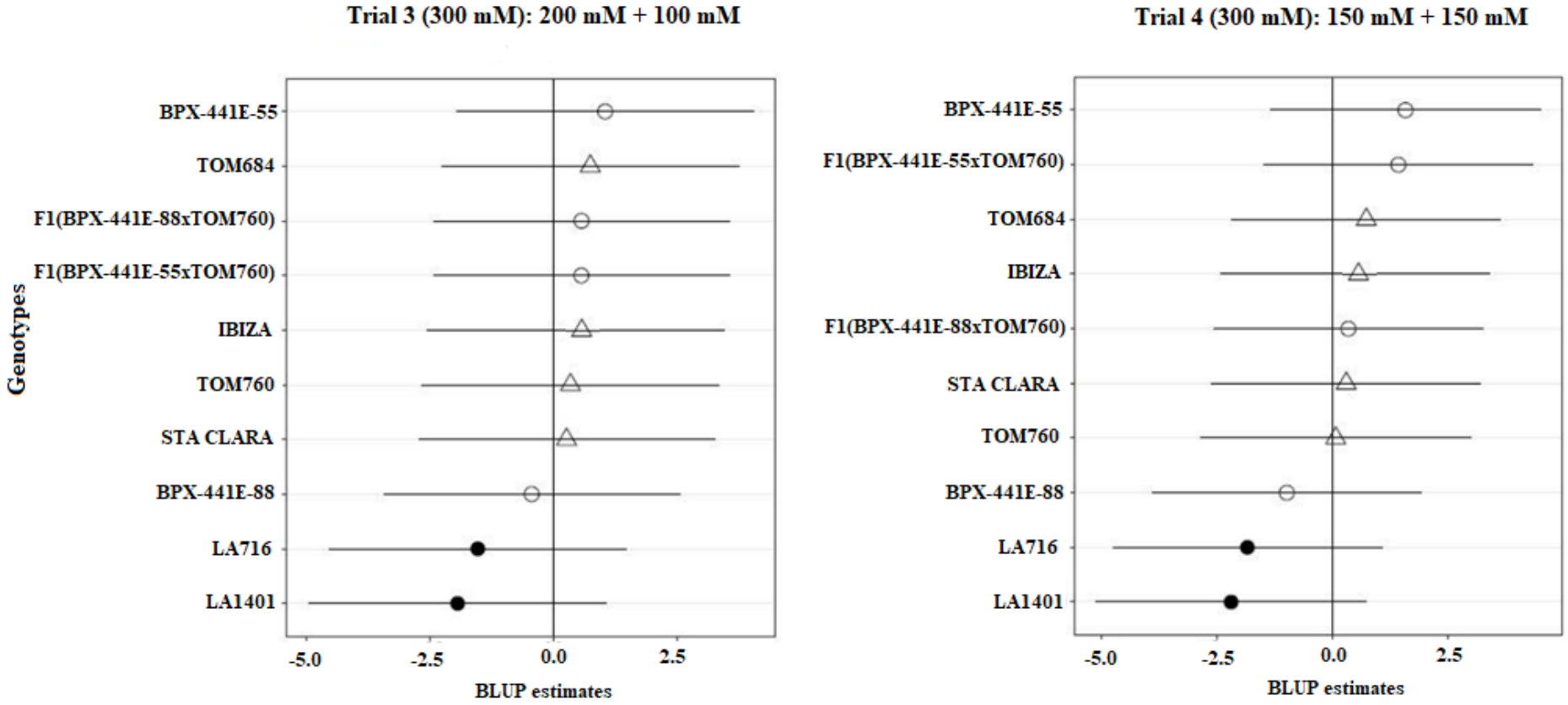
Prediction error variance $VAR(BLUP)$, is the square of the deviations between the predicted genetic values, therefore, smaller $VAR(BLUP)$ indicates better experimental accuracy. The order of efficiency of the screening criteria, based on the σ_{BLUP}^2 was Trial 4 > Trial 3 > Trial 1 > Trial 2, because Trial 4 presented the lower prediction error variance (0.0464) (Table 3).

Fig. 3 Caterpillar plot of the genotypes indicating their salt tolerance ranking, in each trial, considering all the assessments.



Black circle - Salt-tolerant checks; White circle - Genotypes not tested before for salt tolerance; Triangle - Salt-Sensitive checks.
The trials had different NaCl concentrations and different number of assessments.
Trial 1: 12 assessments, 400 mM into four doses: 100 mM + 50 mM + 50 mM + 200 mM.
Trial 2: 3 assessments, 400 mM in a single dose.

Fig. 3 (Contin.) Stress symptoms progress over time in each trial.



Black circle - Salt-tolerant checks; White circle - Genotypes not tested before for salt tolerance; Triangle - Salt-Sensitive checks.
Trial 3: 8 assessments, 300 mM into two doses: 200 mM + 100 mM.
Trial 4: 17 assessments, 300 mM into two doses: 150 mM + 150 mM.

4 DISCUSSION

The floating raft hydroponic system, as indicated in Trials 1, 3 or 4, was a useful and efficient tool to induce and assess salt stress in tomato plants. It allowed for testing of a large number of genotypes in a small area, at the seedling stage, making it viable to be deployed in breeding routine. Other advantages of using a protected hydroponic system to screen salt tolerance are the possibility to perform trials in any season and location, faster plant growth because a complete nutrient mixture, water availability throughout the trial, and no weed and pests problems (Jensen, 1997; Kang, 2013).

Indeed, the majority of the work on selection criteria for improved salt tolerance has been done using solution culture, either in hydroponic or supported hydroponic systems (Tavakkoli et al., 2012), which is considered the best method currently available (Lee et al., 2008). Studies using this system were used to screen salt tolerance in different crops as rocket (Jesus et al., 2015), soybeans (Lee et al., 2008), barley (Tavakkoli et al., 2012), faba bean (Tavakkoli; Rengasamy; McDonald, 2010), and cucumber (Chen et al., 2013).

Saline concentrations of 300 mM or up (Table 2) can trigger stress symptoms a few days after stress induction, because these saline concentrations are extreme, as is the saline concentration of seawater, i.e., approximately 460 mM (Rush; Epstein, 1976) or 32 dS m^{-1} (Tyler et al., 2017). However, when inducing salt stress with high saline concentrations ($\geq$ 300 mM), it was found necessary to split the total amount of NaCl preferably into at least two parts, similar to the fourth trial (Table 3).

Shalata and Neumann (2001) exposed 14-days tomato seedlings to a salt stress of 300 mM NaCl (in hydroponic system), and noticed a permanent wilting of 100% of the seedlings induced by 9 h of salt treatment, similar to what happened during Trial 2, where a total amount of 400 mM (Table 2) was applied in the nutrient solution of 30-days tomato seedlings. The accuracy of Trial 2 was notoriously compromised, when comparing the genetic variance (σ_G^2), the variance within plots (σ_w^2), heritability (h^2), and the prediction error variance VAR(BLUP), with the other trials (Table 3). Besides that, the salt-sensitive check "Santa Clara" was within the three most salt-tolerant genotypes, showing a complex GxE interaction (Fig. 3), confirming its inconsistency. Thus, this screening criteria should not be considered in future trials.

The ranking of the three most salt-tolerant genotypes with regard to salt-tolerance level was consistent in Trial 1, Trial 3, and Trial 4 (Fig. 3). Despite Trials 1 and 4 were very similar (Table 2, Fig. 3), the screening criteria deployed in the fourth trial allowed for smaller prediction error variance VAR(BLUP) (0.0464; Table 3), and smaller VAR(BLUP) indicates higher accuracy of prediction. Furthermore, Trial 4 had the greatest number of assessments (17 assessments) compared with trials 1 (12 assessments), 2 (3 assessments), and 3 (8 assessments), which may have increased its experimental accuracy. Higher accuracy may be a result of more repeated assessment, and in this case, Trial 4 had a larger number of repeated assessments over time (Table 3).

Therefore, Trial 4 was chosen as the best screening criteria for salt-tolerance because of its accuracy VAR(BLUP) (Table 3).

Genotypes reaction to salt stress

S. galapagense 'LA1401', one of the salt-tolerant checks, clearly stood out for salt tolerance in all trials (Fig. 3); confirming previous findings by Rush and Epstein (1981, 1976), Tal and Shannon (1983), and Foolad (2004).

The other salt-tolerant check, *S. pennellii* 'LA716', was the second most salt-tolerant genotype in trials 1, 3 and 4, confirming previous reports of its salt-tolerance (Tal and Shannon, 1983; Frary et al., 2011; Bolger et al., 2014).

BPX-441E-88 also stood out for salt tolerance in trials 1, 2 and 3 (Fig. 3), being the third most salt-tolerant genotype. BPX-441E-88 has *S. pennellii* 'LA716' as ancestor (Morales et al., 2015), and is believed to bear the locus WELL, which is associated with high water use efficiency (Zsögön et al., 2017). In previous studies, BPX-441E-88 was reported to have low stomatal density, higher gas exchange under drought stress conditions (Oliveira, 2016), and low incidence of blossom end rot (BER) (Morales, 2012; Morales et al., 2015; Millones-Chanamé, 2016). Both salinity and drought stresses induce osmotic stress, and thus, cross-tolerance responses and mechanisms may occur in plants (Leksungnoen, 2012), which may explain BPX-441E-88's salt tolerance (Trials 1, 3, and 4; Fig. 3).

Visual stress symptoms

Stress symptoms were reliable as they were consistent in all trials except in Trial 2, and therefore, the descriptive scale developed might be used as a screening criteria for

salt tolerance. Furthermore, their easy measurement and their efficiency in discriminating among genotypes (Fig. 2; Fig. 3), allows for screening of a great number of genotypes in a relatively short-time which are additional reasons to deploy these screening criteria in future studies of salt tolerance. Similar conclusions were made by Asins et al. (1993), Shalata and Neumann (2001), and Chen et al. (2013), in salt tolerance studies using stress symptoms as screening criteria in tomato and cucumber.

In trials 1, 3, and 4, in which the total amount of NaCl was split into two or more doses, a reversible wilting was observed in the early stages (up to five days after salt stress induction), probably due to plant acclimation to salt stress. In these trials permanent wilting was only observed after a long-term exposure to salt stress (about one month after salt stress induction), and may be related to the uptake and toxicity of NaCl, which damaged plasma membranes, due to lipid peroxidation (Shalata and Neumann, 2001). This process could interfere with essential tissue capacity to retain water and exclude sodium ions thereby inhibiting recovery from wilting (Shalata and Neumann, 2001).

Stunted growth was observed in all trials, and in all genotypes, even in the wild accessions *S. galapagense* 'LA1401' and *S. pennellii* 'LA716'. Stunting and reduced expansion of new leaves are generally accepted as adaptive mechanisms of plants exposed to osmotic stress due to salinity (Najla et al., 2009; Chen et al., 2013; Cabot et al., 2014; Bai et al., 2018; Rouphael et al., 2018), as photosynthesis and cell growth are the first processes to be affected by salt stress (Chaves et al., 2009).

In addition, high NaCl concentrations induced chlorosis (Hura and Szewczyk-Taranek, 2017) and leaf curling (Benjamin et al., 1999). The symptoms observed, including wrinkles and a few leaves with abnormal shape, might be resultant of salinity–boron interactions, as several studies have shown that salt stress may increase B toxicity symptoms (Grieve et al., 2016).

Also, a gradual loss of the older leaves of all genotypes submitted to saline stress, especially those plants with scores equal or superior to 4, was observed in the final stages of the trials. The accelerated senescence of the older leaves, also reported by Asins et al. (1993), is due to ion compartmentation, a tolerance mechanism in response to salt stress. Ion compartmentation protects the salt sensitive tissues, so excess ions are sequestered in vacuoles of the older leaves where they accumulate (Chen, 2018). When the vacuoles

reach the maximum concentration of salts, the leaf falls, eliminating them with it (Chen, 2018).

In all trials, plants with scores equal or superior than 4 became increasingly yellowish. The higher the score and the longer the time elapsed after the application of NaCl, the more yellowish became the plant. This yellowing evolved to brown until the plants became completely dried out. The yellowing observed, may be a consequence of photosynthesis reduction due to salinity (Cuartero and Fernández-Muñoz, 1999; Munns, 2002; Sairam et al., 2002; Munns and Tester, 2008; Gupta and Huang, 2014; Tonetto de Freitas et al., 2014; Zhai et al., 2015; Zhai et al., 2016; Hura and Szewczyk-Taranek, 2017; Alves et al., 2018; Morton et al., 2019). The reduction in photosynthetic rate may be caused by reduced CO_2 availability due to diffusion limitations through the stomata and mesophyll (Chaves et al., 2009), reduction of membrane permeability to CO_2 (Kumar et al., 2018), reduction of nitrogen uptake (Leksungnoen, 2012), stomatal closure (Moradi and Ismail, 2007; Roy et al., 2014; Acosta-Motos et al., 2017; Rouphael et al., 2018; Morton et al., 2019) or reduction on enzyme activity (Hura and Szewczyk-Taranek, 2017; Martinez et al., 2018).

5 CONCLUSIONS

Stress symptoms observed in tomato genotypes exposed to high saline concentrations are good parameters to screen salt tolerant genotypes. The best screening criteria for salt tolerance was to assess stress symptoms induced by 300 mM NaCl (split into two doses of 150 mM with a couple days interval between each application) up to one month after sowing, when plants show two totally expanded leaves. This methodology is reliable, fast and simple, and viable to be deployed in breeding routines.

