Accession-specific Parent-of-origin Dependent and Independent Genome Dosage Effects on Salt Tolerance in *Arabidopsis Thaliana*

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Research Article

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Abstract

Improving the salt stress tolerance of crops is an important goal in plant breeding. Changes in the number of chromosome pairs (i.e. ploidy level) cause genome dosage effects which can result in improved traits or emergence of novel traits. The genetic and epigenetic contribution of maternal or paternal chromosomes can differentially affect physiological and metabolic characteristics of F1 offspring. Hence genome dosage effects can be parent-of-origin independent or dependent. The model plant *Arabidopsis thaliana* displays both genome dosage and parent-of-origin effects on plant growth under normal, non-stress conditions. Using an insogenic ploidy series of diploid, triploid and tetraploid lines we investigate the extent of genome dosage effects and their parent-of-origin dependency on *in vitro* salt stress tolerance of seedlings across ten different *A. thaliana* accessions (genetic backgrounds). We demonstrate genome dosage effects on salt stress tolerance in five accessions, and using reciprocal triploid lines demonstrate parent-of-origin dependent genome dosage effects on salt stress tolerance in three accessions. Our results indicate that epigenetic genome dosage and genome dosage balance effects can have significant impacts on abiotic stress tolerance in plants.

Introduction

Polyploidy is the phenomenon where an organism possesses more than two sets of chromosomes per cellular nucleus. Polyploidy can result from whole genome duplication events, which are a major mechanism of plant evolution and speciation (Blanc and Wolfe 2004; Jiao, et al. 2011; Li, et al. 2015). Genome dosage increases in newly formed polyploids can give rise to new or accentuated phenotypes, while genetic redundancy within polyploid genomes can allow duplicated genes to take on a new function (neofunctionalization) or retain different components of an original function (subfunctionalization) (Blanc and Wolfe 2004; Comai 2005; Jiao, et al. 2011; Roulin, et al. 2013).

Gene and genome dosage effects on plant growth have been reported in allopolyploids (polyploids with divergent genomes) (Chen 2010; Wang, et al. 2006). However, allopolyploids are by definition genetic hybrids, wherein determining the contribution of genome dosage *versus* genetic hybridity is difficult to disentangle. In contrast, by using autopolyploids (polyploids with genomes of the same type) it is possible to create an isogenic ploidy series which differ only in the number of chromosome copies in the nucleus. Autopolyploid research in *Arabidopsis thaliana* (Donoghue, et al. 2014; Duszynska, et al. 2013; Fort, et al. 2017; Miller, et al. 2012) and maize (*Zea mays*) (Guo, et al. 1996; Yao, et al. 2013) has shown genome dosage effects on plant growth and development that can be either parent-of-origin independent or dependent.

The induction of autopolyploidy in commercial crops for improvement in yield and quality is used in potato (*S. tuberosum*) (Jansky 2009), sugarcane (*Saccharum officinarum*) Ming et al. 2001 (Ming, et al. 2001), perennial ryegrass (*Lolium perenne*) (Wilkins and Humphreys 2003), blueberry (*Vaccinium corymbosum*) (McCallum, et al. 2016), and alfalfa (*Medicago sativa*) (Brouwer and Osborn 1999) amongst other crops. In addition to increasing yield and quality, improving the salt stress tolerance of

crops is an important objective in plant breeding, particularly for soils subject to salinization and saline agricultural systems (Pitman and Läuchli 2002). There has been limited research investigating genome dosage effects on salt stress tolerance, with no reports that differentiate between parent-of-origin dependent vs independent genome dosage effects.

To date, research on the abiotic stress response of polyploid plants has revealed both positive, negative, and neutral genome dosage effects. In chrysanthemum (*Chrysanthemum indicum*), for a single genotype, it was demonstrated that cold, salt and drought tolerance were improved upon induction of tetraploidy, but heat tolerance was greater at the diploid level (Liu, et al. 2011). Using field transplant experiments of wild yarrow (*Achillea borealis*), hexaploid plants are more likely to survive sand dune environments than tetraploid plants, although population effects are also significant (Ramsey 2011). Enhanced drought tolerance of wild willowherb (*Chamerion angustifolium*) at the tetraploid level over the diploid level has been demonstrated (Maherali, et al. 2009). In *A. thaliana* it has been reported, for a small number of accessions, that plants are more salt tolerant at the tetraploid level than at the diploid level, as measured by days-to-death, seed yield, and levels of anthocyanin (Chao, et al. 2013; Del Pozo and Ramirez-Parra 2014). Taken together, data from diverse plant species of across different growing habits suggests that genome dosage effects on abiotic stress response can occur. However, there has been no significant investigation of parent-of-origin dependent genome dosage effects on abiotic stress responses.

