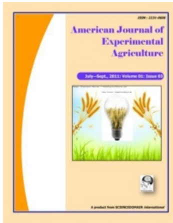

American Journal of Experimental Agriculture
4(12): 1500-1516, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Morpho-Physiological Characterization of Cold- and Pre-flowering Drought Tolerance in Grain Sorghum (*Sorghum bicolor* L. Moench) Inbreds

Yves Emendack[1*], Dariusz Malinowski[1], John Burke[2], Gloria Burow[2] and Zhanguo Xin[2]

[1]Texas A&M AgriLife Research, 11708 Highway 70 South, Vernon, Texas 76385, USA.
[2]Department of Agriculture, Agriculture Research Service, Cropping System Research Laboratory, 3810 4th Street, Lubbock, Texas 79415, USA.

Authors' contributions

This work was carried out as part of a collaborative project between the above institutions led by the collaborating author YE. Experimental design, management, data collection, analyses, and drafting of manuscript was done by author YE. All authors read and approved the final manuscript.

Original Research Article

Received 29th May 2014
Accepted 20th June 2014
Published 5th July 2014

ABSTRACT

Aim: The relationships between early-season cold temperature germination and pre-flowering drought stress in eight grain sorghum inbreds were assessed using morpho-physiological traits.
Study Design: Field was laid out in a randomized complete block design.
Place and Duration: The experiment was conducted at Vernon, Texas (34º9'4"N 99°17'26"W) during the month of April to August, 2013.
Methodology: Lines were sown in the field in early April to determine early-season cold temperature germination. At the pre-booting stage, lines were subjected to limited and no irrigation treatments, to establish a correlation between cold temperature germination and pre-flowering drought stress tolerance. Pre-flowering drought tolerance was assessed at five defined phenological phases of the reproductive growth stage, using morpho-physiological traits.
Results: Final germination percent (FGP) differed among lines (range 30-80%) and was positively correlated with leaf area index, plant height (HGT), and harvest index (HI). These three traits and single plant biomass (SPB) also positively correlated with grain yield

**Corresponding author: Email: yyemendack@ag.tamu.edu, emendacky@yahoo.com;*

(SPY) under pre-flowering drought. Most significant correlations among measured traits were observed at the heading to flowering phase. HI explained 75 and 91% variability in SPY for the limited and no irrigation treatments respectively. Other predictors for SPY were; FGP, HGT and SPB under limited irrigation, and FGP and HGT under no irrigation.
Conclusion: The ability to cease growth, maintain LAI, and retain high CLC and high HI, will be important selection traits to develop sorghum cultivars for temperate and highland regions, with possibility of pre-flowering drought occurrence.

Keywords: Early-season cold temperature germination; pre-flowering drought tolerance; grain sorghum inbreds.

1. INTRODUCTION

Predicted changes in climatic conditions will likely create combinations of various abiotic stresses and make it necessary to adopt novel breeding approaches to develop new crop varieties for modern agriculture [1]. Physiological responses to drought, cold, and salt stress are similar and include; reduced shoot growth and photosynthetic activity, accumulation of reactive oxygen species, alterations in ion transport and compartmentalization, and changes in metabolite profiles [2]. Numerous physiological and molecular changes occur during exposure to cold and drought stress, indicating complex responses involving more than just one pathway or cell compartment. The complexity of traits involved in abiotic stress responses, low genetic variance of yield components, and lack of efficient selection criteria are few of the factors to be considered in the conventional breeding for abiotic stress tolerance in sorghum.

When grown under minimum night temperatures from 4 to 10ºC, sorghum cultivars display delayed floral initiation [3] and infertile pollen [4]; responses observed when water stress was imposed during the pre-flowering stage [5]. These responses limit sorghum production in high altitudes, latitudes, and in regions with sub-optimal temperatures during germination, emergence, and early growth stages [6]. Improved cold tolerance in sorghum could extend its cultivation to the highlands and temperate regions, ensuring grain and forage production in marginal environments, serve as alternative crop in frost free mild winter areas, avoid heat and water deficiency stress later in the season, provide possibility of double crop cycles (early-season plus regular planting season), avoid diseases and pests prevalent during the warm season, and provide an early establishment to outgrow warm-season competitive weeds. Early planting may serve as a water-saving approach in sorghum grown under limited irrigation, by more efficient use of soil moisture accumulated during the winter-and spring-season, thus reducing the amount of irrigation compared to that needed during a regular growing season.