BIBLIOGRAPHY

Acosta-Motos, J.R., M.F. Ortuño, A. Bernal-Vicente, P. Diaz-Vivancos, M.J. Sanchez-Blanco, et al. 2017. Plant Responses to Salt Stress: Adaptive Mechanisms. Agronomy 7(18): 1–38. doi: 10.3390/agronomy7010018.

Agresti, A. 2013. Ordinal Responses: Cumulative Logit Models. Categorical data analysis. Third Edit. Wiley series in probability and statistics. p. 301–307

Amjad, M., S.S. Akhtar, A. Yang, J. Akhtar, and S.E. Jacobsen. 2015. Antioxidative Response of Quinoa Exposed to Iso-Osmotic, Ionic and Non-Ionic Salt Stress. J. Agron. Crop Sci. 201(6): 452–460. doi: 10.1111/jac.12140.

Amjad, M., N. Ameen, B. Murtaza, M. Imran, M. Shahid, et al. 2019. Comparative Physiological and Biochemical Evaluation of Salt and Nickel Tolerance Mechanisms in Two Contrasting Tomato Genotypes. Physiol. Plant. doi: 10.1111/ppl.12930.

Andrade, M.C., A.A. da Silva, I.P. Neiva, I.R.C. Oliveira, E.M. De Castro, et al. 2017. Inheritance of type IV glandular trichome density and its association with whitefly resistance from Solanum galapagense accession LA1401. Euphytica 213(2). doi: 10.1007/s10681-016-1792-1.

Asins, M.J., M.P. Bretó, and E.A. Carbonell. 1993. Salt tolerance in Lycopersicon species. II. Genetic effects and a search for associated traits. Theor. Appl. Genet. 86(6): 769–774. doi: 10.1007/BF00222668.

Bai, Y., C. Kissoudis, Z. Yan, R.G.F. Visser, and G. van der Linden. 2018. Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. Plant J. 93(4): 781–793. doi: 10.1111/tpj.13800.

Benjamin, K.J., D.I. Walker, A.J. McComba, and J.K. C. 1999. Structural response of marine and estuarine plants of Halophila ovalis (R. Br.) Hook. f. to long-term hyposalinity. Aquat. Bot. 64(1): 1–17. doi: https://doi.org/10.1016/S0304-3770(98)00103-X.

Bolger, A., F. Scossa, M.E. Bolger, C. Lanz, F. Maumus, et al. 2014. The genome of the stress-tolerant wild tomato species Solanum pennellii. Nat. Genet. 46(9): 1034–1038. doi: 10.1038/ng.3046.

Cabot, C., J. V. Sibole, J. Barceló, and C. Poschenrieder. 2014. Lessons from crop plants struggling with salinity. Plant Sci. 226: 2–13. doi: 10.1016/j.plantsci.2014.04.013.

Millones- Chanamé, C.E.M. 2016. Herança da tolerância ao estresse hídrico em tomateiro.

Chaves, M.M., J. Flexas, and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Ann. Bot. 103(4): 551–560. doi: 10.1093/aob/mcn125.

Chen, N. 2018. Leaves accumulate excess salt. AskNature. https://asknature.org/strategy/leaves-accumulate-excess-salt/#.XIVeRChKjIU.

Chen, J., J. Shen, J. Xu, G.M. Kere, and Q. Guo. 2013. Heritability and gene effects for salinity tolerance in cucumber (Cucumis sativus L.) estimated by generation mean analysis. Sci. Hortic. (Amsterdam). 159: 122–127. doi: 10.1016/j.scienta.2013.04.020.

Christensen, R.H.B. 2018. Ordinal - Regression Models for Ordinal Data. R Packag. version 2018.4-19. http://www.cran.r-project.org/package=ordinal.

Cuartero, J., and R. Fernández-Muñoz. 1999. Tomato and salinity. Sci. Hortic. (Amsterdam). 78: 83–125.

Cuartero, J., M. C. Bolarín, M.J. Asíns, and V. Moreno. 2006. Increasing salt tolerance in the tomato. J. Exp. Bot. 57(5): 1045–1058. doi: 10.1093/jxb/erj102.

Easlon, H.M., and J.H. Richards. 2009. Drought response in self-compatible species of tomato (Solanaceae). Am. J. Bot. 96(3): 605–611. doi: 10.3732/ajb.0800189.

Faquin, V. 2008. Solução Nutritiva para Folhosas. Lavras.

Flowers, T.J., and A.R. Yeo. 1995. Breeding for salinity resistance in crop plants: Where next? Aust. J. Plant Physiol. 22(6): 875–884. doi: 10.1071/PP9950875.

Foolad, M.R. 2004. Recent advances in genetics of salt tolerance in tomato. : 101–119.

Frary, A., D. Keleş, H. Pinar, D. Göl, and S. Doğanlar. 2011. NaCl tolerance in Lycopersicon pennellii introgression lines: QTL related to physiological responses. Biol. Plant. 55(3): 461–468. doi: 10.1007/s10535-011-0111-x.

FURLANI, P.R.., D.. SILVEIRA, L.C.P.; BOLONHEZI, and V. FAQUIN. 1999. Boletim técnico 180: Cultivo hidropônico de plantas. Campinas.

Gizaw, S.A., K. Garland-Campbell, and A.H. Carter. 2016. Use of spectral reflectance for indirect selection of yield potential and stability in Pacific Northwest winter wheat. F. Crop. Res. 196: 199–206. doi: 10.1016/j.fcr.2016.06.022.

Grieve, C.M., S. Grattan, and E. V. Maas. 2016. Plant Salt Tolerance. Agricultural Salinity Assessment and Management

Gupta, B., and B. Huang. 2014. Mechanism of Salinity Tolerance in Plants : Physiological , Biochemical , and Molecular Characterization. Int. J. Genomics.

Hura, T., and B. Szewczyk-taranek. 2017. Physiological Responses of Rosa rubiginosa to Saline Environment. Water Air Soil Pollut 228(81): 1–11. doi: 10.1007/s11270-017-3263-2.

IPCC, I.P. on C.C. 2014. Climate Change 2014: Impact Adaptation and Vulnerability. Geneva, Switzerland. p. 151

Ismail, A.M., and T. Horie. 2017. Genomics, Physiology, and Molecular Breeding Approaches for Improving Salt Tolerance. Annu. Rev. Plant Biol. 68(1): 405–434. doi: 10.1146/annurev-arplant-042916-040936.

Jensen, M.H. 1997. Hydroponics. HortScience 32(6): 1018–1021.

Jesus, C.G., F.J.S. Júnior, T.R. Camara, Ê.F.F. Silva, and L. Willadino. 2015. Production of rocket under salt stress in hydroponic systems. Hortic. Bras.: 493–497.

Kang, S.A. 2013. Breeding for salinity tolerance in hydroponic systems of crop plants. Slide Share. https://www.slideshare.net/Shehzadkang/breeding-for-salinity-tolerance-in-hydroponic-systems-of-crop-plants.

Kumar, V., S.H. Wani, P. Suprasanna, and L.-S.P. Tran. 2018. Salinity Responses and Tolerance in Plants, Volume 1: Targeting Sensory, Transport and Signaling Mechanisms. Springer.

Lee, J., S.L. Smothers, D. Dunn, M. Villagarcia, C.R. Shumway, et al. 2008. Evaluation of a

Simple Method to Screen Soybean Genotypes for Salt Tolerance. Crop Sci. 48(December): 2194–2200. doi: 10.2135/cropsci2008.02.0090.

Leksungnoen, N. 2012. The Relationship Between Salinity and Drought Tolerance in Turfgrasses and Woody Species.

Martinez, V., M. Nieves-Cordones, M. Lopez-Delacalle, R. Rodenas, T.C. Mestre, et al. 2018. Tolerance to stress combination in tomato plants: New insights in the protective role of melatonin. Molecules 23(3): 1–20. doi: 10.3390/molecules23030535.

Moradi, F., and A.M. Ismail. 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann. Bot. 99(6): 1161–1173. doi: 10.1093/aob/mcm052.

Morales, R.G.F. 2012. Resistência ao déficit hídrico em famílias de tomateiro derivados de Solanum pennellii.

Morales, R.G.., L.. Resende, W.. Maluf, L.E.. Peres, and I.. Bordini. 2015. Selection of tomato plant families using characters related to water deficit resistance. Hortic. Bras. 33: 27–33. doi: http://dx.doi.org/10.1590/S0102-053620150000100005.

Morton, M.J.L., M. Awlia, N. Al-tamimi, S. Saade, Y. Pailles, et al. 2019. Salt stress under the scalpel – dissecting the genetics of salt tolerance. : 148–163. doi: 10.1111/tpj.14189.

Munns, R. 2002. Comparative physiologyy of salt and water stress. Plant, Cell Environ. 25: 239–250. doi: 10.1046/j.0016-8025.2001.00808.x.

Munns, R. 2005. Genes and Salt Tolerance. New Phytol. 167(3): 645–663.

Munns, R., and M. Tester. 2008. Mechanisms of Salinity Tolerance. Annu. Rev. Plant Biol. 59(1): 651–681. doi: 10.1146/annurev.arplant.59.032607.092911.

Murphy, L.R., S.T. Kinsey, and M.J. Durako. 2003. Physiological effects of short-term salinity changes on Ruppia maritima. Aquat. Bot. 75(4): 293–309. doi: 10.1016/S0304-3770(02)00206-1.

Najla, S., G. Vercambre, L. Pagès, D. Grasselly, H. Gautier, et al. 2009. Tomato plant architecture as affected by salinity: Descriptive analysis and integration in a 3-D simulation model. Botany 87(10): 893–904. doi: 10.1139/B09-061.

Oliveira, A.M.S. de. 2016. Resistência de tomateiros a geminivírus e a estresse hídrico.

Rivero, J., D. Álvarez, V. Flors, C. Azcón-Aguilar, and M.J. Pozo. 2018. Root metabolic plasticity underlies functional diversity in mycorrhiza-enhanced stress tolerance in tomato. New Phytol. 220(4): 1322–1336. doi: 10.1111/nph.15295.

Rouphael, Y., G. Raimondi, L. Lucini, P. Carillo, M.C. Kyriacou, et al. 2018. Physiological and Metabolic Responses Triggered by Omeprazole Improve Tomato Plant Tolerance to NaCl Stress. Front. Plant Sci. 9(February): 1–18. doi: 10.3389/fpls.2018.00249.

Roy, S.J., S. Negrão, and M. Tester. 2014. Salt resistant crop plants. Curr. Opin. Biotechnol. 26: 115–124. doi: 10.1016/j.copbio.2013.12.004.

Rush, D.W., and E. Epstein. 1976. Genotypic Responses to Salinity. Plant Physiol. 57: 162–166. doi: 10.1104/pp.57.2.162.

Rush, D.W., and E. Epstein. 1981. Comparative studies on the sodium, potassium, and chloride

relations of a wild halophytic and a domestic salt-sensitive tomato species. Plant Physiol. 68(6): 1308–1313. doi: 10.1104/pp.68.6.1308.

Sairam, R.K., K.V. Rao, and G.C. Sri. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidati v e stress , antioxidant acti v ity and osmolyte concentration. Plant Sci. 163: 1037–1046.

Shalata, A., and P.M. Neumann. 2001. Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J. Exp. Bot. 52(364): 2207–2211. doi: https://doi.org/10.1093/jexbot/52.364.2207.

Silva, A.K.P. de M., R.Q. Cavalcanti, J.B.M. Coelho, and E. Bezerra Neto. 2013. Relação Entre Concentração Molar , Condutividade Elétrica e Potencial Osmótico De Soluções Salinas. Recife.

Spooner, D.M., I.E. Peralta, and S. Knapp. 2014. New nomenclature for Lycopersicon. Sol Genomics Netw. (SGN)—from genotype to phenotype to Breed. https://solgenomics.net/about/solanum_nomenclature.pl (accessed 5 October 2018).

Sun, W., X. Xu, H. Zhu, A. Liu, L. Liu, et al. 2010. Comparative transcriptomic profiling of a salt-tolerant wild tomato species and a salt-sensitive tomato cultivar. Plant Cell Physiol. 51(6): 997–1006. doi: 10.1093/pcp/pcq056.

Tal, M., and M. Shannon. 1983. Salt Tolerance in the Wild Relatives of the Cultivated Tomato: Responses of Lycopersicon esculentum, L. cheesmanii, L. peruvianum, Solanum pennellii and F 1 Hybrids to High Salinity. Funct. Plant Biol. 10(1): 109–117. doi: 10.1071/PP9830109.

Tavakkoli, E., F. Fatehi, P. Rengasamy, and G.K. McDonald. 2012. A comparison of hydroponic and soil-based screening methods to identify salt tolerance in the field in barley. J. Exp. Bot. 63(2): 3853–3868. doi: 10.1093/jxb/err313.

Tavakkoli, E., P. Rengasamy, and G.K. McDonald. 2010. High concentrations of Na+ and Cl– ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. J. Exp. Bot. 61(15): 4449–4459. doi: 10.1093/jxb/erq251.

Team, R.C. 2017. R: A language and environment for statistical computing.

Tonetto De Freitas, S., A.J. McElrone, K.A. Shackel, and E.J. Mitcham. 2014. Calcium partitioning and allocation and blossom-end rot development in tomato plants in response to whole-plant and fruit-specific abscisic acid treatments. J. Exp. Bot. 65(1): 235–247. doi: 10.1093/jxb/ert364.

Tyler, R.H., T.P. Boyer, T. Minami, M.M. Zweng, and J.R. Reagan. 2017. Electrical conductivity of the global ocean. Earth, Planets Sp. 69(1). doi: 10.1186/s40623-017-0739-7.

