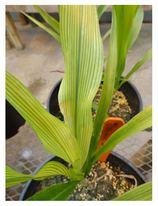

Reduced Seedling Vigor and Interveinal Chlorosis Associated with ALS Mutation in Sorghum Are Affected by Background Genotype

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INTRODUCTION

Acetolactate synthase (ALS) is an enzyme associated with synthesis of branched chain amino acids (valine, leucine and isoleucine) in plants. ALS inhibitor herbicides function through binding to the functional domain of the ALS protein. Susceptible plants die from starvation of these essential amino acids.

While deployment of glyphosate resistance trait has revolutionized weed control in crops such as maize, soybean and cotton, sorghum continues to suffer from weed infestation, especially grass weeds (Al-Khatib et al., 2007). Sustained efforts to develop resistance based weed control technology for sorghum led to identification of a novel resistance source in the wild population. Introgression of the resistance trait into adapted backgrounds yielded highly promising ALS resistant sorghum lines. However, reduced seedling vigor and interveinal chlorosis in many of the lines into which the resistance genes were incorporated has been a source of concern.

OBJECTIVES

1) Quantify the effects of the ALS mutation on seedling vigor and extent of chlorosis in different genetic backgrounds.
2) Assess the impact of altered seedling phenotype on yield potential of ALS resistant hybrids.

MATERIALS AND METHOD

- Both field and greenhouse studies were conducted to examine the effects of ALS mutation on seedling vigor and early season chlorophyll loss. The greenhouse study included 15 advanced ALS resistant families of diverse genetic backgrounds. Each of the families were grown in 5L pots (3 seedlings per pot) filled with a standard potting mix (Sun Gro, Bellevue, WA). The experiment was conducted in a randomized complete block design replicated four times.
- The field study was superimposed on the regular ALS herbicide resistance nursery where large number of families derived from diverse genetic backgrounds were grown. Some 40 different families representing over 15 different genetic backgrounds were selected for this study.
- Three weeks after emergence, seedlings were sprayed with ALS inhibitor herbicide Accent at the rate of 0.75 oz a.i./acre for the greenhouse study and 1.5 oz a.i./acre for the field study. Seedling vigor was rated both before and after the herbicide treatment using a 1-5 scale. Leaf greenness was determined as average of SPAD readings from three and ten plants in a plot for the greenhouse and field experiments, respectively.

RESULTS

- Seedling vigor and interveinal chlorosis rating taken before and after herbicide treatment were significantly different between genetic backgrounds. The difference for both traits, however, was more evident in the field than in the greenhouse (Fig. 1,2,3; Table 1).
- Majority of families carrying the ALS mutation had lower SPAD reading (were less greener) compared to those without the mutation. However, some resistant families had similar SPAD score with non ALS checks (Table 2).
- The average SPAD score for the ALS resistant families was comparable to that of the resistance gene donor Tailwind (TW) but remarkably different between growth stages and genetic backgrounds (Table 2).
- There was positive and significant correlation between pre-herbicide treatment seedling vigor and leaf chlorosis.
- Yield potential of some the ALS resistant hybrids was comparable to the non-ALS hybrid checks (Fig. 4).

Fig.1 Variation in seedling leaf chlorophyll loss associated with ALS inhibitor herbicide resistance mutation in different genetic backgrounds grown in the field (a) and greenhouse (b) conditions.

Table1: Mean squares from ANOVA for SPAD readings and plant vigor before and after the ALS herbicide application on 15 genetic backgrounds with ALS mutation.

Effect	df	Leaf greenness (SPAD scores)		Seedling vigor (1-5)	
		Before	After	Before	After
Block	3	38.07**	0.90	19.99***	4.69
Genotype	14	17.53*	79.64***	3.09*	3.26
Treated vs. untreated	1	NA	40.48*	NA	30.50*
Error	87	7.99	5.13	1.23	2.93

Statistically significant at *P < 0.05; **P < 0.001; ***P < 0.0001

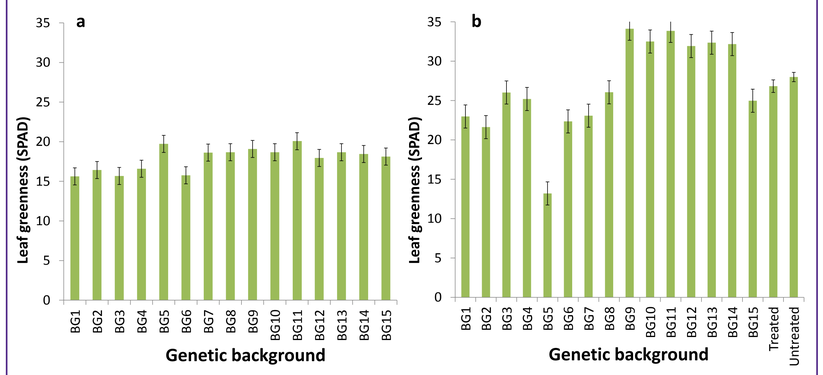

Fig.2 Leaf greenness (SPAD reading) of ALS resistant families scored before (a) and after (b) herbicide treatment as affected by genetic backgrounds.