Genetic variability for cold tolerance exists in sorghum and has been evaluated by both early planting in the field and controlled temperature conditions [7-9]. Cold tolerance in sorghum has been measured by various characteristics, i.e., germination, emergence, vigor, and seedling growth under sub-optimal temperatures [10]. With the current trends in weather patterns and unpredictability of precipitation during the regular planting period (late May to mid-June), early planted cold-tolerant sorghum cultivars will likely be exposed to pre-flowering drought stress. As a result, grain yield could be significantly reduced or even no grain could be produced. Limited research has been conducted to evaluate the relationship between cold and drought stress tolerance in sorghum. Selecting cold tolerant sorghum germplasm for early-season plantings needs to be conducted simultaneously with selection for drought tolerance because such cultivars will likely be exposed to pre-flowering drought stress.

Numerous morpho-physiological traits have been used to evaluate drought tolerance in agricultural crops, including plant gas exchange and remobilization of photosynthetates [11,12], early vigor [13], intrinsic water use efficiency [14,15], root architecture [16], flowering time/anthesis-silking-interval [17], chlorophyll concentrations, stay-green characteristic, delayed senescence [18], dhurrin content [19]. Balota et al. [20] working on twelve grain sorghum cultivars found that respiration rates measured at 10 and 15ºC had the highest association with germination and elongation rates, while respiration at 25ºC was associated with seedling growth characteristics. Unpublished findings by Burke et al. indicate that Ethiopian grain sorghum lines selected for higher dhurrin levels (a characteristic observed in drought tolerant sorghum lines), had better cold germination and vigor than their drought susceptible counterparts. These findings suggest a possible relationship between cold and drought tolerance in sorghum. Identifying traits to select for pre-flowering drought stress tolerance from early-season cold tolerant sorghum germplasm, will facilitate understanding of the morpho-physiological responses of these materials to pre-flowering drought stress, and identify traits that may be used for selection of germplasm tolerant of both stresses. The objectives of this study were to determine early-season cold tolerance of a number of grain-type sorghum recombinant inbred lines (RILs), and establish a correlation between early-season cold tolerance and pre-flowering drought stress tolerance using some selected morpho-physiological traits.

2. MATERIALS AND METHODS

2.1 Plant Material and Experimental Design

The experiment was conducted at Vernon, Texas (34º9'4"N 99º17'26"W) during the month of April to August, 2013. Eight sorghum lines were evaluated in this study. The inbred lines were developed through crossing maternal cold tolerant line BTx623 and other germplasm (Kaoliang, Hong Ke Er Jiao, and Niu Sheng Zui) selected for cold tolerance in China. The eight lines used in this study have been assigned B1((BTx623 x NSL51071)-F2-18-1), B2 ((BTx623 x NSL51071)-F2-46-1), B3 ((BTx623 x PI563998)-F2-69-1), B4 ((BTx623 x PI563998)-74-1), B5 ((BTx623 x PI568016)-F2-99-1), B6 ((BTx623 x PI563998)-F2-119-1), B7 ((BTx623 x PI568016)-F2-191-1), and B8 ((BTx623 x PI563998)-F2-248-1). The experimental design was a randomized complete block with two sub-blocks serving as irrigation treatments (Limited-irrigation; LI and No-irrigation; NI). Each plot (sorghum line) was replicated three times. Plots had four diked rows at 10.67m length and 1.0m row spacing. Irrigation treatments were separated by eight diked rows of BMR-type forage sorghum as a buffer for any furrow irrigation overflow. Seeds were sown on 4 April 2013, at seeding rate of 3.36kg ha^{-1}, which corresponded to 40 seeds m^{-1} row, or 1707 seeds per plot. NPK fertilizer at 40-30-20 composition was applied at 84kgha^{-1} eight weeks after planting. Broad-leaf weeds (predominantly careless weeds; *Amaranthus palmeri*) were controlled with 2,4-dinitrophenylhydrazine herbicide.

