Physiological & Transcriptional Analyses of Two Alfalfa Cultivars with Different Salt Tolerance

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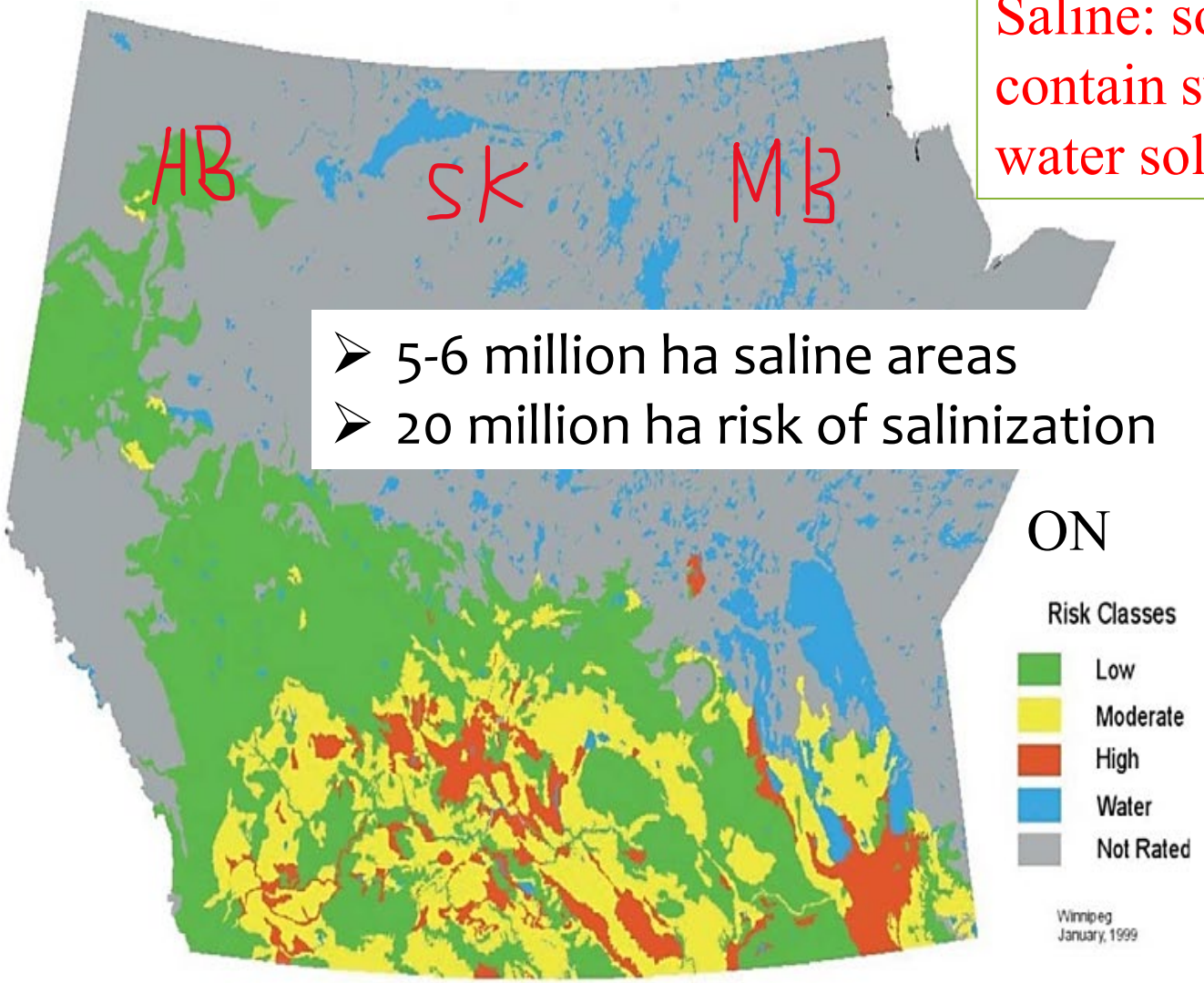
Canadian Prairie Salinity Map

Saline: soils contain sufficient water soluble salts



- 5-6 million ha saline areas
- 20 million ha risk of salinization

Fig. 1. View of the Canadian prairies with the 1996 soil salinity risk evaluation based on five physical and biological factors including the existence of root-zone salts [taken from Wiebe et al. (2007)].