Yang, H., M.K. Shukla, X. Mao, S. Kang, and T. Du. 2019. Interactive Regimes of Reduced Irrigation and Salt Stress Depressed Tomato Water Use Efficiency at Leaf and Plant Scales by Affecting Leaf Physiology and Stem Sap Flow. Front. Plant Sci. 10(February). doi: 10.3389/fpls.2019.00160.

Zhai, Y., Q. Yang, and M. Hou. 2015. The effects of saline water drip irrigation on tomato yield, quality, and blossom-end rot incidence - A 3a case study in the South of China. PLoS One 10(11): 1–17. doi: 10.1371/journal.pone.0142204.

Zhai, Y., Q. Yang, and Y. Wu. 2016. Soil salt distribution and tomato response to saline water

irrigation under straw mulching. PLoS One 11(11): 1–17. doi: 10.1371/journal.pone.0165985.

Zsögön, A., M.H. Vicente, D. Reartes, and L.E. Peres. 2017. Understanding and improving water-use efficiency and drought resistance in tomato. Achieving sustainable cultivation of tomatoes. Edition: First. Chapter: 5. Publisher: Burleigh Dodds Science Publishing. Editors: Autar Mattoo, Avtar Handa. DOI: 10.19103/AS.2016.0007.01

CAPÍTULO 3

Salt tolerance in *S. galapagense*: Genetic control of stress symptoms in a hydroponic system

SUMMARY

To elucidate the genetic control of stress symptoms caused by salinity in *Solanum galapagense*, segregating populations were obtained from the interspecific cross *Solanum lycopersicum* 'TOM-684' x *Solanum galapagense* 'LA1401'. Thirty-five days after sowing, the plants were exposed to a 300 mM salt stress (split into two doses of 150 mM each), and were evaluated based on stress symptoms observed on the leaves. The area under the curve of the stress symptoms over time was used to test the hypothesis of monogenic inheritance under different presumed degrees of dominance, and genetic models were also tested using maximum likelihood tests. Stress symptoms in *S. galapagense* 'LA1401' are controlled by more than one locus, i.e., oligogenic inheritance. Broad-sense heritability was 0.66 and narrow-sense heritability was 0.27.

1 INTRODUCTION

The cultivated tomato, *Solanum lycopersicum* (formerly *Lycopersicon esculentum*), is considered worldwide one of the most economically important horticultural crops (RAZALI et al., 2018), and has been described as sensitive to salt stress (ZHAI; YANG; HOU, 2015). Soil salinity is rapidly increasing on a global scale and is one of the major environmental factors that limit crop production (ALVES et al., 2018). It may decrease average yields for most major crops by more than 50 percent (BARTELS; SUNKAR, 2005) and therefore threaten food security (EGEA et al., 2018) .

The cultivated tomato belongs to an economically important clade that consists of 14 species (RAZALI et al., 2018) and its within-species variability for salinity tolerance is limited. Breeding for salt tolerance should make use of related *Solanum* species adapted to marginal environments (VILLALTA et al., 2008). Accessions of some tomato wild species such as *S. pimpinellifolium* (Asins; Bretó; Carbonell, 1993; Cuartero; Fernández-Muñoz, 1999), *S. galapagense* (Rush & Epstein, 1976; Cuartero; Fernández-Muñoz, 1999; Spooner; Peralta; Knapp, 2014; Andrade et al. 2017), and *S. pennellii* (Cuartero; Fernández-Muñoz, 1999; Frary, Keleş, Pinar, Göl, & Doğanlar 2011) have already been reported as salt tolerant, and could be used as genetic resources to improve salt tolerance in tomato elite lines.

S. galapagense (formely *Lycopersicon cheesmannii* var. *minor*) is native to the Galapagos Islands, where it is distributed in lower elevations, and extends into the littoral

zone, subject to marine influences as salt spray and salt accumulation in the soil (RICK, 1973c). It has been found to be more salt tolerant than cultivated tomatoes (Rush & Epstein, 1981, 1976; Darwin et al., 2003; Darwin, 2009), since it can survive in full strength seawater nutrient solution while the cultivated tomato cannot in most cases withstand levels higher than 50% of seawater (RICK, 1973c).

The aim of this study was to elucidate the genetic control of salt stress symptoms in leaves of tomato genotypes in segregating populations of the cross *S. lycopersicum* 'TOM684' x *S. galapagense* 'LA1401'.

2 MATERIAL AND METHODS

The experiment was carried out in a hydroponic system in a greenhouse of the HortiAgro Sementes S.A. Research Station, Ltda. (alt 842 m, lat 21º 10' 12" S and long 44º 55' 31" W), Ijaci - Minas Gerais, Brazil, following a protocol established by Rezende et al. (2019, unpublished).

2.1 Plant material

Segregating populations were obtained from the interspecific cross *Solanum lycopersicum* 'TOM-684' x *Solanum galapagense* 'LA1401'. LA1401 (= P_2) is a wild accession characterized by a high salt tolerance (Rush & Epstein, 1976; Rush & Epstein, 1981; Darwin, 2009); (Rezende et al., 2019, unpublished). TOM-684 (=P_1) is a standard line susceptible to salt stress (personal information), and a proprietary fresh-market tomato inbred line from Hortiagro Sementes Ltda. The parents LA1401 and TOM-684 were crossed to obtain the F_1 generation. F_1 plants were self-pollinated and backcrossed with both parents (accession LA1401 and line TOM-684) obtaining respectively the F_2 generation and the backcrosses: $F_1BC_{1(1)}$ [=(F_1 x TOM-684)] and $F_1BC_{1(2)}$ [=(F_1 x LA1401)]. Parents (P_1 and P_2) and the populations F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ were sown in deepots according Rezende et al. (2019, unpublished) in a completely randomized design.

Thirty-five days after sowing (07/07/2017), the plants were exposed to a salt stress induced by the addition of 300 mM of NaCl (≈ 26 dS m^{-1}), split as two additions of 150 mM, the second half being applied three days later than the first (07/10/2017).

Phenotypic evaluations started two days after the total amount of NaCl was applied (07/12/2017). Twenty-two evaluations were made over a 52-day interval (through

08/31/2017). Assessments were based on the stress symptoms observed on the leaves (Asins et al. 1993; Rezende et al., 2019, unpublished), and the data collected was used to estimate the area under the stress symptoms progress curve (area). The experiment consisted of 70 plants from each parental line, 70 plants from the F_1, 276 individuals from the F_2 and 135 individuals from each backcross. All plants were disposed in a completely randomized design.

2.2 Rating scale based on stress symptoms in leaves due to salt stress

The rating scale used to evaluate this trial was adapted from Rezende et al. (2019, unpublished).

0- Plant without symptoms + normal growth.
1- No apparent stress symptoms on leaves + stunted growth;
2- Plants with stunted growth + leaves slightly wrinkling;
3- Plants with stunted growth + moderate leaf wrinkling and curling; light chlorosis of the apical meristem;
4- Plants with stunted growth + moderate leaf wrinkling and curling + leaves showing a lighter green and yellowing of the tips or margins + wilting and defoliation of the older leaves + a few leaves might show mosaic, mottling or malformation;
5- Plants with stunted growth + generalized yellowing of the older leaves + intermediate leaves severely wrinkled and curled + the onset of chlorosis in the main vein (close to the petiole) of the leaflets might be observed.
6- Plants with stunted growth + generalized leaf yellowing and wilting + defoliation of the older leaves; a few leaves which were previously yellow might turn brown;
7- Generalized leaf yellowing and irreversible wilting; or plant death.

Right after salt stress induction, in the initial phase, some plants showed a reversible leaf wilting, especially during the hottest hours of the day (Rezende et al. 2019, unpublished).

2.3 Stress symptoms over time

The scores attributed to each plant throughout the assessments were used to estimate the area under the stress symptoms progress curve over time as follows:

$$Area = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where y_i is the score assigned to the stress symptom at the i^{th} observation, t_i is the day of the assessment at the i^{th} observation, and n is the total number of observations (Simko & Piepho 2012).

2.4 Statistical analysis

The statistical analyses were based on the area under the stress symptoms progress curve (area) over time as measured for each of the plants. The mean degree of dominance (MDD), broad-sense (H^2) and narrow-sense (h^2) heritabilities were estimated using the additive-dominant model according to Mather and Jinks (1984) method as follows:

$$MDD = \frac{d}{a};\ H^2 = \frac{\sigma_G^2}{\sigma_P^2} \text{ and } h^2 = \frac{\sigma_a^2}{\sigma_P^2}$$

Being,

d: the dominance deviation from the mean;

a: the additive deviation;

σ_G^2: genotypic variance;

σ_P^2: phenotypic variance;

σ_a^2: additive variance.

2.4.1 Genetic models used to estimate the genetic control of stress symptoms in leaves of tomato plants under saline conditions

2.4.1.1 Chi-square test: hypothesis of monogenic inheritance under different presumed degrees of dominance

Data was used to test hypotheses of monogenic inheritance under different presumed degrees of dominance, as described by (MENEZES et al., 2015). A truncation point (TP) was chosen in the area under stress symptoms progress curve (Area) so that most of P_2 plants were below the TP and most of P_1 plants were above it. The TP used was the midparental value 160.76. Hypothesis of monogenic inheritance was tested under the following assumptions:

a) Data from all generations (P_1, P_2, F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$) have normal distributions.

b) Means and variances of P_1 and P_2 are equal to the respective estimates obtained from the experimental data.

c) Based on normal distribution, frequencies of P_1 and P_2 plants with scores equal or lower than the truncation point were estimated for each plant population tested.

d) The mean of F_1 generation was admitted as being: $\underline{F}_1 = \frac{P_1+P_2}{2} + MDD\frac{P_1-P_2}{2}$, where $\underline{P}_1$ and $\underline{P}_2$ are the respective parental means, where MDD is the mean degree of dominance presumed.

e) The variance of the F_1 population is equal to the respective variance estimate obtained from the experimental data.

f) The expected frequencies (f) of F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ population, based on a monogenic model of inheritance, were estimated as functions of P_1, P_2 and F_1 frequencies, as follows:

$f(F_2) = \frac{[f(P_1)+2f(F_1)+f(P_2)]}{4}$; $f(BC_{11}) = \frac{[f(P_1)+f(F_1)]}{2}$ and $f(BC_{12}) = \frac{[f(P_2)+f(F_1)]}{2}$.

g) The expected number of P_1, P_2, F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ plants ≤ TP were calculated by multiplying the expected frequencies by the total number of plants tested per generation.

h) Expected numbers of plants ≤ TP were compared with their respective observed values in each generation. The frequency of expected plants in P_1 was added to that of P_2, in order to avoid expected frequencies equal to zero. The significance of the deviations was estimated with a χ^2 test, with 4 degrees of freedom.

i) Significant χ^2 values would lead to the rejection of the hypothesis of monogenic inheritance under the degree of dominance presumed. On the other hand, a non-significant χ^2 value would lead to non-rejection of such hypothesis. Values of χ^2 for each simulation were plotted against their respective hypothetical MDD's. The interval of MDD values of which χ^2 values are below the $\alpha = 0.05$ critical value represents the MDD interval in which the monogenic hypothesis was not rejected.

2.4.2.2 Genetic models using maximum likelihood functions

Genetic models assuming monogenic inheritance plus modifier polygenes were tested using maximum likelihood in mixtures of normal densities, as proposed by Gonçalves,

Bearzoti & Ferreira (2004), Menezes et al. (2005), Azevedo et al. (2012), Menezes et al. (2015).

For the analyses, the full genetic model admitted a major gene with additive and dominance effects, and polygenes, also with additive and dominance effects. From the complete genetic model, simpler models containing less parameters were generated (Table 1).

Environmental variances were considered equal for all generations, and gene segregation was considered independent (both major genes and polygenes). Hypothesis tests of the genetic parameters were carried out based on likelihood ratio between two models (GONÇALVES; BEARZOTI; FERREIRA, 2004). The tests were carried out using the statistical software 'Monogen v.0.1'.

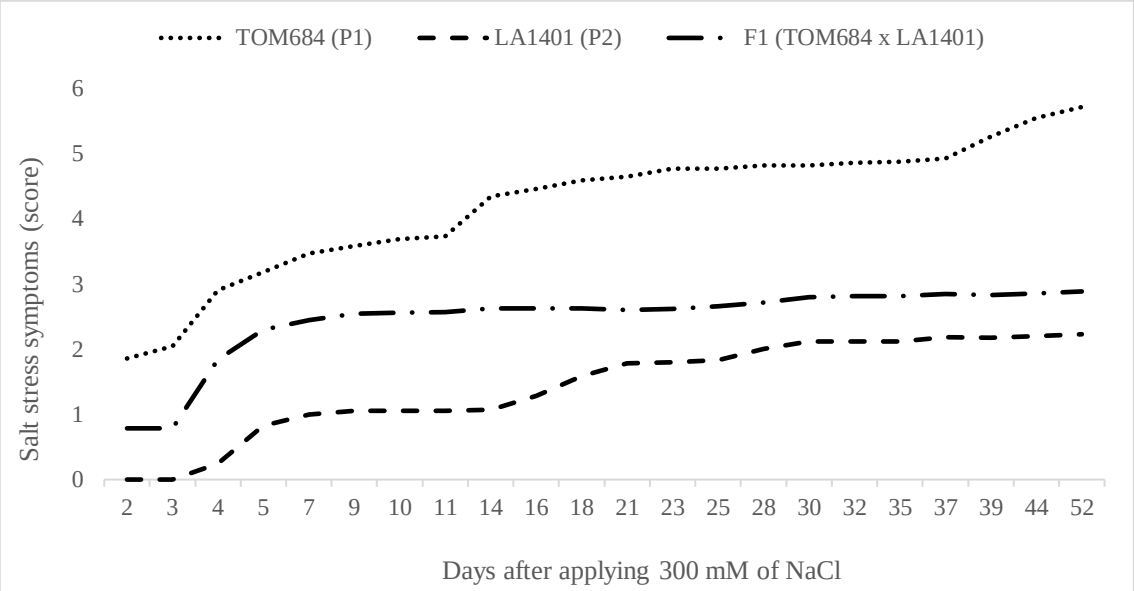
Table 1 Genetic inheritance models according to Gonçalves et al. (2004) tested for salt tolerance in tomato, adapted from Menezes et al. (2015).

