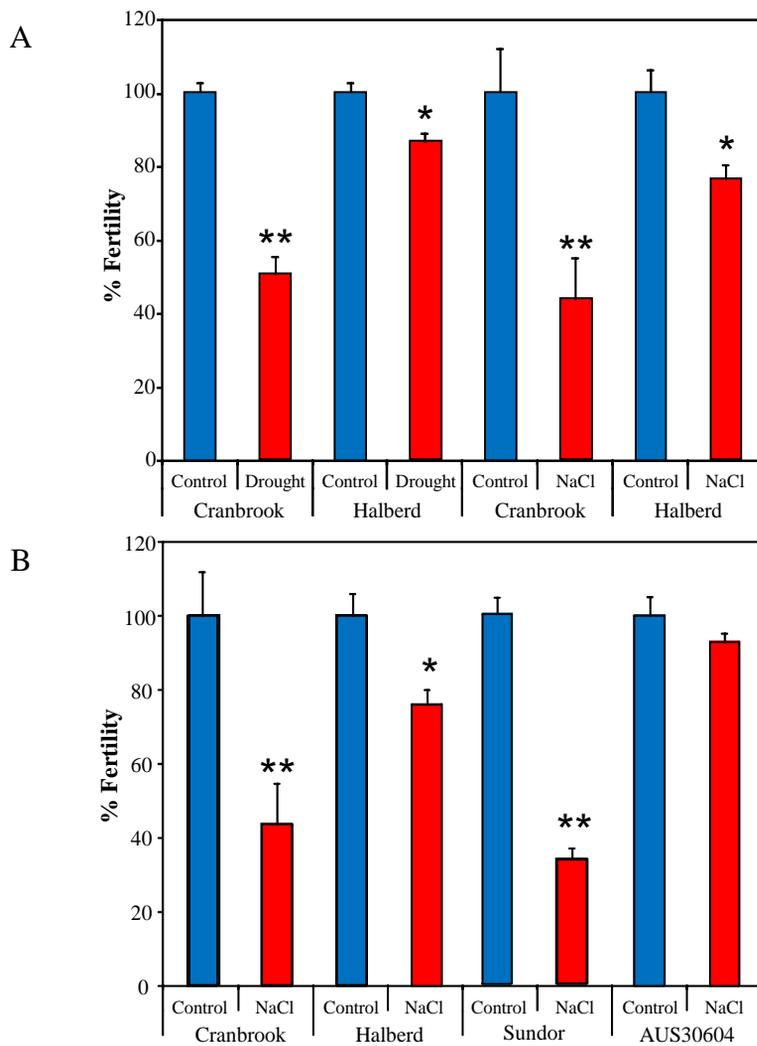
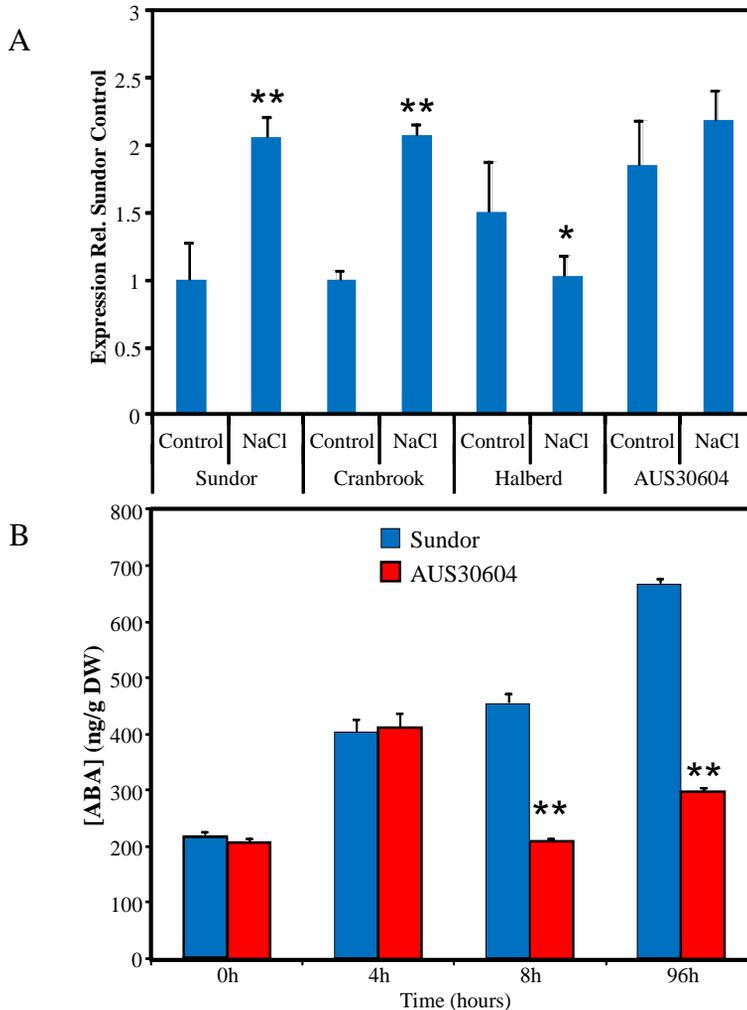