In this study, we utilized NaCl as the ionic stress, where we defined the stress as a major imbalance between the environment and physiology (Jansen 2017), but which did not allow the stress to lead to plant death. We sought to determine if tolerance to salt stress in *Arabidopsis thaliana* (across a range of genetic backgrounds) was subject to genome dosage effects, and whether any such effects were parent-of-origin dependent or independent.

Methods

Plant material and crossing design: Ten *A. thaliana* accessions were kindly provided both at the diploid and tetraploid level. C24, Col-0, L*er*-0, Zu-0 were the kind gift of Luca Comai (UC Davis, CA, USA) and Bur-0, Cvi, Sorbo, T910, TAL07, Wilna were the kind gift of Ortrun Mittelsten Scheid (Gregor Mendel Institute, Vienna, Austria). These accessions have their ecological origin assigned to eight different countries (Supplementary File 1). Plants were grown in 7 X 7 X 6.5 cm pots (Modiform, Leusden, Netherlands) in soil (5:1:1 mix of peat:vermiculite:perlite). Growth room (Cambridge HOK, East Yorkshire, UK) conditions were 16/8 hr light/dark @22/20°C. Plants were maintained for at least six generations before crossing. Reciprocal triploids were generated by manually emasculating flowers on lateral stems with a Dumont no. 5 tweezers (Electron Microscopy Sciences, PA, USA) and reciprocally crossing the diploid and tetraploid lines in both directions: 2x ♀ X 4x ♂ crosses produced paternal-excess triploids – labelled 3x(P), while 4x ♀ X 2x ♂ crosses produced maternal-excess triploids – labelled 3x(M). A single maternal and single paternal plant were used for all crosses, except for Col-0 and Zu-0, where three maternal plants were used to generate sufficient 3x(P) F1 seed due to the strong triploid block. Self-pollinated diploid and

tetraploid siliques were harvested from the maternal parent on the same lateral stem used for generating triploids.

Growth media: All chemicals were obtained from Sigma Aldrich, Ireland. Growth media was prepared by adding ½ strength Murashige and Skoog Basal Medium and sucrose at 0.5% w/v to distilled water. The required amount of NaCl was added. The solution was brought to pH 5.7 using 1 M KOH dropwise. Lastly, agar at 0.8% w/v was added before sterilization.

Plant stress experiments: To quantify different levels of salt stress tolerance, we take as a proxy the plant biomass produced in saline conditions *versus* non-saline conditions (Munns 2010). Plants were grown in artificial growth media *in vitro* supplemented with NaCl as follows. Seeds were first surface sterilized with 70% methanol, then a seed sterilization solution consisting of 5% sodium hypochlorite solution (NaClO) with 0.01% v/v Triton X-100, followed by five washes in sterile, distilled water. Seeds were stratified for three days in the dark at 4°C. Seeds were sown to fresh, stress-free media in 100 X 100 X 20 mm petri-dishes (Sarstedt, Nümbrecht, Germany) sealed with Micropore™ tape (3M, MN, USA). Plates were horizontally positioned in a growth chamber (CLF Plant Climatics, Emersacker, Germany) with 16/8 hr light/dark @22/20°C. After 2 days, plates were positioned vertically. 9-day old plants (growth stage 1.02 (Boyes, et al. 2001)) were transferred to fresh media with or without NaCl.

To determine the relative salt stress tolerance of all ten accession at their basal ploidy level and identify the optimal salt concentration that did not induce bleaching, 9-day old diploid plants were grown in media supplemented with either 0, 50, 75, 100, 125 or 150 mM NaCl. Subsequently, to determine genome dosage and parent-of-origin effects on salt stress tolerance, all ten accessions were investigated at the diploid, tetraploid, and reciprocal triploid level (i.e. an isogenic ploidy series). Diploid and tetraploid plants of the same accession were grown together in the same vertical plates to minimize any plate effect, as were reciprocal triploid plants. Stress plates contained a maximum of five biological replicates per ploidy level while stress-free plates contained a maximum of three biological replicates. After seven days, plants were destructively harvested for fresh weight measurements: plants were dried on a paper towel and weighed on a NewClassic MF weighing scale (METTLER TOLEDO, Greifensee, Switzerland) to the ten-thousandth decimal value. The term shoot is used in this study to mean all plant biomass from the hypocotyl upwards, while the term root means all biomass everything below the hypocotyl. Plants were snipped in two parts (above and below ground) using a Dumont no. 5 tweezers. The percentage plant biomass produced in saline conditions *versus* non-saline conditions was calculated. Statistical differences were determined with either a two-tailed independent samples t-test or one-way Analysis of Variance followed by Tukey's HSD test.