Models	Estimated Parameters
1 = major gene with additive and dominance effects + polygenes with additive and dominance effects + environmental effects	μ, A, D, $[a]$, $[d]$, V_A, V_D, S_{AD}, σ^2
2 = major gene with additive and dominance effects + polygenes with only additive effect + environmental effects	μ, A, D, $[a]$, V_A, σ^2
3 = major gene with only additive effect + polygenes with additive and dominance effects + environmental effects	μ, A, $[a]$, $[d]$, V_A, V_D, S_{AD}, σ^2
4 = major gene with only additive effect + polygenes with only additive effect + environmental effects	μ, A, $[a]$, V_A, $\sigma 2$
5 = polygenes with additive and dominance effects + environmental effects	μ, $[a]$, $[d]$, V_A, V_D, S_{AD}, σ^2
6 = polygenes with only additive effect + environmental effects	μ, $[a]$, V_A, σ^2
7 = major gene with additive and dominance effects + environmental effects	μ, A, D, σ^2
8 = major gene with only additive effect + environmental effects	μ, A, σ^2
9 = only environmental effects	μ, σ^2

3 RESULTS

The average behavior of populations TOM684 (P_1), LA1401 (P_2), F_1 (TOM684 x LA1401) in response to salt stress over time are shown in Fig. 1.

Fig 1 Stress symptoms evolution through the experiment in the populations derived from the cross *S. lycopersicum* 'TOM684' x *S. lycopersicum* 'LA1401'.



From the 2nd day on salt stress, the differences between populations TOM684 (P_1), LA1401 (P_2), and F_1 (P_1 x P_2) were clear, and became more evident over time. Stress symptom evolution for TOM684 (P_1) was quicker than for LA1401 (P_2) (Fig. 1). F_1 showed intermediate values between the parental lines TOM684 (P_1) and LA1401 (P_2) (Fig. 1). The greatest differences between them was observed on the 52th day (Fig. 1).

The area under the stress symptoms progress curve (area) in leaves, 52 days after 300 mM of NaCl was applied, was calculated for P_1, P_2, F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$, and are shown in Table 2.

Table 2 Area under the stress symptoms progress curve over time in leaves of the populations derived from the cross *S. lycopersicum* 'TOM684' x *S. lycopersicum* 'LA1401', 52 days after the total application of 300mM of NaCl.

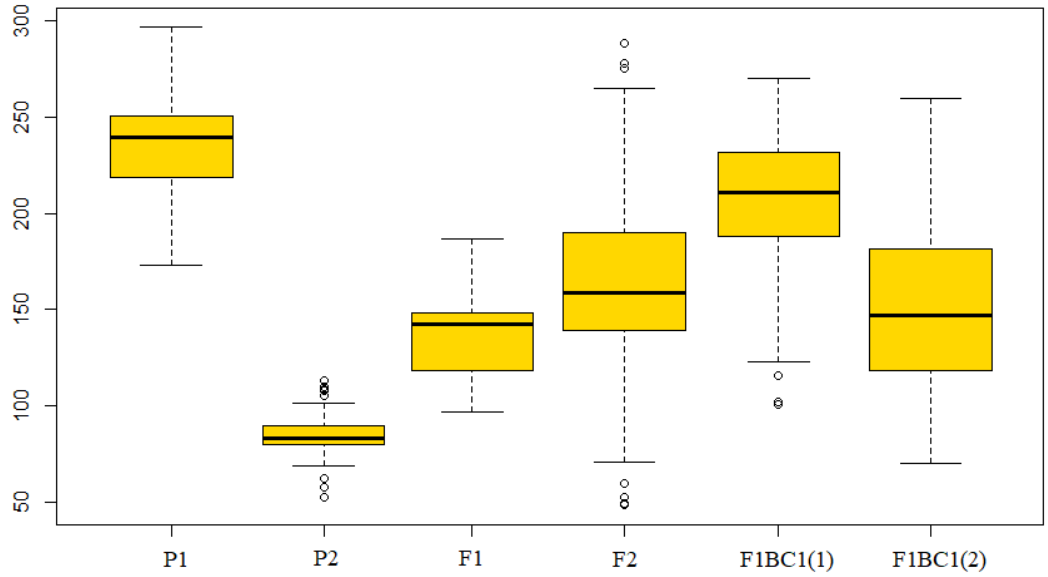
Populations	Area ± SD*	Total Nº Plants	Number of Plants with	
			Area<=160.76	Area>160.76
TOM684 (P_1)	236.38 ± 30.05	70	0	70
LA1401 (P_2)	85.14 ± 11.60	70	70	0
F_1	134.35 ± 22.17	70	65	5
F_2	163.72 ± 42.42	276	140	136
$F_1B_{1(1)}$ [F_1 x TOM684]	206.76 ± 35.79	134	14	120
$F_1BC_{1(2)}$ [F_1 x LA1401]	152.46 ± 42.62	135	80	55

SD*: Standard deviation

The average value for the area in the salt-tolerant parent LA1401 (P_2) (*S. galapagense*) was lower than for the salt-sensitive parent TOM-684 (P_1) (*S. lycopersicum*) (Fig. 2). The F_1 and F_2 populations showed values for the area intermediate between the values found in the parents, however, they were not similar. The area found in the F_1 was lower than in the F_2 (Fig. 2), indicating the existence of non-additive genetic effects. The backcross $F_1BC_{1(1)}$ [(TOM684 x LA1401) x TOM684] had an area greater than the backcross $F_1BC_{1(2)}$ [(TOM684 x LA1401) x LA1401], and closer to the salt-sensitive parent (P_1) TOM684 (Fig. 2). In contrast, $F_1BC_{1(2)}$ showed an average value for the area closer than that of the salt-tolerant parent (P_2) LA1401(Fig. 2).

Boxplots of the area under the stress symptoms progress curve indicate less variation for the population LA1401 (P_2) than for the others (Fig. 2), as can also be indicated by the standard deviations in Table 2.

Fig. 2 Boxplot of areas of populations P_1, P_2, F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$, derived from *S. lycopersicum* 'TOM684' x *S. galapagense* 'LA1401', 52 days after the total application of 300mM of NaCl.



P_1: TOM684; P_2: LA1401; F_1: TOM684 x LA1401; F_2: (F_1 x F_1); $F_1BC_{1(1)}$: [F_1 x TOM684]; $F_1BC_{1(2)}$: [F_1 x LA1401].

The mean degree of dominance was estimated in -0.35 (Table 3), reinforcing that there is a degree of heterosis for stress symptoms in the direction of lower areas, i.e., higher salt tolerance, and the differences found between F_1 and F_2 (Table 3 and Fig. 2) are due to non-additive effects. The broad-sense heritability was estimated in 0.6609 and in the narrow-sense was estimated in 0.2782 (Table 3).

Table 3 Mean degree of dominance (MDD), broad-sense and narrow-sense heritability (H^2 and h^2) for stress symptoms in leaves of tomato plants from populations derived from the cross *S. lycopersicum* 'TOM684' x *S. galapagense* 'LA1401' for salt tolerance in tomato, 52 days after exposure to salt stress.

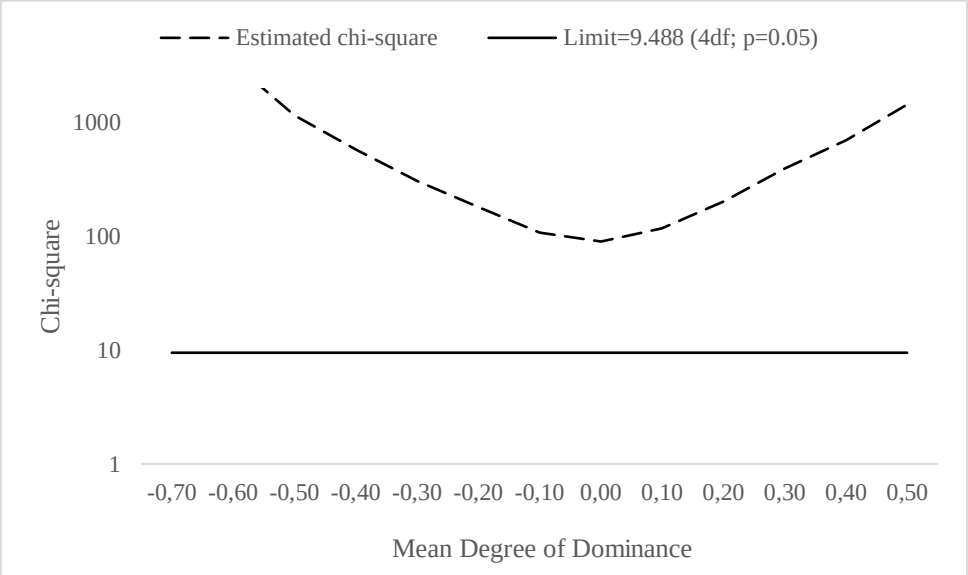
Parameters	52 days
MDD	-0.35
H^2	0.6609
h^2	0.2782

MDD: Mean Degree of Dominance.
H^2: Broad-Sense Heritability
h^2: Narrow-Sense Heritability
All parameters were estimated according to Mather and Jinks (1984), using the additive-dominant model.

3.1 Chi-square test: a hypothesis of monogenic inheritance under different presumed degrees of dominance

To perform the chi-square tests, the genotypes of each population were separated into two classes (≤160.76 and >160.76), using the parental average (160.76) as the truncation point. The observed frequency was based on the raw data, and the expected frequency was based on the total number of plants, from each population, considering different presumed mean degrees of dominance, varying from -1 to 1 (Fig. 3). However, all estimated chi-squares were superior to the critical chi-square p-value at the significance level of 0.05 of 9.488, therefore, the monogenic hypothesis should be discarded in this case (Fig. 3).

Fig. 3 Monogenic hypotheses tested under different presumed degrees of dominance of stress symptoms in leaves due to salt stress in *S. galapagense* "LA1401", 52 days after the application of 300 mM of NaCl.



3.2 Monogen: Testing the hypothesis of monogenic inheritance using maximum likelihood models

The genetic models tested were shown on Table 1, and inheritance tests carried out with maximum likelihood tests are presented in Table 4.

When model 1 was confronted with model 5, the existence of a major gene plus polygenic effects is compared to the occurrence of polygenic effects only (MENEZES et al., 2015) (Table 1). No significant monogenic effects were detected (P=0.173), indicating that there is not a clear effect of a major gene in the control of the trait (Table 4). When

model 1 was confronted with model 7 (Table 1), the hypothesis was rejected, indicating the evidence of polygenic effects (Table 4).

Tests confronting model 5 with model 9, and model 7 with model 9 (MENEZES et al., 2015) (Table 4) reinforce that the control of stress symptoms in *S. galapagense* 'LA1401' is more complex than what is expected from a single major gene alone, and there may likely be both a major gene effect and polygenic effects in the control of this trait.

Table 4 Hypothesis of inheritance tested by using maximum likelihood for salt tolerance in populations derived from the interspecific cross *S. lycopersicum* 'TOM684' x *S. galapagense* 'LA1401' for salt tolerance in tomato.

Models	χ_c^2	Degrees of Freedom	Probability
1 vs 5	3.5069	2	0.173
1 vs 7	94.335	5	0.000
5 vs 9	541.011	5	0.000
7 vs 9	450.183	1	0.000

4 DISCUSSION

4.1 Stress Symptoms

The methodology deployed in this study to induce salt-stress, proposed by Rezende et al. (2019, unpublished), was efficient in distinguishing the salt tolerance level of the parental lines (Fig. 1; Table 2; Fig. 2). The longer the plants were exposed to salt stress, the more severe the stress symptoms became, and the differences in the salt tolerance level of the populations TOM684 (P_1), LA1401 (P_2) and F_1 (TOM684 x LA1401) became evident (Fig. 1).

Even though the parental line *S. galapagense* 'LA1401' (P_2) (= *Lycopersicon cheesmanii* ssp. *minor*) was clearly more salt-tolerant than the parental line, TOM-684 (P_1), it also showed stress symptoms that became more severe over time (Fig. 1). According to Rush and Epstein (1976), LA1401 can survive in full strength seawater nutrient solution, while the *S. lycopersicum* cannot in most cases withstand levels higher than 50% seawater; nevertheless LA1401 growth rates are reduced under saline conditions, which was confirmed in this study.

As expected, from a report by Rezende et al. (2019, unpublished), the longer the plant was exposed to salt stress in this study, the higher was the damage and more visible were the stress symptoms. Even though stress symptoms observed during the experiment and reported in this paper seem simple, they arise from complex mechanisms of tolerance, involving a range of morphological, physiological, biochemical, and even molecular changes (Acosta-Motos et al., 2017).