**Supplementary Fig. S1:** schematic representation of the hydroponics phenotyping method for osmotic stress tolerance

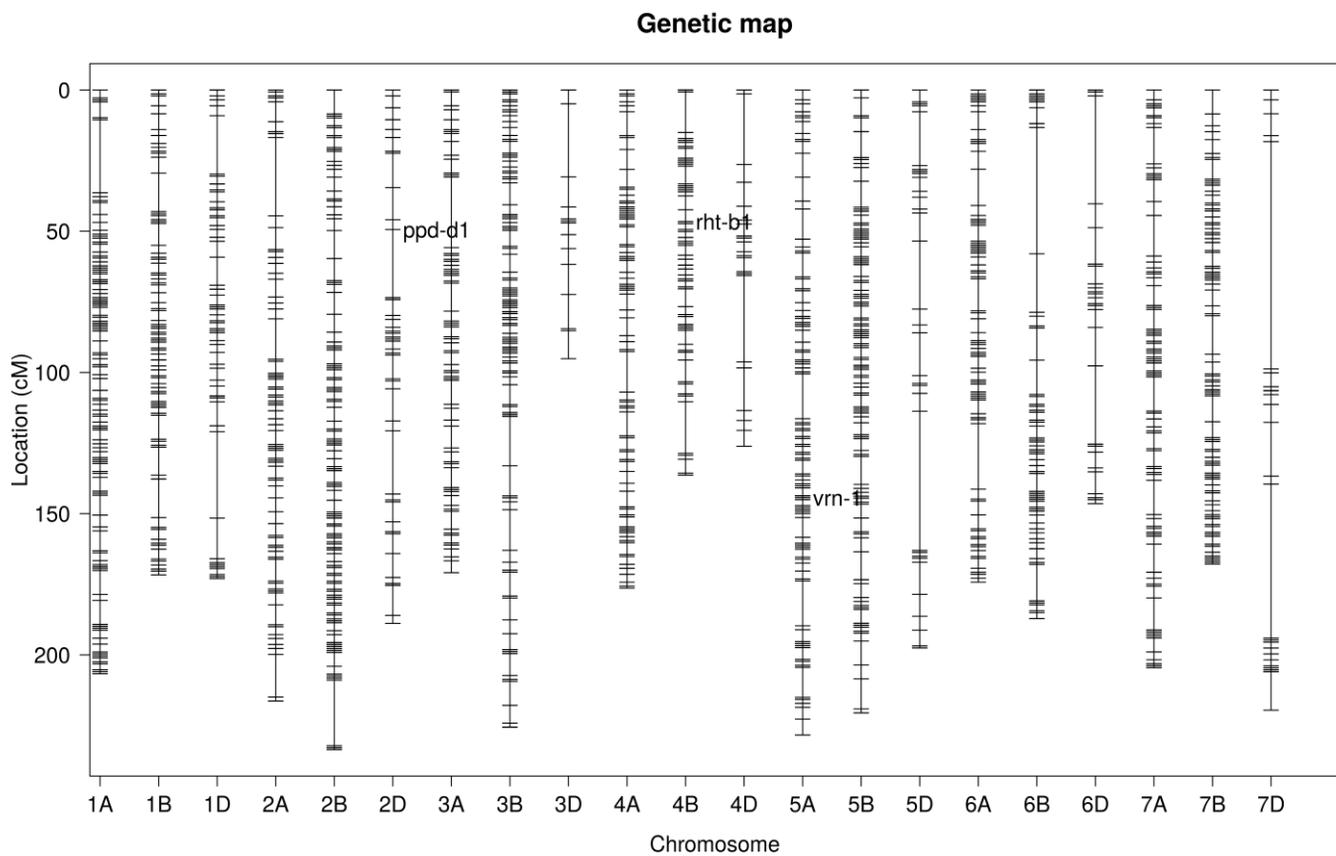


**Supplementary Fig. S2:** comparison of the response to drought (A) and osmotic stress (B) for two drought-sensitive (Sundor, Cranbrook) and two drought-tolerant (Halberd, AUS30604) wheat lines. Data were collected for at least 20 spikes per treatment and error bars represent standard errors. Single and double asterisks indicate significance thresholds at the 5% and 1% level respectively.



**Supplementary Fig. S3:** ABA accumulation in wheat spikes under osmotic stress conditions.

- A. Expression of the ABA biosynthetic gene *TaZEP1* encoding zeaxanthin epoxidase in YM stage wheat spikes shows that the gene is induced in drought-sensitive lines Sundor and Cranbrook, but not in drought-tolerant lines Halberd and AUS30604. Wheat spikes were harvested at the end of the 5-day osmotic stress treatment, before plants were returned to normal conditions. Real-time PCR expression studies were carried out on three repeat samples, each consisting of a mix of three YM stage spikes. These results are similar to those previously observed for the same wheat lines under drought stress conditions (Ji et al., 2011).
- B. Measurement of ABA levels in wheat spikes exposed to osmotic stress for 0, 4, 8 and 96h. While ABA levels steadily increase in drought-sensitive line Sundor, ABA levels for the tolerant line AUS30604 show a temporary increase after 4 hours treatment, but return to untreated levels for the rest of the treatment. Asterisks show significant differences ( $p < 0.01$ ) between Sundor and AUS30604. Three repeat measurements were carried of per time point and each samples consist of a mix of three YM stage spikes. The results are again similar to those observed for drought stress (Ji et al., 2011)



**Supplementary Fig. S4:** Linkage map of the Cranbrook x Halberd DH population constructed with 1,383 SNP markers.