Economic return

Salinity degree	Electrical Conductivity of 0-60 cm depth (mS/cm)	Effect on Crop Growth and Estimate of Potential Yield Loss
Nonsaline	0 - 2	There are no visible effects of salts on the growth of crops. No yield loss.
(W) Weak	2 - 4	Yields of very sensitive crops may be restricted. Cereals are generally unaffected.
(M) Moderate	4 - 8	Yields of many crops are restricted. Wheat yields may be reduced by 30%.
(S) Strong	8 - 16	Only tolerant crops yield satisfactorily. Wheat yields may be reduced by 60%.
(V) Very Strong	16+	Only a few very tolerant crops yield satisfactorily. Wheat yields may be reduced by 80-100%.

Source: https://soilsofsask.ca/soil-survey-soil-characteristics/salinity-class.php

Weed source:

Foxtail barley

Alfalfa Genetic Improvement

- >4 million ha in the Canadian Prairies
- Genetically diverse
- Current cultivars for salt tolerance:
  - 50+ alfalfa cultivars (NAFA 2020)
  - Fall dormancy categories >= 4
  - Higher tolerant, adapted cultivars are needed

- **Objectives**
  - Understand responses of different salt tolerant alfalfa cultivars
  - Identify associated traits and markers for plant selection

- **Germination/greenhouse study**
- **RNA-Seq study**
- **Breeding update**

Germination study

Alfalfa cultivars

Halo (Tolerant)

Vernal (Intolerant)