4.2 Genetic control of stress symptoms in leaves

All chi-squares, estimated under different degrees of dominance, varying from -1 to 1, were significant at the significance level of 5% (χ_t^2= 9.487) (Fig. 3), therefore, the hypothesis of monogenic inheritance should be discarded. These results obtained from the chi-square tests (Fig. 3) point out that there is more than one locus controlling stress symptoms due to salt stress in *S. galapagense* 'LA1401', which are confirmed by the inheritance tests carried out with maximum likelihood functions presented in Table 1, especially by the models 1 vs 5 (Fig. 3; Table 4). However, comparison of models 7 vs 9, which also tests a monogenic hypothesis was significant ($p<0.001$), indicating that a gene with major genetic effects may also be involved in the stress symptoms control. Tests confronting model 5 to model 9, and model 7 to model 9 (Table 4) reinforce that stress symptoms in *S. galapagense* 'LA1401' are affected by polygenes. Therefore, there is indication of an oligo or polygenic inheritance, but with one of the genes involved showing genetic effects that are large enough to be considered major effects.

The substantial difference found between broad-sense (66.09%) and narrow-sense (27.82%) heritability estimates in this study (Table 3) are due to non-additive gene effects, confirming that non-additive effects also have an important role on salt tolerance as reported by Asins et al. (1993) and Chen et al. (2013).

Broad-sense heritability estimated in this study (0.66, Table 2) was lower than that reported by Asins et al. (1993) (0.93), studying the salt tolerance in segregant populations from the interspecific cross S. *lycopersicum* and *S. pimpinellifolium*. Heritability estimates are not constant, they can change over time because the variance in genetic values, the variation due to environmental factors, and/or the correlation between genes and environment can change (WRAY, 2008). Furthermore, the target species for these inheritance studies are different, as a result, they may have different tolerance mechanisms, and different genes to cope with salt stress.

Finally, selection of salt-tolerant genotypes was expected to be effective at 52 days after stress induction (h^2=27.82%). After 52 days, the stress symptoms evolve rapidly, reducing the efficiency on distinguishing the tolerance levels between the genotypes.

5 CONCLUSIONS

Salt-tolerance in *S. galapagense* "LA1401" is a complex polygenic trait, with significant additive and non-additive genetic effects, with some evidence that one of the genes involved may have genetic effects large enough to be considered major gene effects.

BIBLIOGRAPHY

Acosta-Motos, J.R., M.F. Ortuño, A. Bernal-Vicente, P. Diaz-Vivancos, M.J. Sanchez-Blanco, et al. 2017. Plant Responses to Salt Stress: Adaptive Mechanisms. Agronomy 7(18): 1–38. doi: 10.3390/agronomy7010018.

Alves R de C, de Medeiros AS, Nicolau MCM, et al (2018) The partial root-zone saline irrigation system and antioxidant responses in tomato plants. Plant Physiol Biochem 127:366–379. doi: 10.1016/j.plaphy.2018.04.006

Amjad M, Akhtar SS, Yang A, et al (2015) Antioxidative Response of Quinoa Exposed to Iso-Osmotic, Ionic and Non-Ionic Salt Stress. J Agron Crop Sci 201:452–460. doi: 10.1111/jac.12140

Amjad M, Ameen N, Murtaza B, et al (2019) Comparative Physiological and Biochemical Evaluation of Salt and Nickel Tolerance Mechanisms in Two Contrasting Tomato Genotypes. Physiol Plant. doi: 10.1111/ppl.12930

Andrade MC, da Silva AA, Neiva IP, et al (2017) Inheritance of type IV glandular trichome density and its association with whitefly resistance from Solanum galapagense accession LA1401. Euphytica 213:. doi: 10.1007/s10681-016-1792-1

Asins MJ, Bretó MP, Carbonell EA (1993) Salt tolerance in Lycopersicon species. II. Genetic effects and a search for associated traits. Theor Appl Genet 86:769–774. doi: 10.1007/BF00222668

Azevedo SM de, Maluf WR, Faria MV, et al (2012) Inheritance of resistance to the Papaya ringspot virus-watermelon strain (PRSV-W) from watermelon accession "PI 595201." Crop Breed Appl Biotechnol 12:67–75. doi: 10.1590/S1984-70332012000100009

Bartels D, Sunkar R (2005) Drought and salt tolerance in plants. CRC Crit Rev Plant Sci 24:23–58. doi: 10.1080/07352680590910410

Beserra Júnior JEA, Maluf WR, Figueira AR, Barguil BM (2006) Herança da Resistência ao Watermelon mosaic virus em Melancia (Citrullus lanatus L .)*. Fitopatol Bras 31:1–4

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Ann Bot 103:551–560. doi: 10.1093/aob/mcn125

Chen J, Shen J, Xu J, et al (2013) Heritability and gene effects for salinity tolerance in cucumber (Cucumis sativus L.) estimated by generation mean analysis. Sci Hortic (Amsterdam) 159:122–127. doi: 10.1016/j.scienta.2013.04.020

Cuartero J, Fernández-Muñoz R (1999) Tomato and salinity. Sci Hortic (Amsterdam) 78:83–125

Darwin SC (2009) The systematics and genetics of tomatoes on the Galápagos Islands (Solanum , Solanaceae). 1–261

Darwin SC, Knapp S, Peralta IE (2003) Taxonomy of tomatoes in the Galápagos Islands: Native and introduced species of Solanum section Lycopersicon (Solanaceae). Syst Biodivers 1:29–53. doi: 10.1017/S1477200003001026

Egea I, Albaladejo I, Meco V, et al (2018) The drought-tolerant Solanum pennellii regulates leaf water loss and induces genes involved in amino acid and ethylene/jasmonate metabolism under dehydration. Sci Rep 8:1–14. doi: 10.1038/s41598-018-21187-2

Frary A, Keleş D, Pinar H, et al (2011) NaCl tolerance in Lycopersicon pennellii introgression lines: QTL related to physiological responses. Biol Plant 55:461–468. doi: 10.1007/s10535-011-0111-x

Gonçalves RP, Bearzoti E, Ferreira DF (2004) Avaliação da razão de verossimilhança generalizada em teste de hipóteses sobre o controle genético de características contínuas. Semin Ciências Agrárias 281–292. doi: 10.5433/1679-0359.2004v25n4p281

Mather, K; Jinks, JL (1984) Introdução à genética biométrica. Tradutor DUARTE, F. A. M. et. al. Ribeirão Preto - SP: Sociedade Brasileira de Genética. 242 p.

Menezes CB, Maluf WR, Faria M V, et al (2015) Inheritance of resistance to papaya ringspot virus-watermelon strain (PRSV-W) in 'Whitaker' summer squash line. Crop Breed Appl Biotechnol 15:203–209. doi: http://dx.doi.org/10.1590/1984-70332015v15n4a36

Menezes CB de, Maluf WR, Azevedo SM de, et al (2005) Inheritance of parthenocarpy in summer squash (Cucurbita pepo L.). Genet Mol Breed 4:39–46.

Munns R (2002) Comparative physiologyy of salt and water stress. Plant, Cell Environ 25:239–250. doi: 10.1046/j.0016-8025.2001.00808.x

Munns R (2005) Genes and Salt Tolerance. New Phytol 167:645–663

Murphy LR, Kinsey ST, Durako MJ (2003) Physiological effects of short-term salinity changes on Ruppia maritima. Aquat Bot 75:293–309. doi: 10.1016/S0304-3770(02)00206-1

Razali R, Bougouffa S, Morton MJL, et al (2018) The Genome Sequence of the Wild Tomato Solanum pimpinellifolium Provides Insights Into Salinity Tolerance. Front Plant Sci 9:1–21. doi: 10.3389/fpls.2018.01402

Rick CM (1973) Potential Genetic Resources in Tomato Species: Clues from Observations in Native Habitats. In: Genes, Enzymes, and Populations. Springer US, Boston, MA, pp 255–269

Rush DW, Epstein E (1976) Genotypic Responses to Salinity. Plant Physiol 57:162–166. doi: 10.1104/pp.57.2.162

Rush DW, Epstein E (1981) Comparative studies on the sodium, potassium, and chloride relations of a wild halophytic and a domestic salt-sensitive tomato species. Plant Physiol

68:1308–1313. doi: 10.1104/pp.68.6.1308

Simko I, Piepho H (2012) The Area Under the Disease Progress Stairs : Calculation , Advantage , and Application. Phytopathology 102:381–389. doi: 10.1094/PHYTO-07-11-0216

Spooner DM, Peralta IE, Knapp. S (2014) New nomenclature for Lycopersicon. In: Sol Genomics Netw. (SGN)—from genotype to phenotype to Breed. https://solgenomics.net/about/solanum_nomenclature.pl. Accessed 5 Oct 2018

Villalta I, Reina-Sánchez A, Bolarín MC, et al (2008) Genetic analysis of Na+and K+concentrations in leaf and stem as physiological components of salt tolerance in Tomato. Theor Appl Genet 116:869–880. doi: 10.1007/s00122-008-0720-8

Wray NR (2008) Estimating Trait Heritability. Nat Educ 1:

Zhai Y, Yang Q, Hou M (2015) The effects of saline water drip irrigation on tomato yield, quality, and blossom-end rot incidence - A 3a case study in the South of China. PLoS One 10:1–17. doi: 10.1371/journal.pone.0142204

Zsögön A (2011) Identification and characterization of a tomato introgression line with reduced wilting under drought. Thesis. Australian National University. Available on: https://openresearch-repository.anu.edu.au/handle/1885/11216

CAPÍTULO 4

Genetic control of salt tolerance in drought-tolerant advanced tomato line, obtained from S. pennellii 'LA716'

SUMMARY

The cultivated tomato, *Solanum lycopersicum*, is a glycophyte species and is severely damaged even at low salinity levels. Even though genotypic variation for salt tolerance has already been described, all the tolerance sources are found in related wild species. *S. lycopersicum* 'BPX-441E-88', a drought tolerant genotype has been recently described as salt tolerant (data unpublished), and therefore is a potential salt tolerance source for development of salt tolerant cultivars. In this study, populations P_1 (BPX-441E-88), P_2 (TOM760), F_1 (P_1 x P_2), F_2 (P_1 x P_2), $F_1BC_{1(1)}$ (F_1 x P_1), and $F_1BC_{1(2)}$ (F_1 x P_2) were grown under saline conditions (300 mM NaCl) in a hydroponic system, and assessed for stress symptoms, in order to study the inheritance of salt tolerance in BPX-441E-88. This trait was found to be controlled by a major gene with polygenic modifiers with a mean degree of dominance between +0.60 and + 0.70. Estimates of broad-sense and narrow-sense heritability were 26.28% and 10.34%, respectively.

1 INTRODUCTION

The cultivated tomato has a narrow genetic base due to its domestication process (Bauchet; Causse, 2012), however, important sources of variation for salt tolerance exist in wild relatives (Razali et al., 2018). *S. pennellii* Correll, a wild relative of cultivated tomato, is adapted to the arid conditions of the Andean region in South America and stands out for salt (Tal & Shannon, 1983; Frary et al., 2011; Bolger et al., 2014), and drought tolerance (Yu, 1972; Rick, 1973; Kahn, Fender, Bray, & O'Connell, 1993; Zsögön, 2011; Egea et al., 2018), offering a valuable breeding potential for drought (Egea et al., 2018) and salt tolerance in the species (Razali et al., 2018).

Drought and salinity show a high degree of similarity with respect to physiological, biochemical, molecular and genetic effects (Leksungnoen, 2012). Under drought stress, a finite amount of water under decreased water potential would have to be obtained from the soil profile by the plant. Under salt stress, a large amount of water is available, but under low water potential. As a result, a drought tolerant genotype could also be salt tolerant or vice versa, since they might have similar mechanisms to cope with these stresses (Leksungnoen, 2012).

The controlled introgression of delayed wilting upon water deprivation from *S. pennellii* 'LA716' into tomato cv. Micro-Tom led to the characterization of a line, with

semi-determinate growth habit and increased long-term water-use efficiency (WUE) named WELL ("*Water Economy Locus in Lycopersicon*") (Zsögön, 2011; Vicente et al., 2015; Vicente and Reartes, 2017). Because this line did not have desirable agronomic-traits for large-scale tomato production, self-fertilizations of the cross {TOM-684 x (WELL x M-82)} (TOM-684 and M-82 being commercial tomato lines) were performed and the advanced inbred line BPX-441E-88 was obtained.

BPX-441E-88 was tested under drought conditions and was considered drought tolerant due to the low incidence of blossom-end rot (BER) under water deficit (Morales et al., 2015). In BPX-441E-88, BER incidence is known to be controlled by a single major gene (Millones-Chanamé et al., 2019). Because drought and salinity tolerance can be considered related, BPX-441E-88 was tested by Rezende et al. (2019, unpublished) under saline conditions. Among the elite genotypes tested, BPX-441E-88 was one with the highest level of salt tolerance, along with the wild accessions *S. galapagense* 'LA1401' and S. *pennellii* 'LA716'. Thus, this line is a potential valuable source of salt tolerance to the cultivated tomato.

Nonetheless, the mechanisms and the key genes controlling salinity tolerance in tomato are little known (Shah et al., 2018). The aim of this study was to elucidate the genetic control of saline stress symptoms in leaves (Rezende et al. 2019, unpublished), in a segregating population from the cross *S. lycopersicon* 'TOM760' x *S. lycopersicon* 'BPX-441E-88'.

2 MATERIAL AND METHODS

The experiment was set up as a completely randomized design in a floating hydroponic system according to Rezende et al. (2019, unpublished) in a greenhouse of the HortiAgro Sementes Ltda. Research Station, Ijaci, Minas Gerais, Brazil (lat 21^{o} 09' 24'' S, long 44^{o} 55' 34'' W, alt 833m).