2.2 Treatments and Morpho-Physiological Traits Assessed

Originating from the semi-arid sub-tropics, sorghum requires a minimum temperature of 12.8ºC and an annual precipitation of 381-610 mm to complete a growth cycle. Total precipitation during the experimental period (April to mid-August) was 39.80 mm. Precipitation during April was only 28.80 mm compared to a 10-year average precipitation of 63.50 mm. Therefore, available soil water for vegetative growth came mostly from the water stored (117.9mm) in the soil profile from rainfall and snow precipitation during the preceding winter-spring months (November to December, 2012 and January to March, 2013). The effects of pre-flowering drought stress were assessed at five denoted phenological phases of the reproductive growth stage (pre-booting to physiological maturity); (I) pre-booting to booting, (II) booting to heading, (III) heading to flowering, (IV) flowering to anthesis, and (V) physiological maturity. The limited irrigation treatment received irrigation from the pre-booting through flowering phase in the amounts stated in Table 1. The total water (winter-spring rainfall and snow precipitation, and May to August rainfall, plus total irrigation) supplied in the limited irrigation treatment was 344.98mm, compared to 117.90mm (winter-spring rainfall and snow precipitation and May to August rainfall) for the no irrigation treatment.

Table 1. Furrow irrigation and cumulative irrigation (irrigation plus rainfall) applied to limited irrigation treatment from the pre-booting through flowering phenological phases

Phenological phases	Furrow Irrigation (mm)	Cumulative (irrigation + rainfall)
I	51.30	79.30
II	45.00	126.00
II	39.40	170.90
IV	52.30	227.08

Plant morpho-physiological traits evaluated at the five phenological phases were: plant height (HGT; cm), leaf area index (LAI), chlorophyll content (CLC; µmol m^{-2}), total leaf number, the number of fully developed leaves, days to flowering (DTF), days to maturity (DTM), single plant dry biomass (SPB; g), single plant grain yield (SPY; g), and harvest index (HI) (Table 2). Early-season cold temperature germination was evaluated based on final germination percent (FGP). Since the effect of pre-flowering drought stress on sorghum grain yield is most significant at the booting to flowering interval of the reproductive growth stage, CLC, LAI, and HGT were assessed at phenological phases (II and III) of this interval. SPB, SPY, and HI were assessed at physiological maturity (boxes in Table 2). Data from phenological phases I and IV were used to evaluate the initial effects, and extent of the variations of assessed morpho-physiological traits to pre-flowering drought stress, compared to their observed variations in phases II and III.

2.2.1 Final germination percent, days to flowering and to physiological maturity

Final germination was visually assessed every third day following sowing, for four consecutive weeks. Seed was considered germinated once the plumule emerged above the soil surface. The final germination percent was determined at the end of the fourth week, by calculating the final cumulative germination as a percentage of the initial number of seed sown (1707/plot). Days to flowering (50% blooming plants averaged across three replications) and physiological maturity (70% of plants with dark spot on grain averaged across three replications) were also determined for each line.

Table 2. Assessed morpho-physiological traits at various phenological phases of the reproductive stage under limited and no irrigation treatments

Phases	I Pre-booting-Booting		II Booting-Heading		III Heading-Flowering		IV Flowering-Anthesis		V Physiological Maturity	
	LI	NI	LI	NI	LI	NI	LI	NI	LI	NI
FGP										
CLC	x	x	x	x	x	x	x	x		
LAI			x	x	x	x				
HGT	x	x	x	x	x	x	x	x		
SPB	x	x	x	x	x	x	x	x	x	x
SPY									x	x
HI									x	x

FGP: Final germination percent (%); SPB: Single plant dry biomass (g); CLC: Chlorophyll content (µmol m^{-2}); SPY: Single plant yield (g); LAI: Leaf area index; HI: Harvest index; HGT: Plant height (cm); X: Assessed

2.2.2 Plant height, chlorophyll content, and leaf area index

Plant height and chlorophyll content were measured concurrently on the same plant between 9 and 11 a.m. following the 50% booting stage, using a meter-stick and chlorophyll meter (SPAD-502, Minolta, Co., LTD, Japan) respectively. Plant height was measured from the base of plant to the ligules of the flag leaf. CLC readings were taking from the base, middle, and top sections on either sides of the mid-vein of the last two fully-developed leaves. Leaf area index was measured using a Ceptometer (AccuPAR LP-80; Decagon Devices, Inc., USA) from ten random sections in each replication following HGT and CLC measurements.

2.2.3 Single plant biomass, single plant yield, and harvest index

Plants were sampled for dry biomass production at physiological maturity. Six plants were harvested per replication, oven dried at 70ºC for 72 hours, and individually weighed. Single plant biomass (SPB) was calculated per plant. Dried panicles from each of the same six plants were separately hand-threshed and winnowed clean. Grain yield (SPY) was determined for each plant, and harvest index (HI) was calculated as the ratio of SPY to SPB.

