The modulation of cell cycle progression and of histone H3 acetylation in response to salt stress in durum wheat-*Thinopyrum* spp. recombinant lines analysed by a flow cytometric approach.

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Introduction

Plants, as sessile organisms, face several challenging conditions and respond to unfavourable environments through a variety of mechanisms at cytological, molecular and epigenetic levels. Abiotic stresses, such as heat, drought and salinity, considerably affect plant growth and development, determined by cell proliferation through regular progression of the cell cycle (G1, S, G2/M phases) and cell expansion. Previous studies showed that different types of abiotic stresses inhibit cell cycle progression at a specific phase (Qi and Zhang 2020, doi: 10.3389/fpls.2019.01765; Zhao et al 2014, doi:10.1371/journal.pone.0106070.g006). Epigenetic changes, including dynamic histone modifications, are known to influence gene activity and expression by chromatin remodelling and to modulate plant stress response. Here we analyzed the effects of high salt concentration (200mM NaCl) on cell cycle progression in durum wheat-*Thinopyrum* spp. recombinant lines (RLs) vs control lines (CLs) lacking the alien chromatin. In addition we have applied a "flow immunostaining" approach to analyse acetylation changes occurring on Lys9 of histone H3 (H3K9ac) in RLs vs CLs after salt exposure.

Aims

To investigate the effects of high salt concentration in RLS vc CLS lines on:

1. **Cell cycle progression**
2. **Histone H3K9 acetylation**

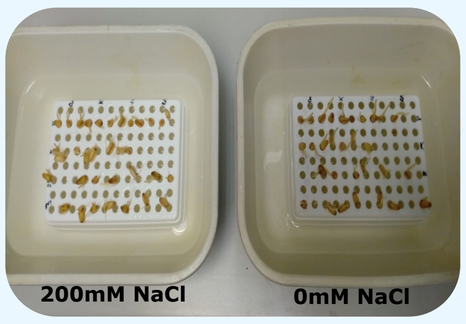
Materials

Wheat-alien (*Thinopyrum* spp.) recombinant and control lines

Line designation	Wheat-*Thinopyrum* recombinant line	Recombinant chromosome	% *Thinopyrum*
R112 Hom+	7AL/7el$_1$L *Th. ponticum*		28 7el$_1$L
R112 Hom-	control line	— (normal 7A)	—
R69-9/R5 Hom+	T. durum 7AL/*Thinopyrum ponticum* 7el$_1$L + *Thinopyrum elongatum* 7EL = 7AL/7el$_1$L/7EL		Total 23%, ~ 10% 7EL
R69-9/R5 Hom-	control line	— (normal 7A)	—
R74-10/R112 Hom+	7AL/7el$_1$L/7EL		Total 28%, ~ 20% 7EL
R74-10/R112 Hom-	control line	—	—

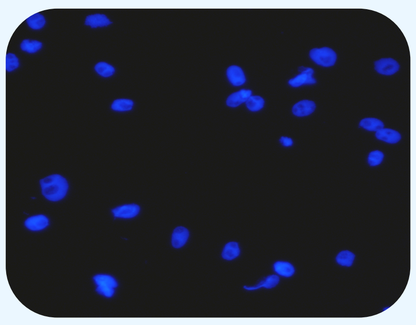
Methods

Seedlings salt treatments



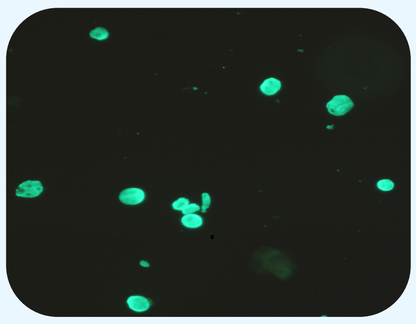
3 days old seedlings of RLs and CLs lines were dipped in an aerated Hoagland nutritive solution and exposed to 0mM or 200mM NaCl for 24h at 22±1°C.

Formaldeide fixation and nuclei isolation



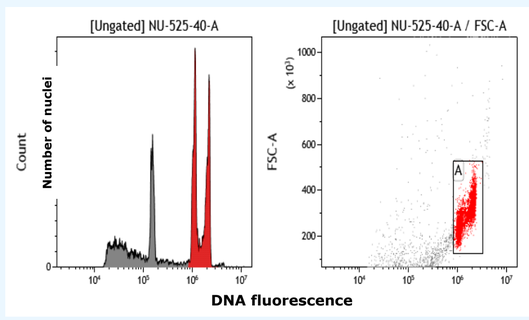
After salt shock, seedlings were fixed in 2% formaldehyde solution in Tris buffer at 5°C for 10 min and washed three times in Tris buffer. Nuclei isolation was performed by homogenization of root tips in LB01 buffer.