Five salinity levels

0 dS m^{-1}

4 dS m^{-1}

8 dS m^{-1}

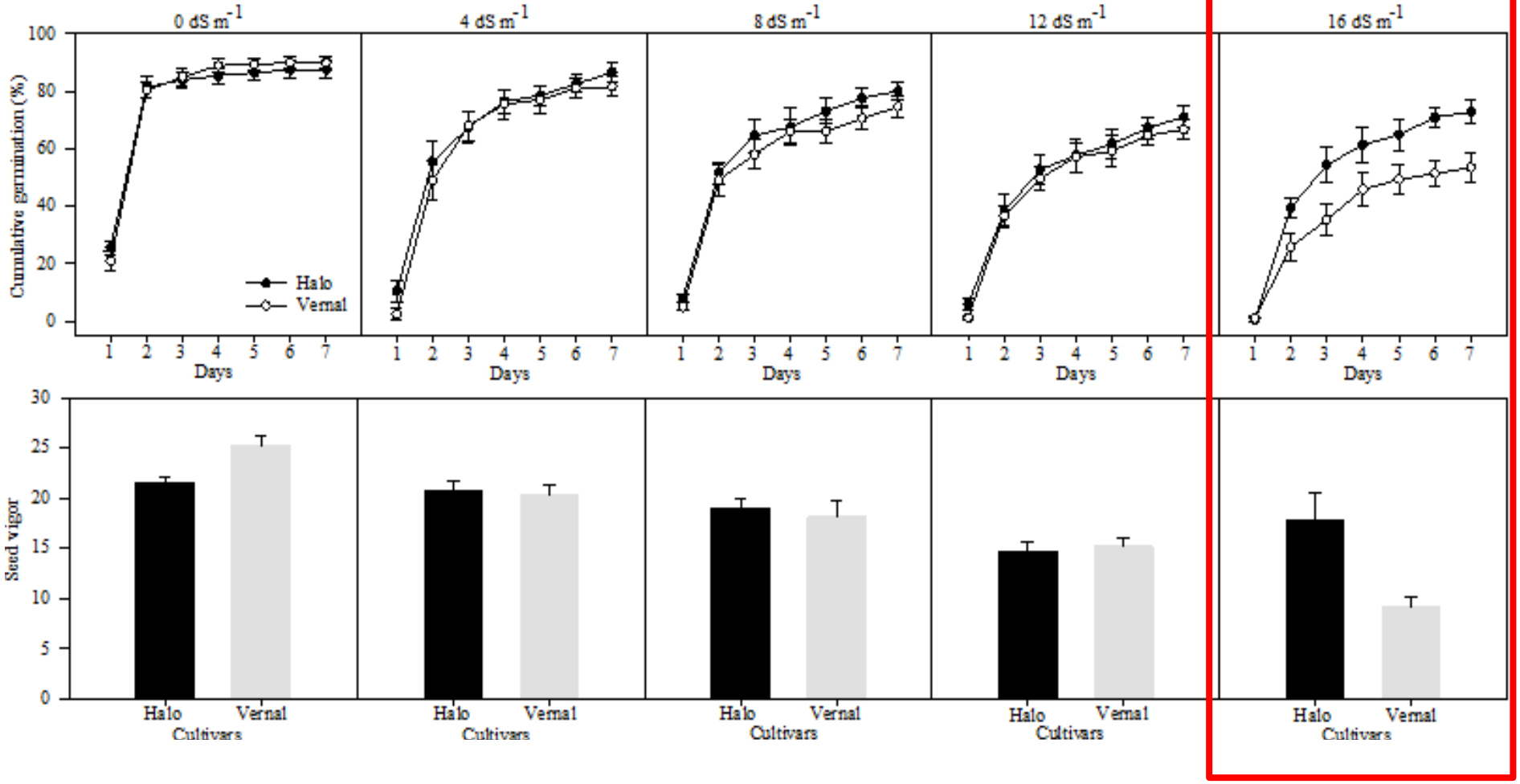
12 dS m^{-1}

16 dS m^{-1}

Salt: NaCl

- ✓ **Cumulative seed germination**
- ✓ **Seed vigor** =germination percentage × seedling length /100

