Nutritional Quality and Grain Yield of *TaNAM*-RNAi Wheat Under Abiotic Stress

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INTRODUCTION

Staple crops, such as wheat, provide a substantial proportion of protein and minerals to human diets. The World Health Organization reports that over 2 billion people are anemic, largely due to iron deficiency. In developing countries. The WHO estimates the global frequency of Zn deficiency to be 31%.

Nitrogen (N) use by cereal crops to produce grain involves multiple processes, including uptake, assimilation, translocation, and remobilization. A substantial percentage of the protein in wheat grain is supplied by amino acids remobilized from vegetative tissue during leaf senescence. Likewise, Fe, Zn and certain other minerals are remobilized from vegetative tissues in several species.

NAC/NAM transcription factors have roles in plant development, abiotic stress, and biotic stress responses. *NAM-B1* and its homeologues are important for regulating the onset of developmentally initiated senescence. Decreased expression of the four endogenous wheat NAM genes (*TaNAM-A1, D1, B2,* and *D2*), using RNA interference (RNAi), delayed senescence and led to decreased N remobilization and lower partitioning of N to developing grain, and lower grain Fe and Zn concentrations (Uauy et al., 2006; Waters et al., 2009).

Objectives

A) To determine whether the delayed senescence phenotype of the NAM-RNAi line would be advantageous under abiotic stresses of heat, low N availability, and post-anthesis drought.
B) To determine the effects of abiotic stress on grain nutritional quality

Results and Discussion

Senescence onset under optimal and stress conditions

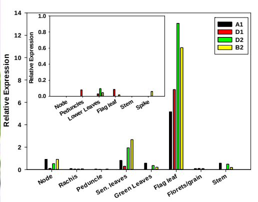

Fig. 1 A previous study showed that *NAM* genes were upregulated in flag leaves rapidly after anthesis, but expression in other tissues was not tested (Uauy et al., 2006). We measured expression of the four NAM homeologues in above-ground tissues in WT hexaploid wheat at pre-anthesis (inset) and during grain fill (main graph). Expression was highest in flag leaves during grain fill.

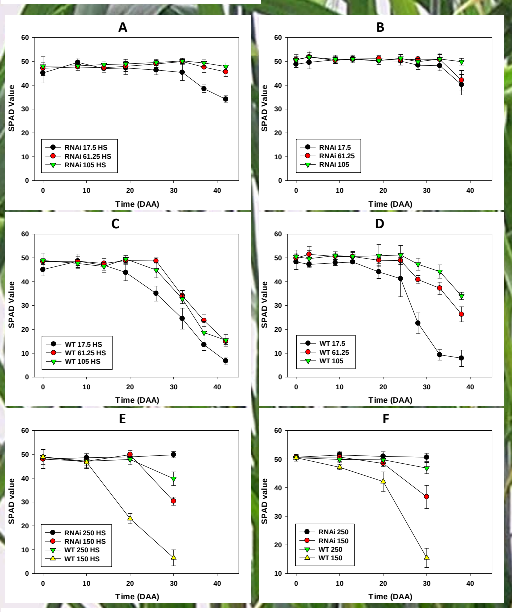

Fig. 2 Monitoring of leaf chlorophyll by Minolta SPAD meter to track onset of senescence. RNAi plants grown under (A) heat stress and (B) optimal greenhouse conditions, WT plants grown under (C) heat stress and (D) optimal greenhouse conditions, and both lines grown under (E) heat stress with water limitation and (F) optimal conditions with water limitation. The RNAi line had delayed senescence under all conditions.

Conditions key:
Heat Stress had day temperatures of 29 C and nights at 22.5 C
Optimal temperatures were 21 C day /15.5 C night
N was supplied at 17.5, 61.25, and 105 mg N per plant
Water limited plants received 0.6 X of well-watered from anthesis onward

Yield advantages of NAM-RANi-induced delayed senescence under abiotic stress?