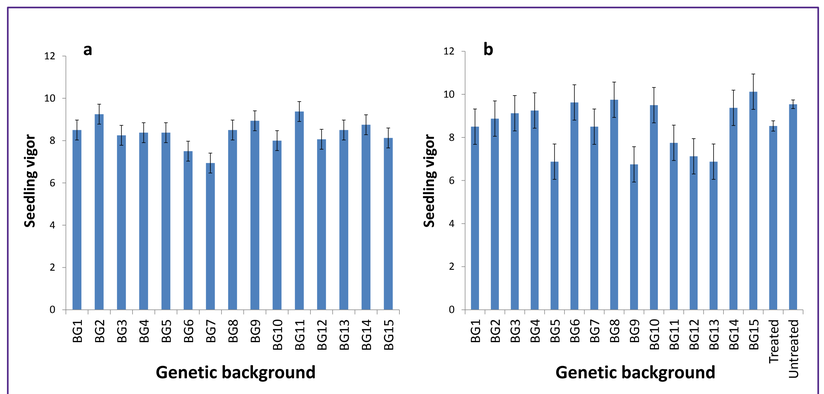

Fig.3 Seedling vigor of ALS resistant families scored before (a) and after (b) herbicide treatment as affected by genetic backgrounds.

Table 2: Mean leaf chlorophyll content and the pattern of chlorophyll change in normal and ALS resistant materials of different genetic backgrounds.

Backgrounds	Generation	Leaf greenness (SPAD)		Change in leaf greenness (+, 0, -)*
		Reading 1 average	Reading 2 average	
Background 1	F_5	33.69	41.56	+
Background 2	F_4	35.28	39.69	+
Background 3	F_4	31.99	38.12	+
Background 4	F_4	27.43	30.91	0
Background 5	F_4	27.98	40.11	+
Background 6	F_3	38.46	33.92	-
Background 7	F_3	39.74	36.83	0
Background 8	F_3	28.10	36.91	+
ALS releases (mixed background)	Line	32.71	41.69	+
Regular genotype	Line	40.82	41.22	0
Tailwind		38.75	35.55	-

*+, 0, and - : SPAD based leaf greenness significantly improved, unchanged and reduced, respectively, after herbicide treatment.

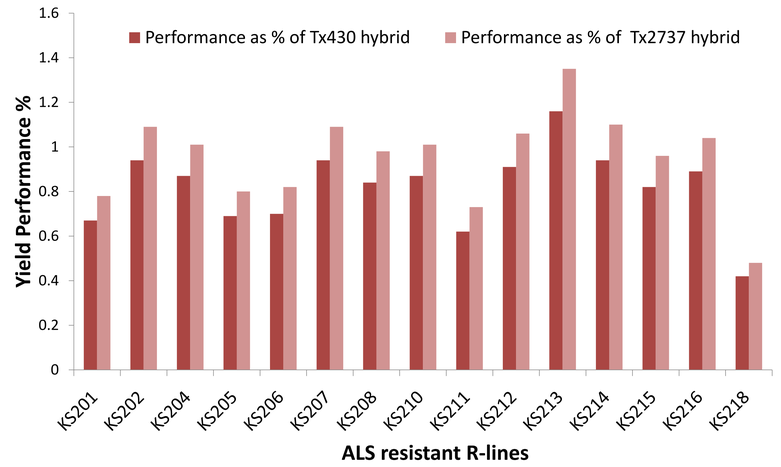

Fig.4 Average performance of hybrids compared to ALS resistant pollinators compared to susceptible hybrids of Tx430 and Tx2737.

DISCUSSION

- There is marked differences in the extent of leaf chlorosis and seedling vigor reduction among ALS resistant families. While the cause of such altered seedling phenotype is the subject of future investigation, the marked difference among families of different genetic background indicates that the traits can be genetically manipulated.
- Leaf chlorosis in the ALS resistance gene donor (TW) was similar to many of the ALS resistant sorghum families. Whereas, some of the resistant families have normal leaf chlorophyll development comparable to those without the ALS resistance trait. While, this makes it difficult to provide definite explanation, such peculiar leaf phenotype in the resistant families may have been caused either by an interaction between the ALS mutation and background genotypes or it may be the result of a linkage drag from the resistance gene donor parent.
- The impact of chlorophyll loss and reduced seedling vigor on grain yield has not been determined. But evaluation of ALS resistant pollinator lines in hybrid combination with standard susceptible females showed some of the ALS hybrids can give comparable yield to public hybrid checks. The variation in yield of ALS hybrids in relation to the checks is comparable with the results of testcross evaluations routinely observed in the non-ALS hybrids and may not be the result of the ALS mutation.

CONCLUSION

- Leaf chlorosis and reduced seedling vigor in ALS resistant sorghum families were significantly different between genetic backgrounds that careful selection of recurrent parent background can minimize these undesirable effects of the mutation.

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