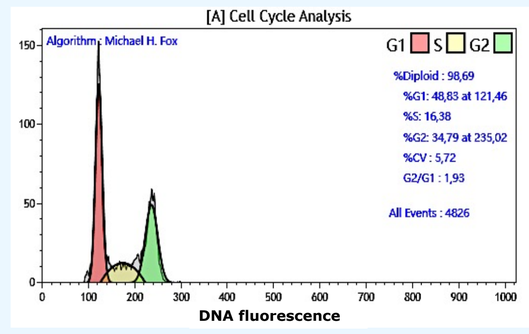
Nuclei immunostaining



Nuclei immuno-staining was performed at 4°C o.n. both on slide and in suspension, using Anti-H3K9ac as the primary antibody (Abcam), followed by 2 h incubation at 37°C with the FITC conjugate secondary antibody (Abcam). Incubation with the secondary antibody only, was also performed as background fluorescence control.

Flow cytometry analysis





Nuclei stained for cell cycle analysis (DAPI-DNA content) or immuno-staining assay (FITC-antiH3K9ac) were run on a CytoFLEX S flow analyzer (Beckman Coulter Flow Cytometry, Milan, Italy). . The % of nuclei at the different cell cycle phases was determined by processing data according to Michael Fox cell cycle modelling algorithm (Beckman Coulter Kaluza software v. 2.1.) Statistical analysis (ANOVA) was performed by JASP software on 5000 cells in 3 replicates, depending on the line.

Results

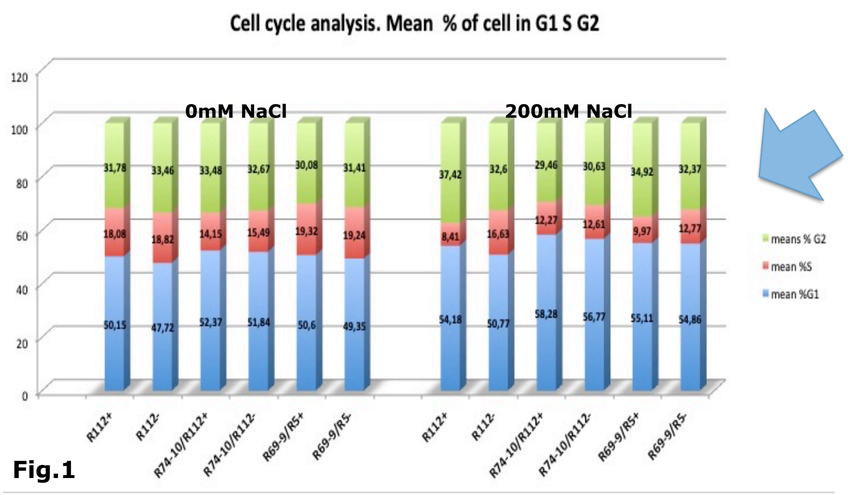


Fig.1

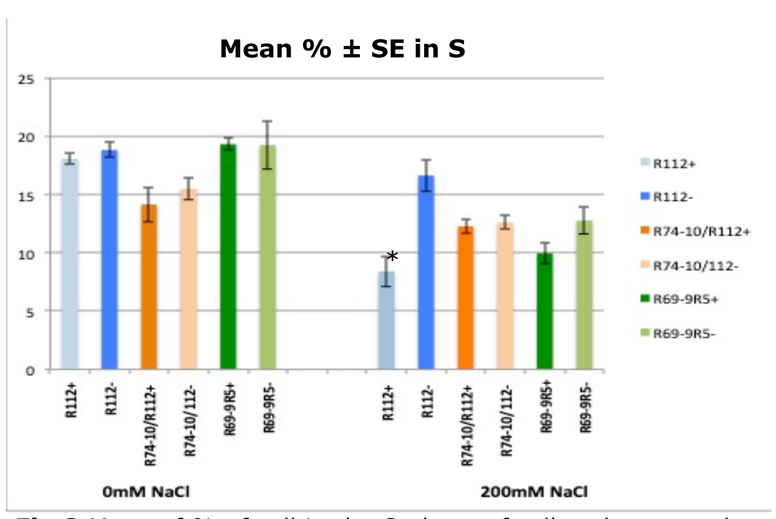


Fig.2 Mean of % of cell in the S phase of cell cycle exposed to 0mM and 200mM NaCl. Significant difference, *p<0,001, has been observed between R112+ vs R112- after salt treatment.