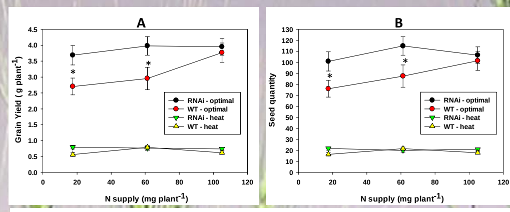

Fig. 3 Grain yield (A) was higher in the RNAi line at low or intermediate N supply under optimal conditions, but not at high N and not under heat stress at any N level. Increased yield was dependent on seed number per spike (B). Plants produced 1 spike under heat stress and 2.7 (WT) or 3.0 (RNAi) spikes under optimal conditions.

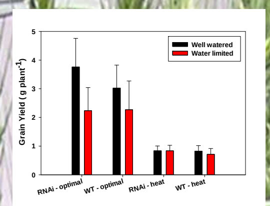

Fig. 4 Under optimal temperatures, water limitation reduced yield of both genotypes significantly, while under heat stress, water limitation did not further reduce yield.

How does abiotic stress affect N utilization and grain protein concentration?

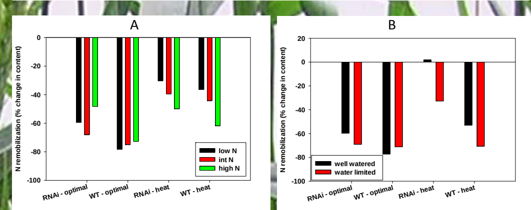

Fig. 5 Between anthesis and maturity, both RNAi and WT genotypes remobilized N from vegetative tissues under optimal temperature conditions and heat stress (A). Well watered RNAi did not remobilize N when combined heat stress (B).

There was no difference between genotypes for vegetative N accumulation pre- or post-anthesis (not shown).

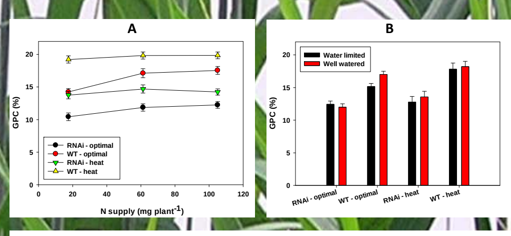

Fig. 6 Grain protein concentration (GPC) was lower in RNAi seeds in all treatments. Under optimal temperature conditions (A), both genotypes had a positive response to increasing N supply. Water limitation (B) under heat stress resulted in higher GPC in WT plants (but fewer seeds).

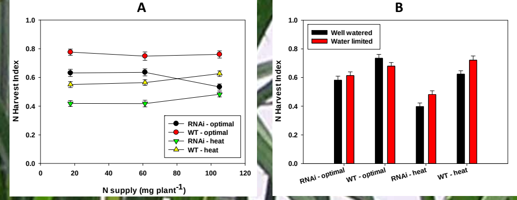

Fig. 7 A greater quantity of total plant N was partitioned into grain in the WT than in RNAi under all conditions. (A) Fertility experiment, (B) water limitation experiment. Partitioning to grain was enhanced under heat stress combined with water limitation.

How does abiotic stress affect mineral partitioning and grain mineral concentration?

Heat stress | **Optimal temperature**

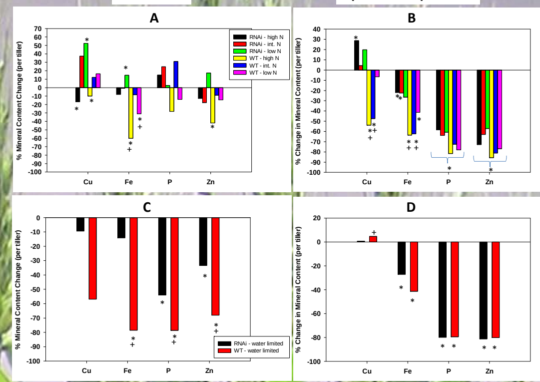

Fig. 8 Minerals that exhibited the most consistent remobilization were Cu, Fe, P, and Zn. Under heat stress (A), remobilization was disrupted as compared to optimal temperatures (B). Water limitation blocked remobilization of Cu. Under optimal conditions and water limitation, RNAi and WT remobilized equivalent percentages of minerals. (* = remobilization different from 0, + = genotypes significantly different)