2.1 Plant material: *S. lycopersicum* "BPX-441E-88" x *S. lycopersicum* "TOM760"

Segregating populations were obtained from the cross *Solanum lycopersicum* 'BPX-441E-88' x *Solanum lycopersicum* 'TOM-760'. BPX-441E-88 (= P_1) is an advanced inbred line originated from the cross [TOM-684 x (Well background from *S. pennellii* "LA716"x M-82)] characterized by high level of drought tolerance (Morales et al., 2015); according to Rezende et al. (2019, unpublished), BPX-441E-88 also stands out

for salt tolerance, so this genotype is a potential source of salt tolerance. TOM-760 (=P_2) is a standard salt sensitive proprietary fresh-market tomato inbred line from Hortiagro Sementes Ltda.

The parents BPX-441E-88 and TOM-760 were crossed to obtain the F_1 (P_1 x P_2) generation. F_1 plants were self-pollinated and backcrossed with both parents (line BPX-441E-88 and line TOM-760), in order to obtain respectively the F_2 generation and the backcrosses: $F_1BC_{1(1)}$ [=(F_1 x BPX-441E-88)] and $F_1BC_{1(2)}$ [=(F_1 x TOM-760)].

2.2 Experiment

This experiment was conducted according to screening procedures for salt-tolerance indicated by Rezende et al. (2019, unpublished). The genotypes BPX-441E-88 (P_1) and TOM760 (P_2), and the populations F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ were sown in 04/12/2017 in boxes filled with the commercial substrate Carolina Padrão®. Seven days later (11/12/2017), when the seedlings had approximately 5 cm in height, the seedlings were transplanted to 110cm^3 deepots filled with vermiculite, and distributed in hydroponic pools under a completely randomized design. The experiment consisted of 70 plants from each parental line (P_1 and P_2), 70 plants from the F_1, 276 individuals from the F_2 and 135 individuals from each backcross ($F_1BC_{1(1)}$ and $F_1BC_{1(2)}$). The deepots were placed in 54-cell trays in two pools (3x0.6x0.2 meters each). The nutrient solution used in this study was the same suggested by Rezende et al. (2019, unpublished) (Chart 1).

After 15 days (26/12/2017), when the plants had two true leaves, the plants were exposed to salt stress induced by the addition of 150mM (approximately 8530 g in 1600 liters) of NaCl, and two days later (28/12/2017) they received the remaining amount of NaCl (150mM), totaling 300mM of NaCl. The amount of NaCl to be applied was based on electrical conductivity, considering the nutritional solution (Chart 1) and were splited according to Rezende et al (2019, unpublished).

Chart 1 Electrical conductivity and pH readings of the water, nutrient solution and saline solution (300 mM) in this experiment.

Solution	EC (µS)	EC (mS)	pH
Pure Water	328	0.328	6.06
Water + Nutrient Solution*	2060	2.06	4.93
Water + Nutrient Solution* + 300 mM of NaCl	31300	31.3	5.66

Nutriente Solution*: Macronutrients (MAXSOL F21 - Jaraguá®: N-8%; P-11%; K-38%; Mg-1.6%; S-2.9%; Fe-0.2%; Zn-0.02%; Mn-0.04%; Cu-0.004%; B-0.02%; Mo-0.004%) = 0.725 Kg / 1000 L + Calcium

Nitrate (Jaraguá®: N-15.5%; Ca-19%) = 0.540 Kg / 1000 L + 0.035 Kg / 1000 L Micronutrients (ConMicros Standard - Conplant®: B- 1,82%; Cu-1,82% ; Fe-7,26%; Mn-1,82%; Mo-0,36%; Ni-0,335%; Zn-0,73%).

The assessments were based on the rating scale for stress symptoms, adapted from Rezende et al. (2019b, unpublished) as follows: (0) Plant without symptoms + normal growth; (1) No apparent stress symptoms on leaves + stunted growth; (2) Plants with stunted growth + leaves slightly wrinkling; (3) Plants with stunted growth + moderate leaf wrinkling and curling; light chlorosis of the apical meristem; (4) Plants with stunted growth + moderate leaf wrinkling and curling + leaves showing a lighter green and yellowing of the tips or margins + wilting and defoliation of the older leaves + a few leaves might show mosaic, mottling or malformation; (5) Plants with stunted growth + generalized yellowing of the older leaves + intermediate leaves severely wrinkled and curled + the onset of chlorosis in the main vein (close to the petiole) of the leaflets might be observed; (6) Plants with stunted growth + generalized leaf yellowing and wilting + defoliation of the older leaves; a few leaves which were previously yellow might turn brown; (7) Generalized leaf yellowing and irreversible wilting; or plant death.

Fifteen assessments were performed, over a period of 33 days, starting on the day that the total amount of NaCl was applied (28/12/2017) and lasting through the 33th day (30/01/2018) when almost all plants of the salt-sensitive checks reached the maximum score (7). The data collected was used to estimate the area under the stress symptoms curve progress (area) over time (Rezende et al. 2019, unpublished).

2.3 Area under the stress symptoms progress curve (Area)

The scores attributed to each plant throughout the assessments were used to estimate the area under the stress symptoms progress curve, according to Rezende et.al. (2019, unpublished) as follows:

$$Area = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} \times (t_{i+1} - t_i)$$

where y_i is the score assigned to the stress symptom at the i^{th} observation, t_i is the day of the assessment at the i^{th} observation, and n is the total number of observations (SIMKO; PIEPHO, 2012).

2.4 Statistical analysis

2.4.1 Testing hypotheses of genetic control of salt stress tolerance

To estimate the genetic control of stress symptoms, statistical analyses based on the area under the stress symptoms curve over time (area) given to each observation were performed in two different ways: (a) testing for monogenic inheritance via different chi-squares under different assumed degrees of dominance and (b) testing hypothesis of monogenic inheritance, plus presence of polygenic modifier genes using genetic models based on maximum likelihood functions.

A. Test of monogenic inheritance hypothesis based on chi-squares assuming different degrees of dominance

The area under the stress symptoms curve progress (area) given to each plant was used to test hypotheses of monogenic inheritance under different presumed degrees of dominance, as described by Menezes et al. (2015) and Rezende et al. (2019, unpublished). A truncation point (TP) was chosen near the midpoint between P_1 and P_2, such that most of the P_1 plants were below the TP and most of the P_2 plants were above it. The TP used for "*S. lycopersicum* 'TOM760' x *S. lycopersicum* 'BPX-441E-88'" was 93.14, which was the midpoint area between the parental lines on the last evaluation date (29 days after the total application of 300mM of NaCl).

B. Test of monogenic inheritance, plus presence of polygenic modifier genes hypothesis based on genetic models using maximum likelihood functions

Some genetic models were tested using maximum likelihood in mixtures of normal densities, as proposed by Gonçalves, Bearzoti & Ferreira (2004). Areas under the curve of stress symptoms over time were taken as indicators of salt tolerance, smaller areas being indicative of higher levels of salt tolerance.

For the analyses, the full genetic model admitted a major gene with additive and dominance effects, and polygenes, also with additive and dominance effects (Table 1). From the complete genetic model, simpler models containing fewer parameters were generated (Table 1). Environmental variances were considered equal for all generations, and gene segregation was considered independent (both major genes and polygenes). Hypothesis tests of the genetic parameters were carried out based on likelihood ratio

between two models (GONÇALVES; BEARZOTI; FERREIRA, 2004). The tests were carried out using the statistical software 'Monogen v.0.1'.

Table 1 Genetic inheritance models according to Gonçalves et al. (2004) tested for salt tolerance in tomato, adapted from Menezes et al. (2015).

Models	Estimated Parameters
1 = major gene with additive and dominance effects + polygenes with additive and dominance effects + environmental effects	μ, A, D, $[a]$, $[d]$, V_A, V_D, S_{AD}, σ^2
2 = major gene with additive and dominance effects + polygenes with only additive effect + environmental effects	μ, A, D, $[a]$, V_A, σ^2
3 = major gene with only additive effect + polygenes with additive and dominance effects + environmental effects	μ, A, $[a]$, $[d]$, V_A, V_D, S_{AD}, σ^2
4 = major gene with only additive effect + polygenes with only additive effect + environmental effects	μ, A, $[a]$, V_A, $\sigma 2$
5 = polygenes with additive and dominance effects + environmental effects	μ, $[a]$, $[d]$, V_A, V_D, S_{AD}, σ^2
6 = polygenes with only additive effect + environmental effects	μ, $[a]$, V_A, σ^2
7 = major gene with additive and dominance effects + environmental effects	μ, A, D, σ^2
8 = major gene with only additive effect + environmental effects	μ, A, σ^2
9 = only environmental effects	μ, σ^2

2.4.2 Heritability and Variances

Broad-sense (H^2) and narrow-sense (h^2) heritability were estimated according to Wright (1968) as follows:

$$H^2 = \frac{\sigma_G^2}{\sigma_P^2}$$

$$h^2 = \frac{\sigma_A^2}{\sigma_P^2}$$

The variances, means and deviation errors of parents BPX-441E-88 (P_1) and TOM760 (P_2), populations F_1, F_2, backcrosses $F_1B_{1(1)}$ [F_1 x BPX-441E-88] and $F_1BC_{1(2)}$ [F_1 x TOM760] were estimated on Excel® (Wright, 1968).

$$\sigma_P^2 = \sigma_{F2}^2$$

$$\sigma_E^2 = \frac{\sigma_{P1}^2 + \sigma_{P2}^2 + 2\sigma_{F1}^2}{4}$$

$$\sigma_G^2 = \sigma_P^2 - \sigma_E^2$$

$$\sigma_A^2 = 2\sigma_{F2}^2 - \left(\sigma_{F1BC1(1)}^2 + \sigma_{F1BC1(2)}^2\right)$$

where σ_E^2 is environmental variance; σ_{P1}^2 is the environmental variance among plants of parent BPX-441E-88 (P_1); σ_{P2}^2 is the environmental variance among plants of parent TOM760 (P_2); σ_{F1}^2 is the environmental variance among plants of F_1 population; σ_{F2}^2 is the variance among individuals of F_2 population; $\sigma_{F1BC1(1)}^2$ is the variance among individuals of the first backcross to the parent BPX-441E-88 (P_1); $\sigma_{F1BC1(2)}^2$ is the variance among individuals of the first backcross to the parent TOM760 (P_2).

3 RESULTS

The areas under the stress symptoms progress curve (area) were estimated based on the scores (0-7) given for each observation throughout the experiment. The assessment performed on January 26th 2017 (= 29 days after the total application of 300mM of NaCl) was the last one considered to estimate the area under the curve symptoms over time. Obtained frequencies of plants with area above and below TP=93.14 were compared with their respective expected frequencies under hypotheses of monogenic inheritance under different assumed degrees of dominance.

The average area under the curve for populations P_1, P_2, F_1, F_2, $F_1BC_{1(1)}$ and $F_1BC_{1(2)}$ are shown in Table 2. Smaller areas are indicative of higher salt tolerance. The

salt-tolerant parental line BPX-441E-88 (P_1) showed the lowest value for the area, while, the salt-sensitive parental line TOM760 (P_2), had the largest area (Table 2).

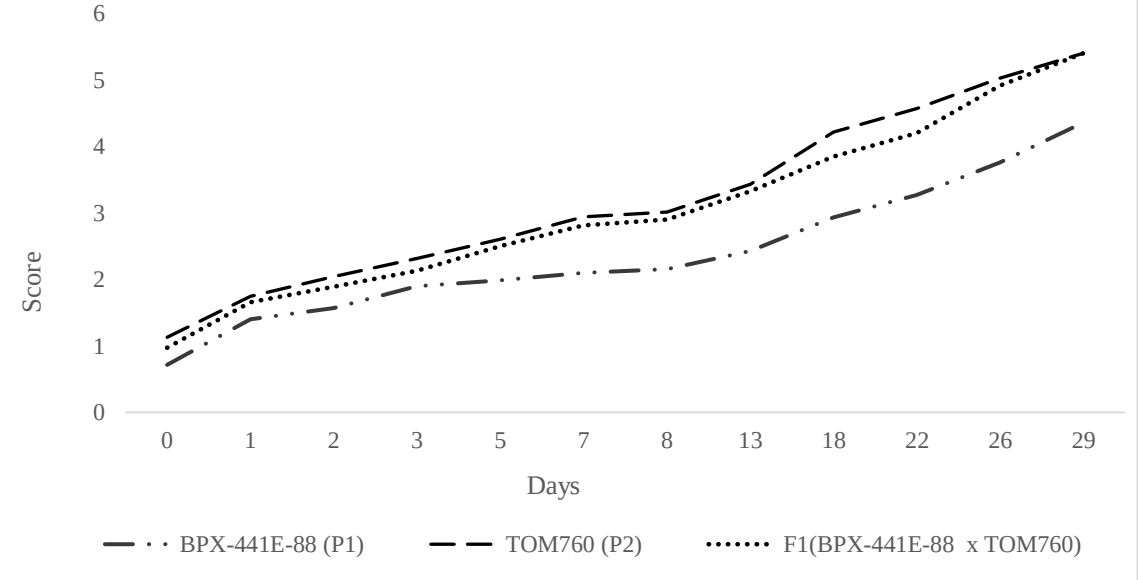
Table 2 Area under the stress symptoms progress curve in leaves of the populations derived from the cross *S. lycopersicum* 'BPX-441E-88' x *S. lycopersicum* 'TOM760', 29 days after the total application of 300mM of NaCl.