Flow cytometry: Plants were grown in a growth room as before. All chemicals were obtained from Sysmex, Kobe, Japan. Approximately 3 cm^2 of leaf material from 1-month old plants was removed and chopped with a razor blade in the presence of 400 µl nuclei extraction buffer. After 5 minutes the mixture was strained into a 3.5 ml Röhren tube through a 30 µm CellTrics® filter. 1 ml of UV-stain was added

before the sample was analyzed on a Partec Ploidy Analyzer (Sysmex, Kobe, Japan). Ploidy levels of diploid, tetraploid and reciprocal triploid plants were confirmed (Supplementary File 1).

Results

A concentration of 125 mM NaCl is optimal to induce salt stress in the majority of *A. thaliana* accessions at the diploid level *in vitro:* All ten accessions, at their basal ploidy level (diploid, apart from Wilna which also has naturally occurring tetraploid populations), were grown in media supplemented with a range of NaCl concentrations. The objective was to determine the highest salt stress concentration that did not stop seedling growth nor induce bleaching. Using these criteria, it was determined that 125 mM NaCl is optimal to induce salt stress in most accessions. The accession Cvi-0 was identified as particularly salt-sensitive, as reported by others (Borsani, et al. 2001), and thus we determined should be tested at 100 mM NaCl (Figure 1).

Genome dosage effect on salt stress tolerance between 2x and 4x isogenic lines: Comparisons between genetically isogenic autopolyploid diploid and tetraploid plants allows to identify genome dosage effects in relation to any differential phenotypes. Both in NaCl stress and NaCl stress-free media tetraploid plants are larger than diploid plants, as measured for both above (shoot)- and below (root)-ground biomass (Figure 2). There is a consistent trend across all accessions at the diploid and tetraploid level that root growth is affected more than shoot growth under salt stress (Figure 3). Of the 10 accession genetic backgrounds analysed, one accession displays a genome dosage effect difference in salt stress tolerance of shoot biomass between diploid and tetraploid plants. In the accession Bur-0, diploid plants accumulate significantly ($P < 0.05$) more above-ground biomass than tetraploid plants i.e. are more salt stress tolerant (Figure 3). For all other accessions, diploid and tetraploid plants accumulate similar above- and below-ground biomass under salt stress. On average diploid and tetraploid plants accumulate equal biomass under salt stress: above-ground biomass is reduced by ~50% and below ground biomass is reduced by ~70% by salt stress, independent of genome dosage under these conditions (Supplementary File 1).

Parent-of-origin independent genome dosage effect on salt stress tolerance: Comparison of the salt tolerance of diploid lines with isogenic triploid counterparts allows for a test of genome dosage effects on salt tolerance. Where such genome dosage effects occur, if both the maternal-excess and paternal-excess triploid lines display an equivalent genome dosage effect this can be considered as a parent-of-origin independent genome dosage effect. There are five cases where both maternal- and paternal-excess triploid plants accumulate less biomass under salt stress than their diploid equivalent indicating a parent-of-origin independent genome dosage effect on salt stress tolerance (Figure 3). In the cases of Col-0 and TAL07, diploid plants accumulate significantly ($P < 0.05$) more above-ground shoot biomass than both maternal- and paternal-excess triploid plants. In the cases of Wilna and Zurich, diploid plants accumulate significantly ($P < 0.05$) more below-ground biomass than both maternal- and paternal-excess triploid plants. In the case of the accession L*er*-0 both above- and below-ground biomass is significantly greater in diploid plants than in maternal- and paternal-excess triploid plants (Figure 3). A pooled analysis

suggests that diploid *A. thaliana* plants accumulate significantly ($P < 0.05$) more root biomass under salt stress than triploids (Supplementary File 1).

Similarly, the comparison of tetraploid lines with their isogenic triploid counterparts also allows for an additional test of genome dosage effects on salt tolerance. We identify three cases where both maternal- and paternal-excess triploid plants accumulate less biomass under salt stress than their tetraploid equivalent, indicative of a parent-of-origin independent genome dosage effect on salt stress tolerance (Figure 3). In the cases of Col-0, L*er*-0 and TAL07 tetraploid plants accumulate significantly ($P < 0.05$) more above-ground biomass than both of the maternal- and paternal-excess triploid plants.