2.3 Statistical Analysis

Data were analyzed using SPSS 17.0 (SPSS Inc., USA) and Sigma Stat 3.1 (Systat Software Inc., USA). Multivariate analysis of variance was used to identify significant effects of experimental treatments (sorghum breeding lines, irrigation levels, and phenological phases) on assessed traits based on Wilks' Lamda test and its associated significant level (P=0.05). The Bonferroni adjustment was used where necessary to avoid type I errors. Means among and within lines were separated by Tukey's HSD test and student T-test respectively at the 5% probability level, except when stated otherwise. Pearson correlation matrix was used to identify correlations between measured traits. The predictive effect of individually assessed traits on yield was analyzed using a forward multi-regression analysis. Due to the small sample size, the variance in yield as explained by each assessed trait was expressed as the adjusted R square value. To ensure a standard distribution of data and improve homogeneity of error variances, the percentage values of germination were transformed using the arcsin function prior to analysis of variance.

3. RESULTS AND DISCUSSION

3.1 Results

The main effects of experimental treatments (sorghum line, irrigation or no irrigation, and phenological phase of the reproductive growth stage) and their interactions on measured morpho-physiological traits are presented in Table 3.

Table 3. Summary of multivariate analysis of variance of phenological phase, irrigation level, and sorghum line effects on assessed morpho-physiological traits

	FGP	SPB	SPY	HI	HGT	LAI	CLC
Phase					*	*	*
Irrigation		*	*	*	*	*	*
Lines	*	*	*	*	*	*	*
Phase x Irrigation							*
Phase x Lines					*	*	*
Irrigation x Lines		*	*	*	*	*	*
Phase x Irrigation x Lines					*	*	*

** denotes significant interaction at P=.05. See legend in Table 2 for abbreviations*

3.1.1 Final germination percent, days to flowering and to physiological maturity

Freezing and below freezing temperatures were recorded post-sowing (4 April, 2013) on the 5 (0ºC), 10 (-1ºC), 11(0ºC), 19 (0ºC), and 24(-1ºC) of April 2013 (Fig. 1).

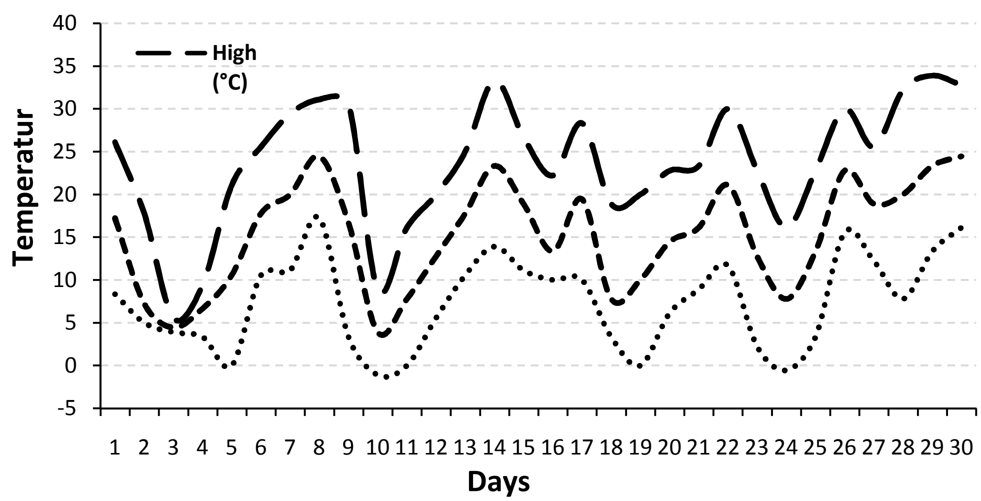

Fig. 1. Daily temperature variations during seeding month of April 2013

FGP was affected only by the main effect of sorghum line (Table 3). FGP significantly varied among lines. FGP for lines B1, B2, B3, B4, B5, and B7 were the highest, followed by line B8, with line B6 having the lowest FGP value at just 30% (Table 4). Lines were of different maturity groups, with different DTF and DTM.