Germination and seed vigor



Greenhouse study: Plant materials and salt treatment

Five alfalfa cultivars

Tolerant

Halo

Intolerant

Vernal

Five salinity levels

0 dS m^{-1}

4 dS m^{-1}

8 dS m^{-1}

12 dS m^{-1}

16 dS m^{-1}

Hoagland solution

Observation in the greenhouse

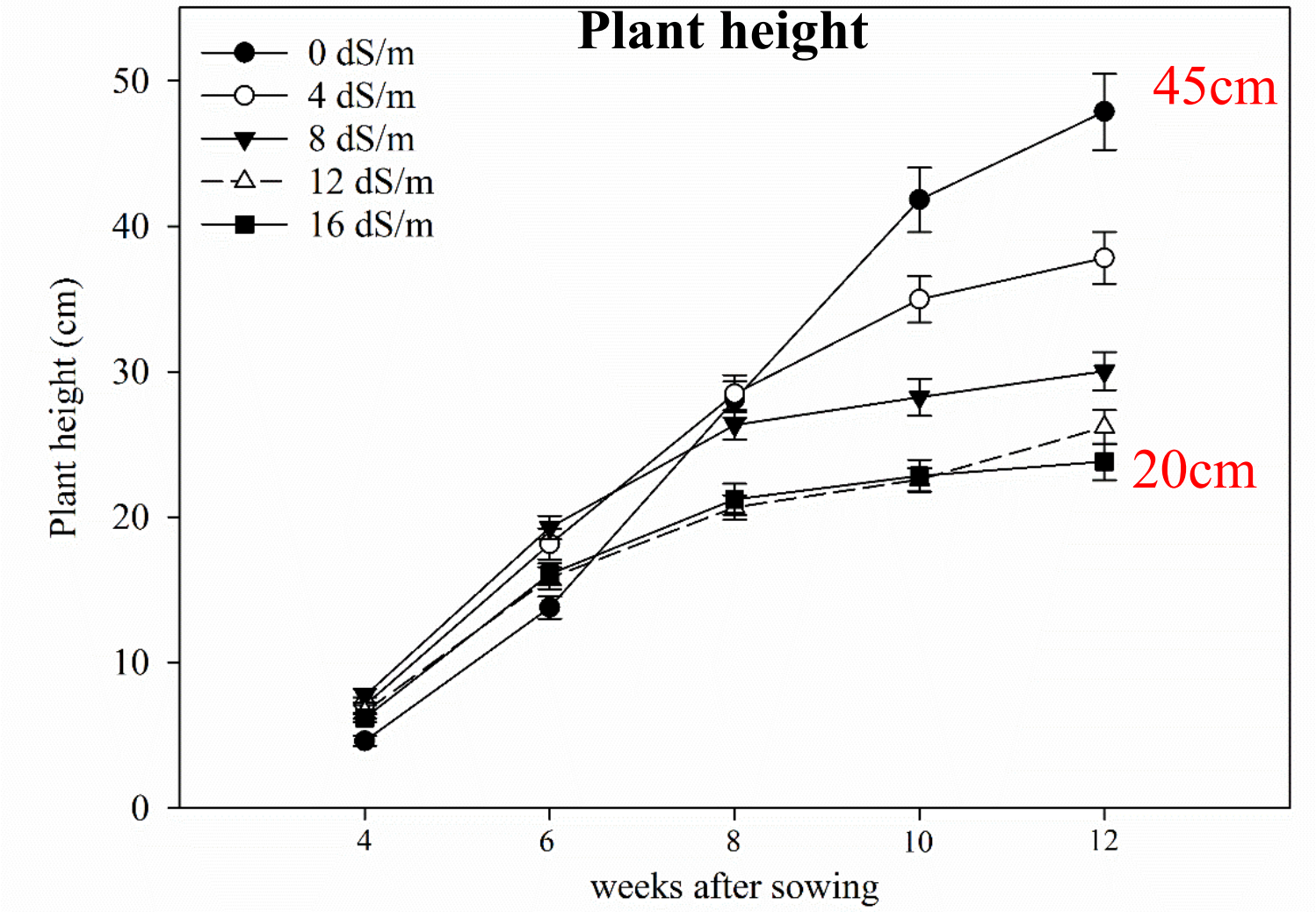


Figure 1. Average plant height (cm) of alfalfa plants at different stages of growth under five gradient of salt stresses