Parent-of-origin dependent genome dosage effects on salt stress tolerance: F1 triploid plants generated via reciprocal crosses between genetically isogenic diploid and tetraploid parents can be used to identify parent-of-origin dependent genome dosage effects. Paternal excess F1 triploid plants have nuclei containing two paternally inherited genome copies and one maternally inherited genome copy, while maternal excess F1 triploid plants have one paternally inherited genome copy and two maternally inherited genome copies. Notably, the chromosome sets in both reciprocal F1 triploids are genetically identical but epigenetically differ according to the parental genome dosage ratio.

In both stress and stress-free media, paternal-excess F1 triploid plants are larger than maternal-excess F1 triploid plants, as measured for both above- and below-ground biomass (Figure 4). For three accessions there is a difference in salt stress response between reciprocal F1 triploid plants, indicating that there is a parent-of-origin dependent genome dosage effects on salt stress tolerance in these accessions (Figure 3). In the case of L*er*-0, maternal-excess triploid plants accumulate significantly ($P < 0.05$) more above-ground (shoot) biomass than paternal-excess triploid plants. In the case of Col-0, paternal-excess triploid plants accumulate significantly ($P < 0.05$) more below-ground (root) biomass than maternal-excess triploid plants. The accession Wilna is the only case where maternal-excess triploid plants accumulate significantly ($P < 0.05$) more above- and below-ground biomass than the genetically isogenic paternal-excess triploid plants. For all other accessions, the reciprocal F1 triploid pairs accumulate similar above- and below-ground biomass under salt stress. On average reciprocal F1 triploid plants accumulate equal biomass under salt stress: above-ground biomass is reduced by ~50% and below ground biomass is reduced by ~75% (Supplementary File 1).

Discussion

Saline conditions impose physiological constraints on plants through, firstly, oxidative stress, and secondly, ionic stress (Shabala 2017). Salt accumulating in the substrate surrounding the roots inhibits the capacity of roots to take up water (an osmotic effect). Lower water availability at this stage will lead to reduced plant growth, as has been shown for *A. thaliana* (Luo, et al. 2017), rice (Yeo, et al. 1991), maize (Frensch and Hsiao 1994; Rodriguez, et al. 1997), and barley (Munns, et al. 2000). Plant roots use the ionic composition of the substrate in which they are grown for turgor recovery, and thus can soon begin to take in water after the initial osmotic stress (Shabala and Lew 2002). However, this water contains very

high Na^+ and Cl^- concentrations leading to the second major physiological constraint on growth: ionic stress. Na^+ and K^+ ions possess similar physio-chemical properties: Na^+ can compete with K^+ for important binding sites within the cell, impairing enzyme activity (Flowers and Colmer 2008; Shabala and Lew 2002). In addition, Na^+ and Cl^- ions can accumulate in the cell wall causing cell dehydration (Munns and Passioura 1984). The inability to compartmentalize/exclude harmful ions inside the cell (e.g. in the vacuole) inhibits regular cell function and leads to cell death from either toxicity or dehydration (Munns 2002). This internal injury inhibits new leaf growth, reduces overall plant photosynthesis and thus reduces supply of carbohydrates to new cells (Shabala 2017). Plants have evolved different ionic stress tolerance mechanisms, which can vary across species, and may depend on local environmental conditions as well as the length of salinity exposure. These are (i) the ability of roots to recognize Na^+ ions and exclude them from accumulating within the plant (Alberico and Cramer 1993; Byrt, et al. 2007; Fortmeir and Schubert 1995; Matsushita and Matoh 1991), and (ii) tissue tolerance of Na^+ and Cl^- ions through compartmentalization (Apse, et al. 1999; Flowers and Colmer 2008; Flowers, et al. 2010; Mühling and Läuchli 2002; Munns and Tester 2008).

Arabidopsis thaliana accessions display variation in tolerance to low concentrations of NaCl, but not high NaCl

The *A. thaliana* accessions used in this experiment originate from across the northern hemisphere (Supplementary File 1). While many *A. thaliana* accessions show only small differences in nucleotide sequence they can display large genetic variation for phenotypic characteristics, e.g. flowering time (Kowalski, et al. 1994). At low concentrations of NaCl there were notable variations between genetically different accessions in this study. Four accessions (Bur-0, Col-0, T910, Wilna) display normal or above-normal above-ground biomass accumulation at 50mM NaCl, while others (L*er*-0, Sorbo, TAL07) display ~50% reduction in above-ground biomass accumulation at this concentration (Figure 1).