Table 4. Final germination percent (FGP), days to flowering (DTF), and days to physiological maturity (DTM) of eight grain sorghum inbred lines (RIL code)

RIL code	Mat. group	RIL Pedigree	FGP (%)	DTF (days)	DTM (days)
B1	Late	(BTx623 x NSL51071)-F2-18-1	77a[z]	87a	113a
B2	Medium	(BTx623 x NSL51071)-F2-46-1	80a	74c	100c
B3	Med.-late	(BTx623 x PI563998)-F2-69-1	78a	80b	106b
B4	Med.-late	(BTx623 x PI563998)-F2-74-1	80a	80b	106b
B5	Med.-late	(BTx623 x PI568016)-F2-99-1	75a	80b	106b
B6	Med.-late	(BTx623 x PI563998)-F2-119-1	30c	83b	109b
B7	Late	(BTx623 x PI568016)-F2-191-1	80a	87a	113a
B8	Medium	(BTx623 x PI563998)-F2-248-1	65b	74c	100c
		s.e.	4.63	1.31	2.13

[z]Means for sorghum lines within an assessed trait. Within columns, means followed by a common letter are not significantly different (P=.05). Standard error, s.e.

3.1.2 Plant height

Plant height was affected by an interaction among phenological phase, irrigation treatment, and sorghum line (Table 3). Under limited irrigation treatment, lines differed in HGT (Table 5). In the booting to heading phase, lines B2 and B8 were significantly taller than lines B5 and B7, with other lines being intermediate. In the heading to flowering phase, lines B1, B2, B3, B4, and B8 were significantly taller than lines B5 and B6, and line B7 was the shortest. Under no irrigation treatment, line B2 was the tallest, followed by lines B1, B8, and B4, then lines B3 and B5, with lines B6 and B7 being the shortest ones in the booting to heading phase. Lines B6, B7, and B8 significantly reduced height in response to lack of irrigation during phase II. Lines B1 and B2 were the tallest under no irrigation in the heading to flowering stage, followed by lines B8 and B4, then lines B3, B5 and B6, and line B7 was the shortest. During phase III, all lines but B1 and B2 significantly reduced height in response to drought.

3.1.3 Chlorophyll content

Chlorophyll content was affected by an interaction among phenological phase, irrigation treatment, and sorghum line (Table 3). Under limited irrigation treatment, lines B1 and B3 had significantly lower CLC than the other lines in the booting to heading phase (Table 6). In the heading to flowering phase, the differences among the lines were more complex, with lines B7 and B8 having the highest CLC and line B3 the lowest CLC. Under no irrigation treatment in the booting to heading phase, line B8 had the highest CLC, followed by lines B2, B4, B6, and B7, with line B3 having the lowest CLC. Similar as in the limited irrigation treatment, lines B7 and B8 had the highest CLC, and line B3 the lowest CLC during the heading to flowering phase. Drought did not affect CLC of any lines in the booting to heading phase. In contrast, drought reduced CLC in all lines but lines B4 and B8 during the heading to flowering phase.

3.1.4 Leaf area index

Leaf area index was affected by an interaction among phenological phase, irrigation treatment, and sorghum line (Table 3). Under limited irrigation treatment, line B3 had the highest LAI during the booting to heading phase (Table 7). In the heading to flowering phase, line B5 had the highest LAI, followed by lines B1, B2, B3, B4, B7 and B8, while line B6 had the lowest LAI. Under no irrigation treatment, lines B4 and B5 had the highest LAI in the booting to heading phase, followed by lines B1, B2, B3, and B8, while lines B6 and B7 had the lowest LAI. In the heading to flowering phase, the highest LAI was noted for line B4, followed by line B2, then lines B1 and B3, then lines B5 and B8, with the lowest LAI expressed by lines B6 and B7. In the booting to heading phase, drought reduced LAI of lines B3 and B7. Reduction in LAI due to drought was more pronounced during heading to flowering phase, affecting lines B3, B5, B7, and B8.