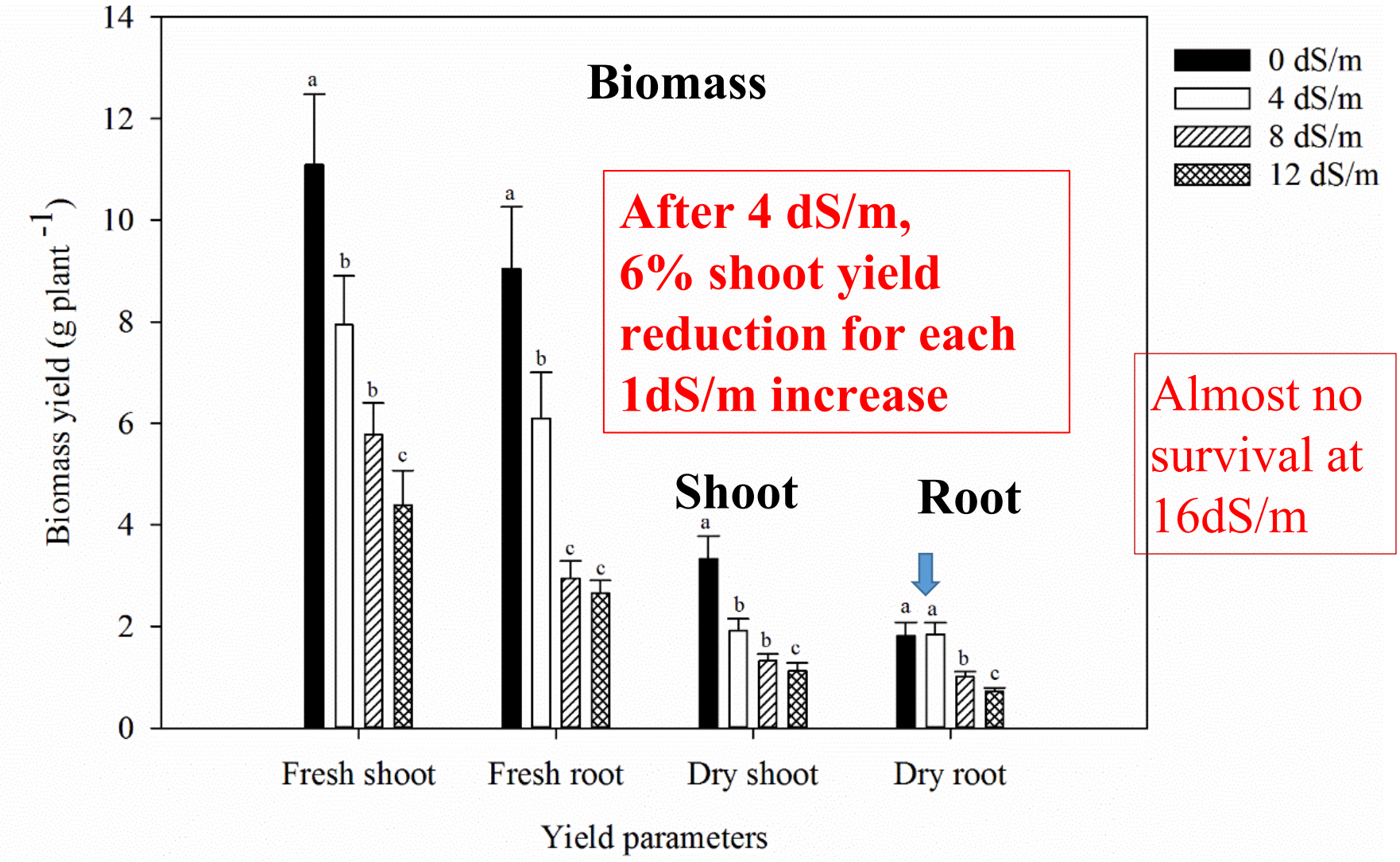


Figure 2. Fresh and dry root and shoot biomass yield of five alfalfa varieties under four gradient of salt stress

Chlorine content in the leaves (integrated absorption peak areas based on XRF spectroscopy)

Cultivar	Salt ($dS\ m^{-1}$)	Chlorine
Halo Tolerant	**0**	**149±28**
	12	**9278±461**
Vernal Intolerant	**0**	**240±16**
	12	**11952±261**

RNA-Seq analysis

Table 2.1. Number of differently expressed genes (DEGs), proteins and their functions in salt tolerant and intolerant alfalfa cultivars.