Previous work has demonstrated large differences between *A. thaliana* accessions for NaCl tolerance, as measured using days-to-death (Katori, et al. 2010) and leaf rosette area coupled with electrolyte leakage (Julkowska, et al. 2016). Our experiments did not identify a large variation in NaCl tolerance between accessions at stressful NaCl concentrations (Figure 1), even though there is some overlap with the accessions used in our study and the previously studies. It is noteworthy that previous experiments used much higher concentrations of NaCl than our study, both in soil and in artificial growth media *in vitro* experiments.

Different accessions of *Arabidopsis thaliana* display parent-of-origin independent and dependent genome dosage effects on salt stress tolerance

We have previously demonstrated that *A. thaliana* tetraploid plants accumulate more above-ground biomass than their diploid equivalents (Fort, et al. 2016), indicating a genome dosage effect. Likewise, paternal-excess F1 triploid plants can also accumulate more above-ground biomass than their diploid equivalent, as well as maternal-excess F1 triploid plants. In this study, we identify genome dosage effects

(and also parent-of-origin dependent genome dosage effects) on salt stress tolerance (Figure 3). We reveal that in some genetic backgrounds (accessions) there are parent-of-origin independent genome dosage effects on salt stress tolerance, that are not evident in other genetic backgrounds (Figure 3). Indeed, five of the ten accessions tested (i.e. Col-0, TAL07, Wilna, Zurich and L*er*-0) displayed significant parent-of-origin independent genome dosage effects on salt stress tolerance where diploids were more stress-tolerant than both of the reciprocal triploids (Figure 3). In addition, in three out of ten accessions (i.e. Col-0, L*er*-0 and TAL07) the tetraploid plants were were more stress-tolerant than both of the reciprocal triploids (Figure 3). If the genome dosage effects on salt tolerance we have identified were linear, we should expect that the salt tolerance value will either be 2x > 3x > 4x or 2x < 3x < 4x. However, there are no cases where such trends are evident. Instead, our results indicate that in five genetic backgrounds, the salt tolerance of both isogenic reciprocal triploid lines is lower than both the diploid and tetraploid parental lines. For Col-0, TAL07 and L*er*-0, the finding that diploid and tetraploid plants perform better under salt stress tolerance than triploid plants is strongly suggestive of a parental-genome dosage balance effect on salt stress tolerance, where plants with an equal parental contribution of chromosome sets (i.e. 2x and 4x) perform better than those with unequal parental genome dosage.

In addition, we demonstrate that there are also parent-of-origin dependent genome dosage effects on salt stress that are accession-specific. The reciprocal triploid lines are genetically identical at the nuclear level differing only in whether the two genome copies of the genome in the triploid plant have been inherited paternally (via pollen) or maternally (via ovule). Any differences in salt stress tolerance between the maternal-excess triploid and the paternal-excess triploid are likely to have an epigenetic basis, either due to parent-of-origin specific epigenetic marks and/or parent-of-origin specific dosage dependent factors. We have identified such parent-of-origin dependent genome dosage effects on salt tolerance in three genetic backgrounds (Figure 3). Such parent-of-origin dependent genome dosage effects on salt tolerance between the reciprocal triploid plants could be due to epigenetic marks at the nuclear genome level (in the embryo or endosperm), cytoplasmic differences between diploid and tetraploid maternal parents, or dosage dependent factors in the maternal seed coat that differ between diploid and tetraploid maternal parents.

Overall, the genome dosage effects between diploids and tetraploids, and the reciprocal F1 triploid pairs, are occurring in plants that are genetically identical (isogenic) apart from their differential genome dosage or whether their genome copies are maternally derived (madumnal) or paternally derived (padumnal). Hence, such accession-specific genome dosage effects are likely epigenetic in nature as they do not involve any changes in DNA sequence.

Conclusion

The effects of soil salinisation are greatest in arid and semi-arid agricultural settings. Response to soil salinisation can be physical (e.g. drainage management) or biological (breeding crops for enhanced salt tolerance) (Acosta-Motos, et al. 2017). The induction of polyploidy is widely used in crop improvement, where some studies suggest abiotic stress tolerance may be influenced by genome dosage. Using the

model plant species *Arabidopsis thaliana*, we demonstrate accession-specific parent-of-origin dependent and independent genome dosage effects on salt tolerance. The parental genome dosage balance effects on salt stress tolerance we have identified in this study likely have an underlying epigenetic basis, which may open up new avenues for harnessing epigenetic genome dosage balance effects to improve plant abiotic stress tolerance.