Cell cycle analysis revealed, as general trend, an increase of nuclei in G1 and a reduction in S phase after salt treatment, more pronounced in some of the RLs vs CLs (Fig. 1). In particular, line R112+, containing *Th. ponticum* chromatin, shows a significant reduction of the S phase (Fig. 2) compared to its CL.

The "Flow immunostaining" unveiled a modulation in the H3K9 acetylation level in the salt-exposed material vs the non-stressed, and in RLs vs CLs. Line R112+ showed an increase in the H3K9ac after salt treatment, while a reduction in FITC fluorescence was observed in CL R112- at 200mM NaCl (Fig. 3). On the contrary, RLs R69-/R5+ and R74-10/R112+ appeared less affected by salt exposure in terms of H3K9 acetylation level variation when compared to their respective CLs. From the first results, the presence of alien chromatin in durum wheat background seems to affect epigenetic processes. .

Flow immunostaining analysis

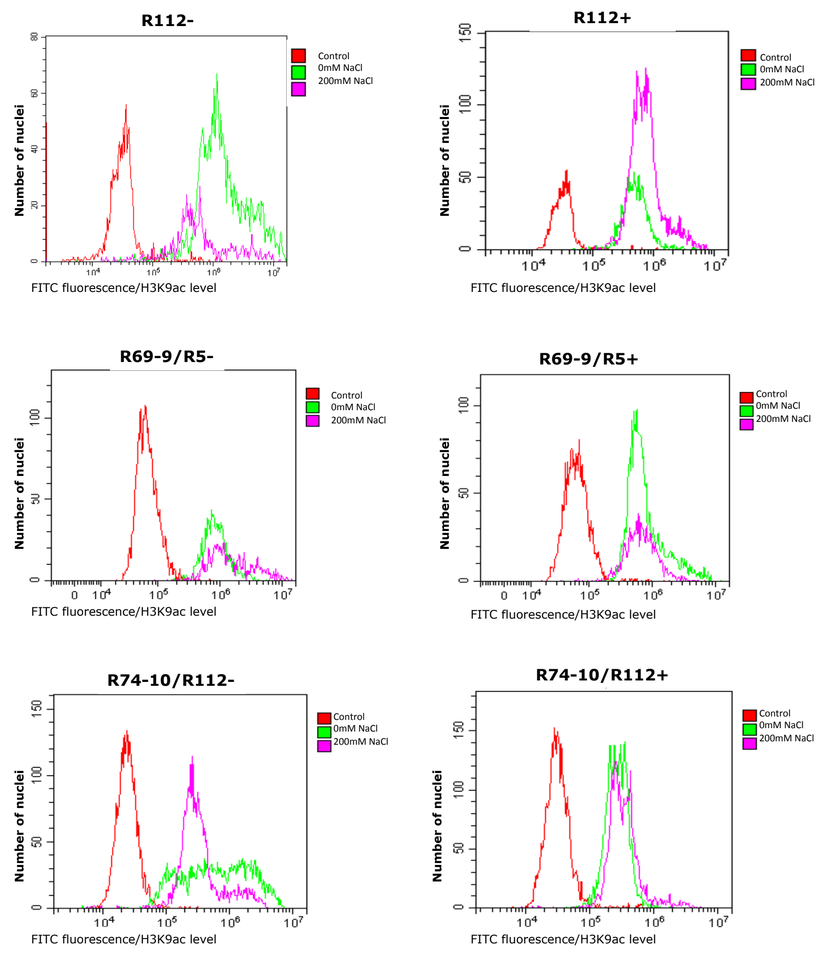


Fig.3 H3K9 acetylation level (X axis) of nuclei from RLS and CLS after exposure to 0 (green curve) and 200mM NaCl (pink curve). Red curve: background fluorescence derived from nuclei incubation with secondary antibody only.

Conclusions

The exposure to 200mM NaCl produced a general increase in the % of cells in G1 and a decrease in the S phase in all lines analyzed, indicating a slowdown in cell cycle progression, but with some significant differences among RLs vs CLs. It can be hypothesized that reducing cell cycle progression during the stress might prevent cell entry into critical stages for potential cell damage (e.g. S phase), thus allowing the cellular defence system to be activated. Plants able of an efficient blocking and of a fast response in term of gene expression modulation could probably be more resilient. Histone acetylation is one of the epigenetic processes which modulate (increase) gene expression through chromatin modification. We observed differences in the overall H3K9 acetylation level among the RLs and between RLs vs CLs after exposure to salt stress. The presence of alien chromatin in wheat background appears to affect wheat response to salt stress. Further analyses with a panel of antibodies against different histones and/or other epigenetic mechanisms (methylation), together with other approaches (e.g. chromatin immunoprecipitation, CHIP) will better elucidate the picture.