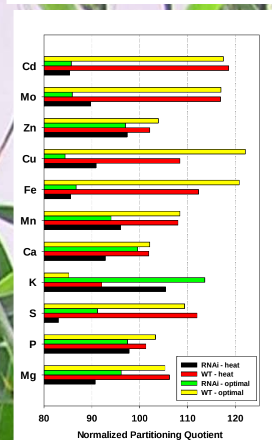

Fig. 9 Mineral seed partitioning quotients (PQ) express mineral partitioning to grain relative to dry matter partitioning to grain, normalized to the mean. For most minerals, the RNAi line had decreased partitioning to grain (all significant under heat stress except P, Ca, Zn; all significant under optimal temperature except Ca). The PQ for K was higher for the RNAi line.

Table 1 (below) Concentration of most minerals was higher in WT grain than in RNAi, under both heat stress and optimal temperatures. In both lines, concentrations were generally higher under optimal conditions.

	Concentration in Seed at Maturity			
	Heat Stress		Optimal Temperature	
	***TaNAM*-RNAi**	**Control**	***TaNAM*-RNAi**	**Control**
Mg	1260 ± 30	1470 ± 30**	1520 ± 35	1586 ± 37
P	3040 ± 100	3880 ± 100**	3791 ± 107	4017 ± 115
S	1520 ± 40	1830 ± 40**	2084 ± 44	2291 ± 48**
K	4900 ± 80	4300 ± 70**	7105 ± 137	5647 ± 147**
Ca	501 ± 10	497 ± 10	685 ± 18	648 ± 19
Mn	42.0 ± 1.5	51.6 ± 1.4**	50.8 ± 1.9	54.8 ± 2.0
Fe	32.5 ± 1.7	48.5 ± 1.6**	34.4 ± 1.3	43.7 ± 1.4**
Cu	3.80 ± 0.14	4.84 ± 0.14**	3.75 ± 0.33	4.91 ± 0.36*
Zn	45.6 ± 2.2	56.2 ± 2.1**	34.9 ± 1.5	36.8 ± 1.6
Mo	0.834 ± 0.046	1.264 ± 0.045**	0.825 ± 0.035	1.123 ± 0.037**
Cd	0.148 ± 0.008	0.216 ± 0 008**	0.201 ± 0.013	0.257 ± 0.014**

***, ** indicate that F-value for genotype effect is significant at *p*< 0.05 and 0.01, respectively**

Conclusions and Future Directions

Conclusions: The knockdown of TaNAM genes in flag leaves did result in delayed senescence in both optimal and stress conditions relative to the WT. This resulted in a yield advantage under low or intermediate N supply, but only under optimal temperature conditions; there was no RNAi advantage under heat stress or water limitation. Remobilization and partitioning of N and most minerals to grain was impaired in the RNAi line, as was GPC and mineral concentration. Thus, the delayed senescence phenotype of the RNAi line may offer yield gains under certain field conditions, but the decreased nutritional quality is not desirable.

Future directions: Since the NAM genes are transcription factors, it may be possible to identify direct or downstream gene targets that will allow separation of the delayed senescence and impaired mineral partitioning.

Acknowledgements

This research was funded in part by a grant to BMW from the UNL Agricultural Research Division, and the USDA-NIFA Triticeae-CAP grant (2011-68002-30029). The authors thank Laura Armbrust, Grace Troupe, Sam McInturf, and Brad Edeal with technical assistance with sample processing.