Declarations

Availability of data and material

The ploidy level of diploid, tetraploid and reciprocal triploid plants is shown in Supplementary File 1.

All fresh weight measurements recorded in this experiment are available in Supplementary File 2.

Conflicts of interests

Not applicable

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Author's contributions

BH and CS conceived the experiment. BH performed the experiments. BH, GB, PMcK and CS wrote the manuscript.

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Figures

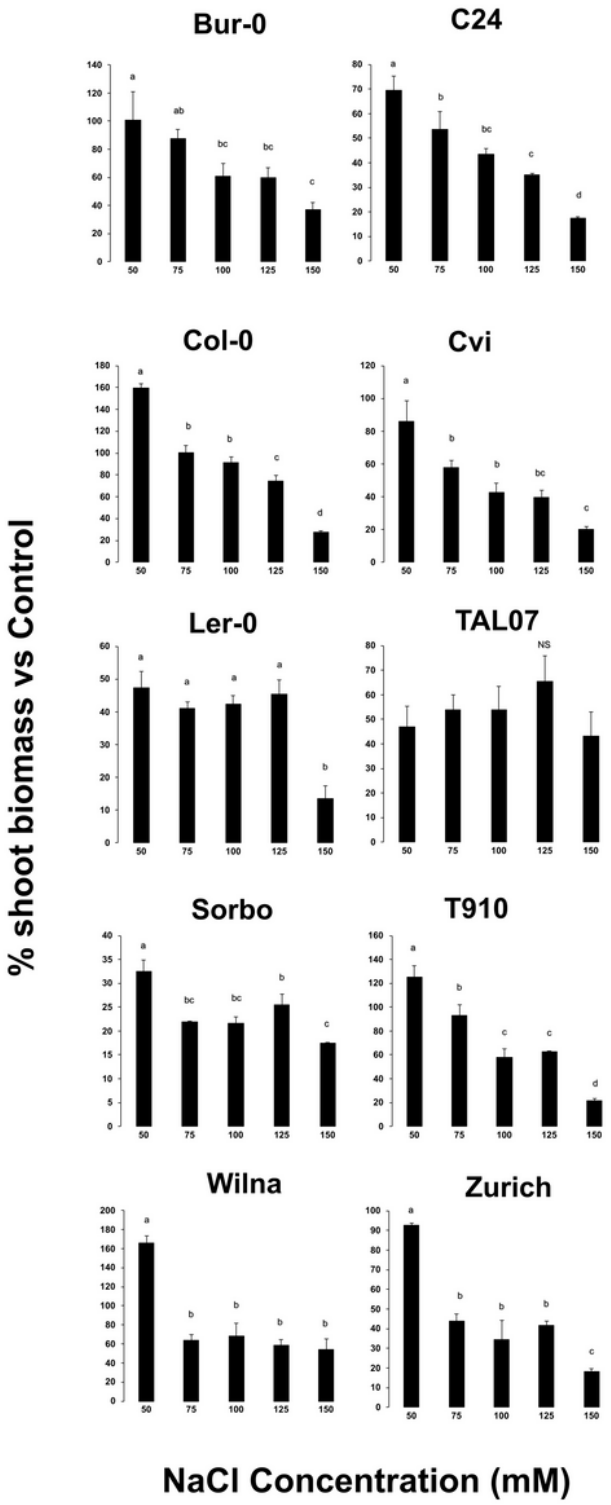

Figure 1

Above-ground biomass accumulation of ten *Arabidopsis thaliana* accessions at the diploid level under a range of salt concentrations. Plants at their basal ploidy level (diploid) were germinated in stress-free artificial growth media and after 9 days transferred to fresh media with or without NaCl. Plants were destructively harvested after 7 days. Each accession was analyzed with a one-way ANOVA and means

assigned different letters are statistically different ($P < 0.05$) according to Tukey's HSD test; NS not statistically different ($P > 0.05$). Error bars represent Standard Deviation.