Populations	Area	TNP	Number of Plantas with	
			Area<=93.14	Area>93.14
BPX-441E-88 (P_1)	78.6 ± 16.3	70	56	14
TOM760 (P_2)	107.7 ± 14.9	70	16	54
F_1	102.1 ± 15.3	70	15	55
F_2	99.4 ± 18.1	275	99	176
$F_1B_{1(1)}$ [F_1 x BPX-441E-88]	90.8 ± 17.5	134	68	66
$F_1BC_{1(2)}$ [F_1 x TOM760]	99.9 ± 17.2	134	41	93

Mean followed by standard error of mean.
TNP = total number of plants.

The stress symptoms evolution over time for P_1, P_2 and F_1 are shown in Fig 1. In the beginning of the experiment (up to 3 days after salt stress induction), there were no clear differences between the populations, but the differences became more clear over time (Fig. 1).

Fig. 1 Stress symptoms progress in populations BPX-441E-88 (P_1), TOM760 (P_2) and BPX-441E-88 x TOM760 (F_1) in days after the initial salt stress induction.

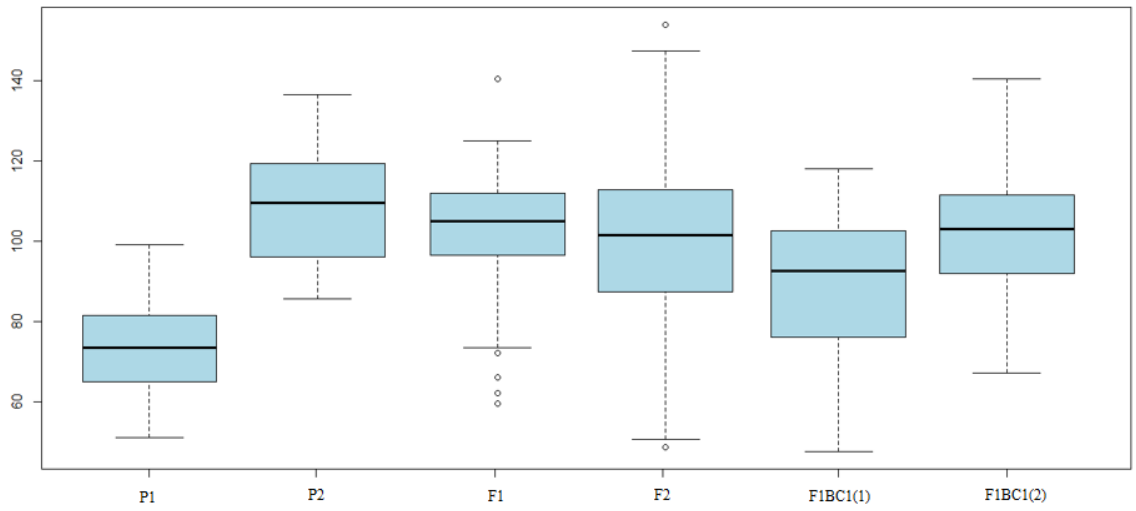


Days = days after the total amount of NaCl, 300 mM (split into two doses of 150 mM), was applied. The first dose (150 mM) was applied in 12/26/2017, and the second dose (150 mM) was

applied two days later (12/28/2017), and because of that, plants started to show stress symptoms in the day zero.

In the boxplot it is possible to notice that the F_1 (BPX-441E-88 x TOM760) population was closer to TOM-760 (P_2) than to BPX-441E-88 (P_1) (Fig. 2), therefore, the dominance is in the direction of higher salt-sensitivity, i.e., reduced salt tolerance (Table 2; Fig 1 and Fig 2).

Fig. 2 Boxplot of the area in populations derived from the cross *S. lycopersicum* 'BPX-441E-88' *S. lycopersicum* 'TOM760' 29 days after the total application of 300mM of NaCl.



P_1: BPX-441E-88; P_2: TOM760; F_1: BPX-441E-88 x TOM760; F_2: (F_1 x F_1); $F_1BC_{1(1)}$: [F_1 x BPX-441E-88]; $F_1BC_{1(2)}$: [F_1 x TOM760].

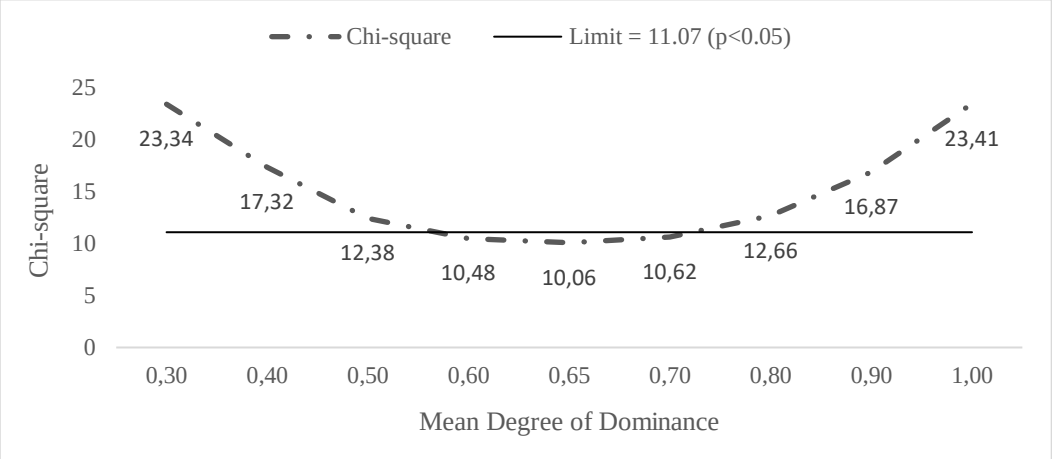
Chi-square testes of monogenic hypotheses under different presumed degrees of dominance indicate that the monogenic inheritance hypothesis can be accepted for mean degrees of dominance presumed between +0.6 and +0.7, with 95% confidence interval (Table 3; Fig 3), with a minimum chi-square indicated by MDD of +0.65, an indication that there is partial dominance in the direction of lower salt stress tolerance.

Table 3 Monogenic hypothesis test, under different presumed degrees of dominance, of the area under the curve of stress symptoms progress in leaves due to salt stress in tomato, accumulated up to 29 days after salt stress induction.

MDD	χ_C^2
0.50	12.38*
0.60	10.4 ^{ns}
0.65	10.0 ^{ns}
0.70	10.62 ^{ns}
0.80	12.66*
0.90	16.87

MDD = mean degree of dominance.
Reference values: $X^2_{(5df;\ 0.05)} = 11.070$
^{ns} Non-significant.
*Significant at 95% of confidence.

Fig. 3 Monogenic hypothesis test under different presumed degrees of dominance of stress symptoms in leaves due to salt stress in tomato, 29 days after salt stress induction).



3.2 Genetic models using maximum likelihood functions

Inheritance tests carried out with maximum likelihood functions are presented in Table 4. When model 7 is confronted with model 9 (Table 1), the hypothesis was rejected (Table 4), which means that there is a major gene in the control of the trait. The same hypothesis was confirmed when confronting models 2 vs 6, 2 vs 4, and 1 vs 8 (Table 4).

Conversely, when model 5 is confronted with model 9 (Table 1), the tested hypothesis was also rejected, an indication that there are modifier polygenic effects in the control of leaf symptoms due to salt stress (Table 4).

Thus, stress symptoms triggered by salt stress in *S. lycopersicum* 'BPX-441E-88' are under control of a single gene locus, with modifying polygenic effects (Table 4).

Table 4 Hypotheses of inheritance tested by using maximum likelihood for salt tolerance in populations derived from the cross *S. lycopersicum* 'BPX-441E-88' *S. lycopersicum* 'TOM760' for salt tolerance in tomato.

Models	χ_c^2	DF	Probability
7 vs 9	123.3255	2	0.000
5 vs 9	124.7332	5	0.000
2 vs 6	16.0256	2	0.0003
2 vs 4	16.0256	1	0.0000
1 vs 8	15.9217	6	0.0141

DF degrees of freedom.
Probability at 95% of confidence.

The significance of the chi-square test for fitness of the additive-dominant model indicates the existence of epistasis, probably between the major gene and the modifier polygenes (Table 5).

Broad-sense (26.28%) and narrow-sense (10.34%) heritabilities were estimated (Table 5).

Table 5 Mean components, mean degree of dominance (MDD), broad-sense and narrow-sense heritability (H^2 and h^2) for stress symptoms in leaves of tomato plants from populations derived from the cross *S. lycopersicum* 'BPX-441E-88' *S. lycopersicum* 'TOM760' for salt tolerance in tomato.

Parameters	**29 days**
M	93.00
[a]	13.09
[d]	8.95
$\chi 2$	124.61
MDD	+0.60 - +0.70
H^2 (%)	26.28
h^2 (%)	10.34

μ: Parental mean.
[a]: Additive mean effect.
[d]: Non-additive (dominance) mean effect.
χ^2: Chi-square test for fitness of the additive-dominant model (Mather; Jinks, 1984).
MDD: Mean Degree of Dominance.
H^2: Broad-Sense Heritability
h^2: Narrow-Sense Heritability

4 DISCUSSION

Stress symptoms triggered by salt stress: genetic control

Crop yields are reported to decline when EC value goes above 4 dS m^{-1} (Sairam; Rao; Sri, 2002). This threshold is much lower than EC used to induce salinity stress in this study (31.3 dS m^{-1}; chart 1), and made it possible to observe stress symptoms within a few days after the stress induction.

The differences on the performances of the populations P_1, P_2, F_1, F_2, $F_1BC_{1(1)}$, and $F_1BC_{1(2)}$ under saline conditions (Table 2; Fig. 2) was not as notorious as those reported by Rezende et al. (2019, unpublished), when evaluating segregant populations of *S. lycopersicum* 'TOM684' and *S. galapagense* 'LA1401'. These differences may be due to the genetic background of the salt-tolerant genitors: BPX-441E-88, even though tracing back to *S. pennellii* 'LA716', is already a pre-commercial line, in contrast to 'LA1401', which is a wild accession. Thus, the differences in salt-tolerance level between BPX-441E-88 and TOM760, both *S. lycopersicum*, and their respective segregant populations (F_1, F_2, $F_1BC_{1(1)}$, and $F_1BC_{1(2)}$) may be lower than the differences found by Rezende et al. (2019, unpublished), with *S. galapagense* 'LA1401', *S. lycopersicum* 'TOM760', and their derived populations. It is possible that not all genetic factors involved in salt tolerance of *S. galapagense* 'LA1401' will be present in *S. lycopersicum* 'BPX-441E-88'.

Another difference between this study and the study performed by Rezende et al. (2019, unpublished) is the assessment interval, counting from the day of stress induction to the day of the last assessment, when most of the salt-sensitive plants reached scores 6 and 7. The assessment interval varies according to the salt tolerance level of the population, thus, the population derived from *S. galapagense* 'LA1401' lasted longer (52 days) under salt stress (Rezende et al., 2019 – unpublished), compared to the population derived from *S. lycopersicum* 'BPX-441E-88' (29 days). *S. galapagense* (= *Lycopersicon cheesmanii* ssp. *minor*) is far more salt-tolerant than the cultivated tomato, being reported to survive in full strength seawater nutrient solution while the cultivated tomato cannot in most cases withstand levels higher than 50% seawater (Rush; Epstein, 1976).

BPX-441E-88 (P_1) and TOM760 (P_2) are both *S. lycopersicum*, but they are nonetheless quite different with regard to salt-tolerance (Table 2; Fig. 1 and Fig. 2), confirming the finds of Rezende et al. (2019, unpublished). The salt-tolerant genotype BPX-441E-88 (P_1) shows the lowest value for the area under the curve of symptoms along time, whereas the salt-sensitive TOM760 (P_2), has the biggest area (Table 2; Fig. 2).

In this study the hypothesis of a major gene controlling salt stress symptoms in *S. lycopersicum* 'BPX-441E-88' was not rejected in both tests, using different statistical approaches (Tables 3 and 4). The estimated mean degree of dominance within the interval +0.6 to +0.7 (Table 5). The control of this same trait in *S. galapagense* 'LA1401' is more complex (Rezende et al., 2019b – unpublished), and is considered predominantly polygenic.

Possibly, the major gene controlling salt tolerance in BPX-441E-88 detected in this study (Table 3; Fig. 3), is the same locus described by Millones-Chanamé (2016) and Millones-Chanamé et al. (2019), controlling both low incidence of blossom-end rot and low stomatal density on the adaxial face of the leaflet, an indication of pleiotropy between drought tolerance and salt tolerance. According to Chanamé (2016) and Millones-Chanamé et al. (2019), there is strong evidence that this locus is the same locus WELL described by Zsögön (2011); Vicente et al. (2015); Zsögön et al. (2017), promoting water-use efficiency (Zsögön, 2011), because genetic background of BPX-441E-88 has the cultivar WELL (Zsögön, 2011) as ancestor (Morales et al., 2015).

Salt tolerance *versus* drought tolerance

This study confirmed that *S. lycopersicum* 'BPX-441E-88' is a potential genetic resource for the cultivated tomato because of its drought (Morales et al., 2015; Oliveira, 2016; Chanamé, 2016; Millones-Chanamé et al., 2019), and salt tolerance (Rezende et al. 2019, unpublished). Salinity and drought stress are similar with respect to physiological, biochemical, and genetic effects (Leksungnoen, 2012). Physiological drought occurs when soluble salt levels in the soil solution are high enough to limit water uptake due to low water potential, thereby inducing drought stress (Leksungnoen, 2012).