Alfalfa genotype	Tissue	Salt stress	Total number of differentially expressed genes/proteins	Major pathway/function	Reference
NM-801 (tolerant), Vernal (intolerant)	root	2-week-old seedlings treated with ~5, ~10 dS m^{-1} NaCl for 3 days	83	Ion homeostasis, protein turnover and signaling, protein folding, cell wall components, carbohydrate and energy metabolism, reactive oxygen species regulation and detoxification, and purine and fatty acid metabolism.	(Rahman et al., 2015)
Zhongmu-1 (*M. sativa*, tolerant), Jemalong A17 (*M. truncatula*, intolerant)	root	1-month-old seedlings treated with ~30 dS m^{-1} NaCl for 8h	93 (tolerant) 30 (intolerant)	Molecule binding and catalytic activity. Defense against oxidative stress, metabolism, photosynthesis, protein synthesis and processing, and signal transduction.	(Long et al., 2016)
AZ-88NDC (intolerant), AZ-GERM SALT-II (tolerant)	root	1-week-old seedlings treated with ~15 dS m^{-1} NaCl for 7 days	288/273 and 468/337 up-/down regulated in intolerant and tolerant, respectively	Response to stress, kinase activity, hydrolase activity, oxidoreductase activity, extracellular region.	(Postnikova et al., 2013)
Zhongmu No. 1 (tolerant)	root	12-day-old seedlings treated with ~25 dS m^{-1} NaCl for 1, 3, 6, 12, 24 h	8861 at one or more time points	Iron ion transport, ion homeostasis, antiporter, signal perception, signal transduction, transcriptional regulation and antioxidative defense.	(Luo et al., 2019)
Zhongmu No. 1 (tolerant)	root, shoot	1-week-old seedlings treated with ~10, ~20 dS m^{-1} NaCl for 7	26 (shoot) 35 (root)	Photosynthesis (31%), and stress and defense (20%) in the shoot. Defense (26%), metabolism (17%), and protein translation, processing, and degradation (17%) in the root.	(Xiong et al., 2017)
CW064027, Bridgeview (tolerant), Rangelander (intolerant)	shoot	4th-cut treated with 1.53, 8, 15.6 dS m^{-1} maintained by sulphate- based sodium, calcium, and magnesium salts	685/527, 368/139 up-/down regulated in CW064027 and Bridgeview at control, 537/949, 375/1045 up-/down regulated in CW064027 and Bridgeview at 8 dS m^{-1}, 1129/1196, 843/1516 up-/down regulated in CW064027 and Bridgeview at 15.6 dS m^{-1}	Redox-related genes, B-ZIP transcripts, cell wall structural components, lipids, secondary metabolism, auxin and ethylene hormones, development, transport, signaling, heat shock, proteolysis, pathogenesis-response, abiotic stress, RNA processing, and protein metabolism.	(Gruber et al., 2017)
Zhongmu-1 (tolerant), Xingjiang Daye (intolerant)	leaf	30-day-old plants treated with ~50 dS m^{-1} NaCl for 7 days	1125 and 2237 between cultivars at control and stress, respectively	Response to stimulus, reactive oxygen species, responding to stress, response to hormone and other stress-responsive processes.	(Lei et al., 2018a)