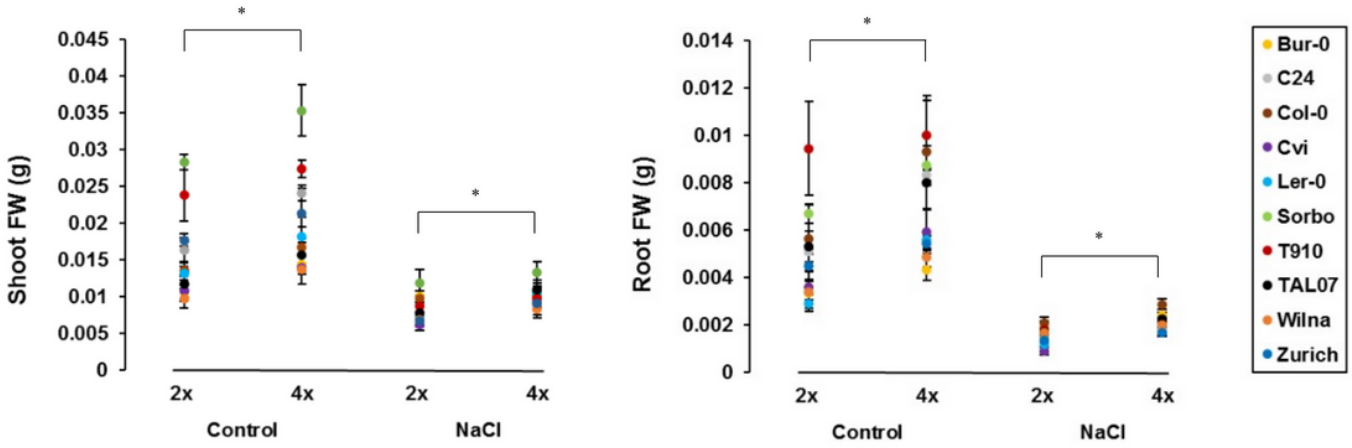

Figure 2

Tetraploid plants are larger than diploid plants in both NaCl stress and NaCl stress-free media. On average, there is a consistent genome dosage effect across accessions that tetraploid plants accumulate more above- and below-ground biomass than diploid plants. Plants were germinated in stress-free artificial growth media and after 9 days transferred to fresh media with or without NaCl. Plants were destructively harvested after 7 days. * statistically different ($P < 0.05$) according to a two-tailed independent samples t-test.