Early responses to water and salt stress are largely identical except for the ionic component in the cells of plants under salt stress (Leksungnoen, 2012). Wilting symptoms observed in this study are commonly associated with drought stress, but they are also the first symptoms after salt stress induction, as reported by Shalata and Neumann (2001).

Addition of salts to water lowers its osmotic potential, resulting in decreased availability of water to root cells (Sairam; Rao; Sri, 2002). Salt stress thus exposes the plant to secondary osmotic stress, which implies that all the physiological responses, which are invoked by drought stress, can also be observed in salt stress (Sairam; Rao; Sri, 2002). Thus, a salinity tolerant genotype could also be drought tolerant or vice versa, and they have similar mechanisms to cope with those stresses (Leksungnoen, 2012).

Photosynthesis, together with cell growth, is among the primary processes to be affected by water or salt stress (Chaves; Flexas; Pinheiro, 2009), which in this study may be observed in terms of yellowing and stunting of the plants. However, when compared with drought, salt stress may affect more genes and more intensely, possibly reflecting the combined effects of osmotic (Chaves et al., 2009) and ionic stress in salt-stressed plants (Acosta-Motos et al., 2017). The defoliation observed after long-term exposure to salt stress is clearly a response to ionic stress, because older leaves accumulate higher concentrations of salts (Chaves et al., 2009).

Water deficit and salinity, especially under high light intensity, may disrupt photosynthesis and increase photorespiration, altering the normal homeostasis of cells and cause an increased production of reactive oxygen species (ROS) (Miller et al., 2010). Similarly to drought stress, salt stress can lead to blossom-end rot (Cuartero and Fernández-Muñoz, 1999; Leksungnoen, 2012), and affects WUE. *S. lycopersicum* 'BPX-441E-88' is highly tolerant to blossom-end-rot (Morales et al., 2015), and in this study, as well as in previous studies (Rezende et al. 2019a, unpublished) it was also considered highly salt-tolerant (Table 2; Fig. 1 and Fig. 2).

Salt stress may affect WUE due to the ion specific toxicity and the decrease in available water, as well as the photosynthetic activity and crop yield (Khataar; Mohammadi; Shabani, 2018). WUE is initially almost constant then sharply reduces with high salinity levels (Khataar; Mohammadi; Shabani, 2018). Thus, plant capability of maintaining high WUE (Munns, 2005; Syvertsen et al., 2010) under saline conditions is an important indicator of salt tolerance. The genotype WELL, "Water Economy Locus in *Lycopersicum*", line that originated 'BPX-441E-88', was selected for higher WUE (Zsögön, 2011).

According to Hasanuzzaman et al. (2018), during severe drought and salt stress conditions, plants reduce stomatal transpiration near to zero by the stomatal closure, increasing the WUE under stress conditions. BPX-441E-88 has low stomatal density in

the adaxial leaf surface (Oliveira, 2016; Millones-Chanamé et al. (2019), which reflects on its higher blossom-end-rot tolerance (Oliveira, 2016), and, as our results suggest, probably on its salt tolerance (Rezende et al. 2019, unpublished).

Stress symptoms triggered by salt stress: heritability and genetic effects

Heritability estimates found in this study (Table 5) are smaller than those found for stress symptoms by Asins et al. (1993) (*S. pimpinelifolium*) and Rezende et al. (2019b, unpublished) (*S. galapagense*). However, the species used in these inheritance studies were different, probably coping with salt stress in different ways (Cuartero; Fernández-Muñoz, 1999). Furthermore heritabilities are not constant, they may be changed by varying the variance contributed by the environmental factors (Wray, 2008), such as time of exposure to salt stress and NaCl concentration (Table 2; Fig. 1), therefore, heritability estimates may be subject to considerable large error variation components, and caution is therefore necessary in their interpretation (Rao; McNeilly, 1999).

Several species examined for salt tolerance suggests that different genetic architectures may be controlling the character. In BPX-441E-88, salt tolerance is controlled by both additive and non-additive gene actions (Table 5), so both are important in controlling the expression of salt tolerance, as previously reported by Rao; McNeilly (1999). This might explain the difference found between broad and narrow-sense heritabilities (H^2=26.28% and h^2=10.34%) (Table 5). However, in this case, the non-additive variance cannot be exploited by the breeder to improve salt tolerance, since it is in the direction of reducing salt tolerance (Fig. 1).

CONCLUSIONS

Salt stress symptoms in *S. lycopersicum* 'BPX-441E-88' are controlled by a major gene, with polygenic modifiers, showing broad and narrow-sense heritabilities of 26.28% and 10.34% respectively, with mean degree of dominance within the interval +0.60 to +0.70. BPX-441E-88 is a potential resource to improve salt-tolerance in tomato genotypes, and may be a viable and advantageous alternative in tomato production regions due to its double aptitude: drought and salt-tolerance.

BIBLIOGRAPHY

Asins MJ, Bretó MP, Carbonell EA (1993) Salt tolerance in Lycopersicon species. II. Genetic

effects and a search for associated traits. Theor Appl Genet 86:769–774. doi: 10.1007/BF00222668

Azevedo SM de, Maluf WR, Faria MV, et al (2012) Inheritance of resistance to the Papaya ringspot virus-watermelon strain (PRSV-W) from watermelon accession "PI 595201." Crop Breed Appl Biotechnol 12:67–75. doi: 10.1590/S1984-70332012000100009

Bauchet G, Causse M (2012) Genetic Diversity in Tomato (Solanum lycopersicum) and Its Wild Relatives. Genet Divers Plants. doi: 10.5772/33073

Bolger A, Scossa F, Bolger ME, et al (2014) The genome of the stress-tolerant wild tomato species Solanum pennellii. Nat Genet 46:1034–1038. doi: 10.1038/ng.3046

Chanamé CEM (2016) Herança da tolerância ao estresse hídrico em tomateiro. Universidade Federal de Lavras

Chaves MM, Flexas J, Pinheiro C (2009) Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. Ann Bot 103:551–560. doi: 10.1093/aob/mcn125

Chen J, Shen J, Xu J, et al (2013) Heritability and gene effects for salinity tolerance in cucumber (Cucumis sativus L.) estimated by generation mean analysis. Sci Hortic (Amsterdam) 159:122–127. doi: 10.1016/j.scienta.2013.04.020

Cuartero J, Fernández-Muñoz R (1999) Tomato and salinity. Sci Hortic (Amsterdam) 78:83–125

Egea I, Albaladejo I, Meco V, et al (2018) The drought-tolerant Solanum pennellii regulates leaf water loss and induces genes involved in amino acid and ethylene/jasmonate metabolism under dehydration. Sci Rep 8:1–14. doi: 10.1038/s41598-018-21187-2

Frary A, Göl D, Keleş D, et al (2010) Salt tolerance in Solanum pennellii: Antioxidant response and related QTL. BMC Plant Biol 10:. doi: 10.1186/1471-2229-10-58

Frary A, Keleş D, Pinar H, et al (2011) NaCl tolerance in Lycopersicon pennellii introgression lines: QTL related to physiological responses. Biol Plant 55:461–468. doi: 10.1007/s10535-011-0111-x

Gonçalves RP, Bearzoti E, Ferreira DF (2004) Avaliação da razão de verossimilhança generalizada em teste de hipóteses sobre o controle genético de características contínuas. Semin Ciências Agrárias 281–292. doi: 10.5433/1679-0359.2004v25n4p281

Hasanuzzaman A, Shabala L, Zhou M (2018) Factors determining stomatal and non-stomatal (residual) transpiration and their contribution towards salinity tolerance in contrasting barley genotypes. Environ Exp Bot. doi: 10.1016/j.envexpbot.2018.05.002

Kahn TL, Fender SE, Bray E a., O'Connell M a. (1993) Characterization of Expression of Drought- and Abscisic Acid-Regulated Tomato Genes in the Drought-Resistant Species Lycopersicon pennellii. Plant Physiol 103:597–605. doi: 10.1104/pp.103.2.597

Khataar M, Mohammadi MH, Shabani F (2018) Soil salinity and matric potential interaction on water use , water use efficiency and yield response factor of bean and wheat. Sci Rep 1–13. doi: 10.1038/s41598-018-20968-z

Leksungnoen N (2012) The Relationship Between Salinity and Drought Tolerance in Turfgrasses and Woody Species. Utah State University

Mather, K, Jinks, JL. (1984) Introdução à genética biométrica. Tradutor DUARTE, F. A. M. et. ai. Ribeirão Preto - SP: Sociedade Brasileira de Genética.242 p.

Menezes CB, Maluf WR, Faria M V, et al (2015) Inheritance of resistance to papaya ringspot virus-watermelon strain (PRSV-W) in 'Whitaker' summer squash line. Crop Breed Appl Biotechnol 15:203–209. doi: http://dx.doi.org/10.1590/1984-70332015v15n4a36

Menezes CB de, Maluf WR, Azevedo SM de, et al (2005) Inheritance of parthenocarpy in summer squash (Cucurbita pepo L.). Genet Mol Breed 4:39–46.

Miller GAD, Suzuki N, Ciftci-yilmaz S, Mittler RON (2010) Reactive oxygen species homeostasis and signallingduring drought and salinity stresses. Plant, Cell Environ 33:453–467. doi: 10.1111/j.1365-3040.2009.02041.x

Millones-Chanamé CE, de Oliveira AMS, de Castro EM, Maluf WR (2019) Inheritance of blossom end rot resistance induced by drought stress and of associated stomatal densities in tomatoes. Euphytica 215:120. doi: 10.1007/s10681-019-2444-z

Morales RG., Resende L., Maluf W., et al (2015) Selection of tomato plant families using characters related to water deficit resistance. Hortic Bras 33:27–33. doi: http://dx.doi.org/10.1590/S0102-053620150000100005

Morales RGF (2012) Resistência ao déficit hídrico em famílias de tomateiro derivados de Solanum pennellii. Universidade Federal de Lavras

Munns R (2005) Genes and Salt Tolerance. New Phytol 167:645–663

Oliveira AMS de (2016) Resistência de tomateiros a geminivírus e a estresse hídrico. Universidade Federal de Lavras

Rao SA, Mcneilly T (1999) Genetic basis of variation for salt tolerance in maize (Zea mays L). 145–150

Razali R, Bougouffa S, Morton MJL, et al (2018) The Genome Sequence of the Wild Tomato Solanum pimpinellifolium Provides Insights Into Salinity Tolerance. Front Plant Sci 9:1–21. doi: 10.3389/fpls.2018.01402

Rick CM (1973) Potential Genetic Resources in Tomato Species: Clues from Observations in Native Habitats. In: Srb AM (ed) Genes, Enzymes, and Populations. Boston MA, pp 255–269

Rush DW, Epstein E (1976) Genotypic Responses to Salinity. Plant Physiol 57:162–166. doi: 10.1104/pp.57.2.162.

Sairam RK, Rao KV, Sri GC (2002) Differential response of wheat genotypes to long term salinity stress in relation to oxidati v e stress , antioxidant acti v ity and osmolyte concentration. Plant Sci 163:1037–1046

Shah LR, Sharma A, Nabi J, Prasad J (2018) Breeding approaches for abiotic stress management in vegetable crops. J Pharmacogn Phytochem 7:1023–1028

Shalata A, Neumann PM (2001) Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J Exp Bot 52:2207–2211. doi: https://doi.org/10.1093/jexbot/52.364.2207

Simko I, Piepho H (2012) The Area Under the Disease Progress Stairs : Calculation , Advantage , and Application. Phytopathology 102:381–389. doi: 10.1094/PHYTO-07-11-0216

Sun W, Xu X, Zhu H, et al (2010) Comparative transcriptomic profiling of a salt-tolerant wild tomato species and a salt-sensitive tomato cultivar. Plant Cell Physiol 51:997–1006. doi: 10.1093/pcp/pcq056

Syvertsen JP, Melgar JC, Garcı F (2010) Salinity Tolerance and Leaf Water Use Efficiency in Citrus. J Am Soc Hortic Sci 135:33–39

Tal M, Shannon M (1983) Salt Tolerance in the Wild Relatives of the Cultivated Tomato: Responses of Lycopersicon esculentum, L. cheesmanii, L. peruvianum, Solanum pennellii and F 1 Hybrids to High Salinity. Funct Plant Biol 10:109–117. doi: 10.1071/PP9830109

Vicente MH, Zsögön A, de Sá AFL, et al (2015) Semi-determinate growth habit adjusts the vegetative-to-reproductive balance and increases productivity and water-use efficiency in tomato (Solanum lycopersicum). J Plant Physiol 177:11–19. doi: 10.1016/j.jplph.2015.01.003

Wray NR (2008) Estimating Trait Heritability. Nat Educ 1:

Wright S (1968) The genetics of quantitative variability. In: Evolution and genetics of populations, 2nd edn. Univeristy of Chicago, Chicago, pp 373–420

Yu AT (1972) The genetics and physiology of water usage in Solanum pennellii Com. and its hybrids with Lycopersicon esculentum Mill. Davis California

Zsögön A (2011) Identification and characterization of a tomato introgression line with reduced wilting under drought. Thesis. Australian National University. Available on: https://openresearch-repository.anu.edu.au/handle/1885/11216

Zsögön A, Vicente MH, Reartes D, Peres LE (2017) Understanding and improving water-use efficiency and drought resistance in tomato. In: Achieving sustainable cultivation of tomatoes. Edition: First. Chapter: 5. Publisher: Burleigh Dodds Science Publishing. Editors: Autar Mattoo, Avtar Handa. DOI: 10.19103/AS.2016.0007.01