RNA-Seq analysis

Cultivars: Halo & Vernal

Four biological replications

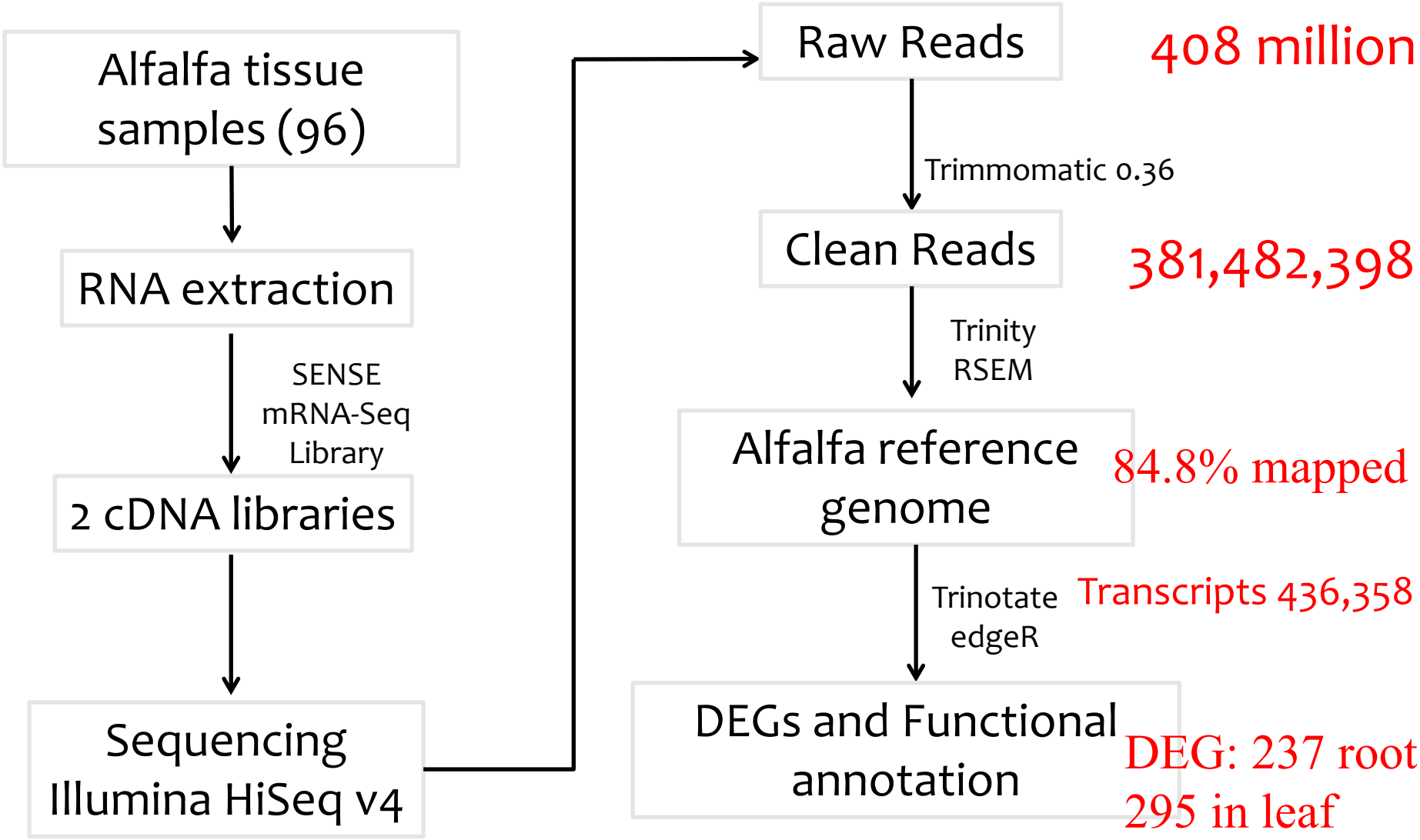
Clones: 6 identical clones

Time: 0h 3h 27h

12ds/m salt stress

Tissue: Leaf root

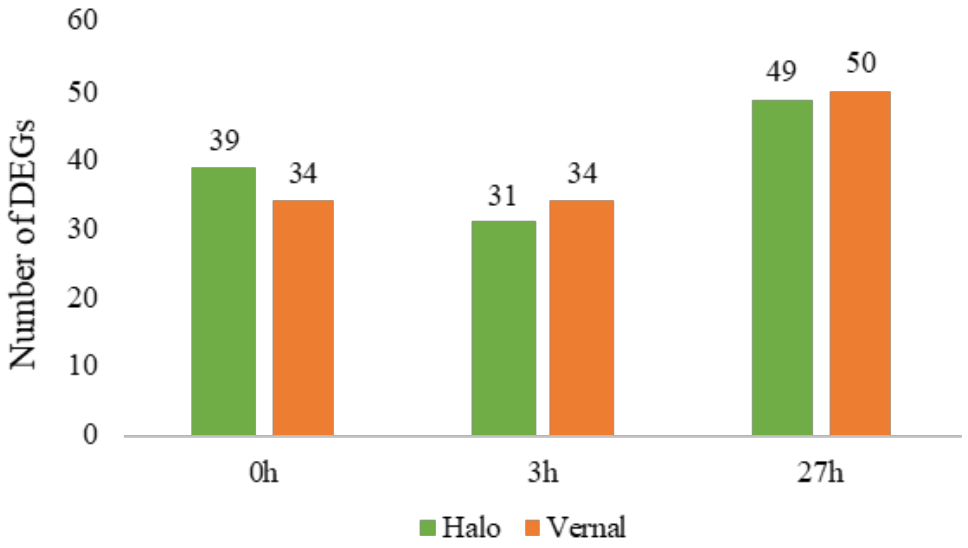
RNA-Seq analysis



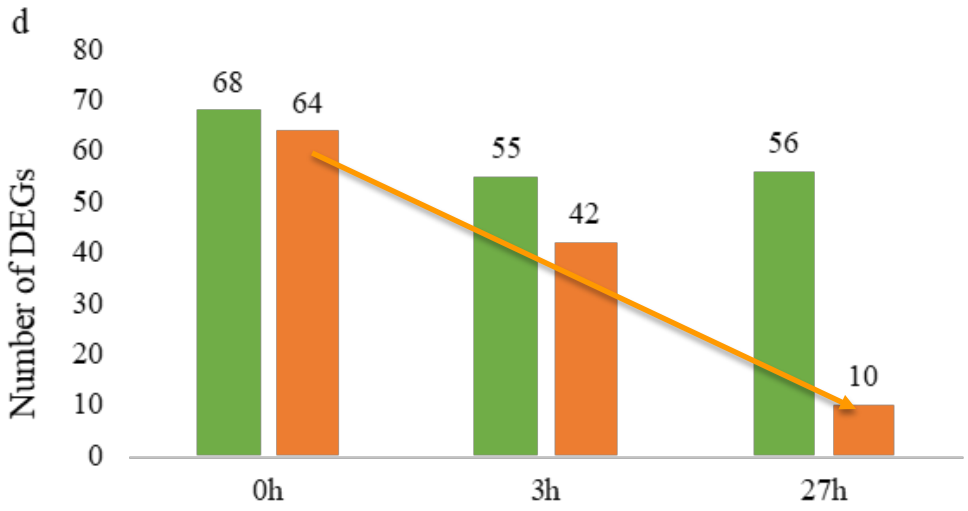
FDR <0.05 & log2 (fold change) ≥2

Number of DEGs

Leaf



Root



Halo

Vernal

Salt responsive candidate genes highly expressed in salt tolerance cultivar ‘ Halo’

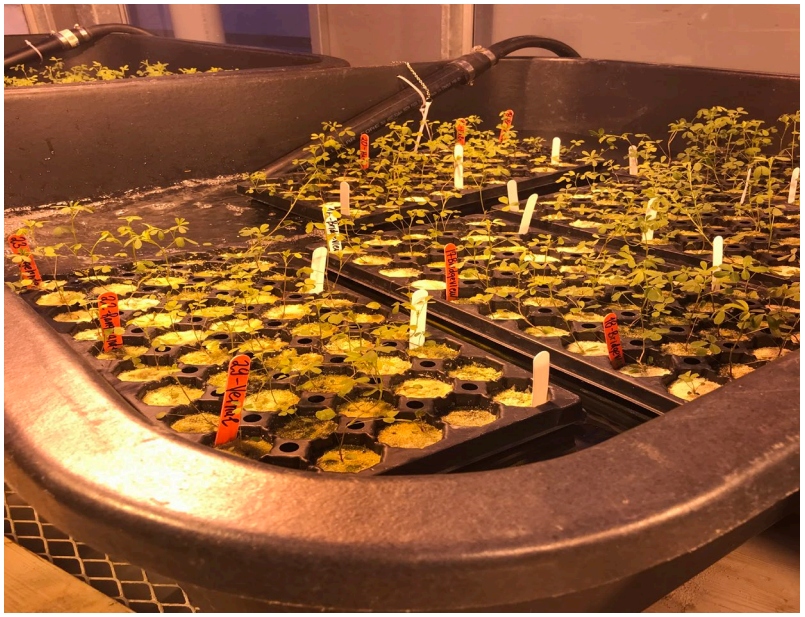
	Gene ID	nr ID[1]	log_2FC^2 (Leaf)			Putative function
			0h	**3h**	**27h**	
Leaf	MS.gene07287	XP_003591401.1	8.8	11.2	10.5	calvin cycle protein CP12-2, cl
Root	MS.gene058673	PNX87529.1	7.9	5.5	9.5	heavy-metal-associated domain-pratense]

- **Halo alfalfa: 15 in leaf and 18 in root**

The genes were involved in transmembrane protein function, photosynthesis, carbohydrate metabolism, defense against oxidative damage, cell wall modification and protection against lipid peroxidation.

Breeding for salt tolerance (progress)

- Diverse genetic background - 5 cycles of selection (C1-C5)
- Winter hardiness
- Validate new selected genotypes using molecular markers
- Test breeding lines in saline areas



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