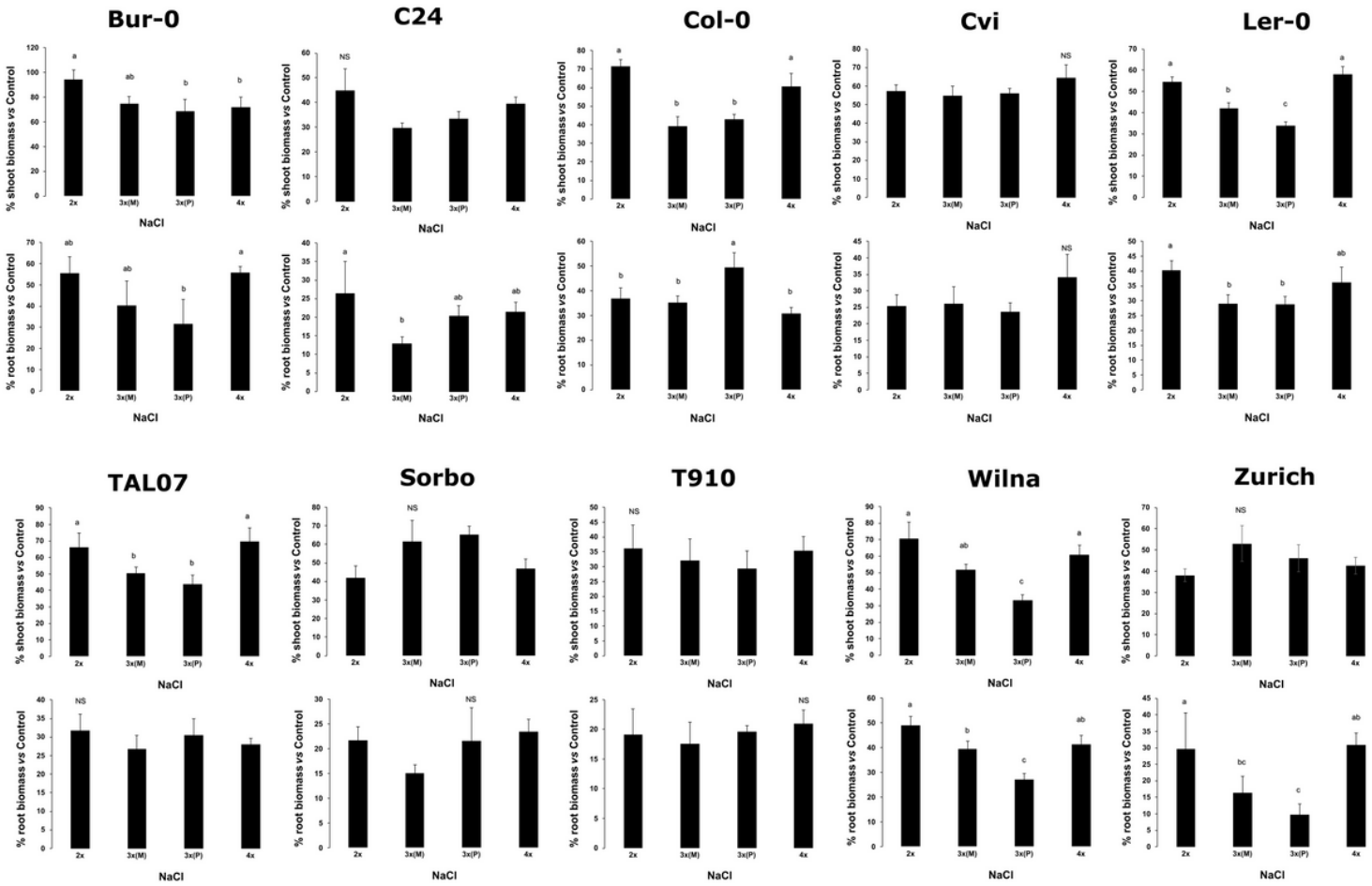

Figure 3

Parent-of-origin dependent and independent genome dosage effects on salt tolerance in *Arabidopsis thaliana*

There is a parent-of-origin independent genome dosage effect on salt stress tolerance in Bur-0, Col-0, TAL07, Wilna, Zurich and L*er*-0. There is a parent-of-origin dependent genome dosage effect on salt stress tolerance in Col-0, L*er*-0 and Wilna. Plants were germinated in stress-free artificial growth media and after 9 days transferred to fresh media with or without NaCl. Plants were destructively harvested after 7 days. Each accession was analyzed with a one-way ANOVA and means assigned different letters are statistically different ($P < 0.05$) according to Tukey's HSD test; NS not statistically different ($P > 0.05$). Error bars represent Standard Deviation.

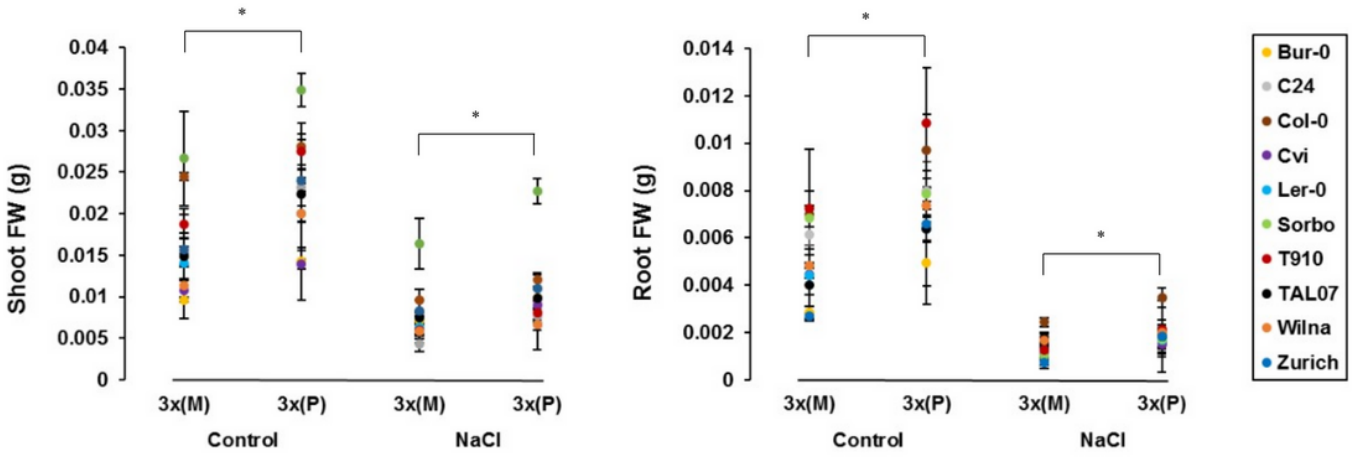

Figure 4

Paternal-excess triploid plants are larger than maternal-excess triploid plants in both stress and stress-free media. On average, there is a consistent genome dosage effect across accessions that paternal-excess triploid plants accumulate more above- and below-ground biomass than maternal-excess triploid plants. Plants were germinated in stress-free artificial growth media and after 9 days transferred to fresh media with or without NaCl. Plants were destructively harvested after 7 days. * statistically different ($P < 0.05$); NS not statistically different ($P > 0.05$) according to a two-tailed independent samples t-test.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile1.pdf
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