Table 5. Effects of an interaction among phenological phase, irrigation treatment, and sorghum line on plant height

	II-Booting-heading		III-Heading-flowering	
	LI	NI	LI	NI
B1	102.53bc[z]	97.79b	110.24a	105.41a
B2	119.63a	116.76a	120.57a	115.82a
B3	90.75cd	81.10cd	*114.63a	87.14bc
B4	98.63bc	90.60bc	*108.46a	91.01b
B5	77.72e	70.79d	*89.74b	77.01bc
B6	*80.01de[y]	55.70e	*95.00b	79.37bc
B7	*71.55e	47.93e	*77.04c	51.13c
B8	*108.28ab	91.01b	*112.09a	98.04b
Mean	*93.29	*81.81	*103.48	*82.00
s.e.	3.48	3.68	3.58	3.63

[z]Means for sorghum lines within an irrigation treatment and phenological phase. Within columns, means followed by a common letter are not significantly different (P=.05). Standard error, s.e.
[y]Within rows, "" denotes a significant difference for each line between irrigation treatments within a phenological phase*

3.1.5 Single plant biomass

Single plant biomass was affected by an interaction between irrigation treatment and sorghum line (Table 3). Under limited irrigation treatment, line B8 had the highest SPB and lines B1 and B7 the lowest SPB, while the rest of the lines had intermediate SPB (Fig. 2). Under no irrigation treatment, line B2 had the highest SPB, followed by lines B4, B5, B6, and B8, while lines B1, B3, and B7 had the lowest SPB. Drought significantly reduced SPB in all lines but less so in line B2 (17% reduction) compared to the other lines (33 to 71% reduction).

3.1.6 Single Plant grain yield

Single plant yield was affected by an interaction between irrigation treatment and sorghum line (Table 3).Under limited irrigation treatment, line B4 had the highest SPY, followed by lines B2, B3, and B8, then lines B5 and B6, with lines B1 and B7 having the lowest SPY (Fig. 3). Under no irrigation treatment, line B2 had the highest SPY followed by line B4, then lines B3, B5, B6, B7, and B8, with line B1 having zero SPY. Drought significantly reduced SPY in all lines but less so in line B2 (52% reduction) compared to the other lines (83 to 100% reduction).

Table 6. Effects of an interaction among phenological phase, irrigation treatment, and sorghum line on plant chlorophyll content

	II-Booting-heading		III-Heading-flowering	
	LI	NI	LI	NI
B1	46.67b[z]	47.43bc	*55.33bcd[y]	44.40bc
B2	53.37a	50.33ab	*59.23b	45.87bc
B3	46.40b	38.87c	*52.43d	37.32d
B4	52.87a	49.30ab	55.80bcd	48.63b
B5	52.13a	44.37bc	*54.53cd	39.17c
B6	51.87a	51.07ab	*58.43bc	45.33bc
B7	55.00a	51.87ab	*60.93a	42.93a
B8	62.03a	56.90a	62.87a	54.93a
s.e.	1.38	2.87	1.03	2.20

[z]Means for sorghum lines within an irrigation treatment and phenological phase. Within columns, means followed by a common letter are not significantly different (P=.05). Standard erro, s.e.
[y]Within rows, "" denotes a significant difference for each line between irrigation treatments within a phenological phase*

Table 7. Effects of an interaction among phenological phase, irrigation treatment, and sorghum line on plant leaf area index

	II-Booting-heading		III-Heading-flowering	
	LI	NI	LI	NI
B1	1.70b[z]	1.54b	2.00b	1.64c
B2	1.93ab	1.54b	2.09b	1.82b
B3	*2.12a	1.57b	*2.07b	1.54cd
B4	1.99ab	1.92a	2.30b	2.17a
B5	1.97ab	1.80a	*2.41a	1.50d
B6	1.58b	1.34c	1.60c	1.31e
B7	*1.76b[y]	1.27c	*2.07b	1.21e
B8	1.66b	1.55b	*2.00b	1.51d
s.e.	0.01	0.03	0.12	0.05

[z]Means for sorghum lines within an irrigation treatment and phenological phase. Within columns, means followed by a common letter are not significantly different (P=0.05). Standard error, s.e.
[y]Within rows, "" denotes a significant difference for each line between irrigation treatments within a phenological phase*

3.1.7 Harvest index

Harvest index was affected by an interaction between irrigation treatment and sorghum line (Table 3).Under limited irrigation treatment, lines B3 and B4 had the highest HI, followed by line B2, then lines B6 and B8, and lines B1 and B5, with line B7 having the lowest HI (Fig. 4). Under no irrigation treatment, HI was highest for line B2, followed by lines B4 and B8, then lines B3, B5, B6, and B7, with line B1 having HI value of zero. Like single plant yield and single plant biomass, drought significantly reduced HI in all lines but less so in line B2 (43% reduction) compared to the other lines (83 to 100% reduction).

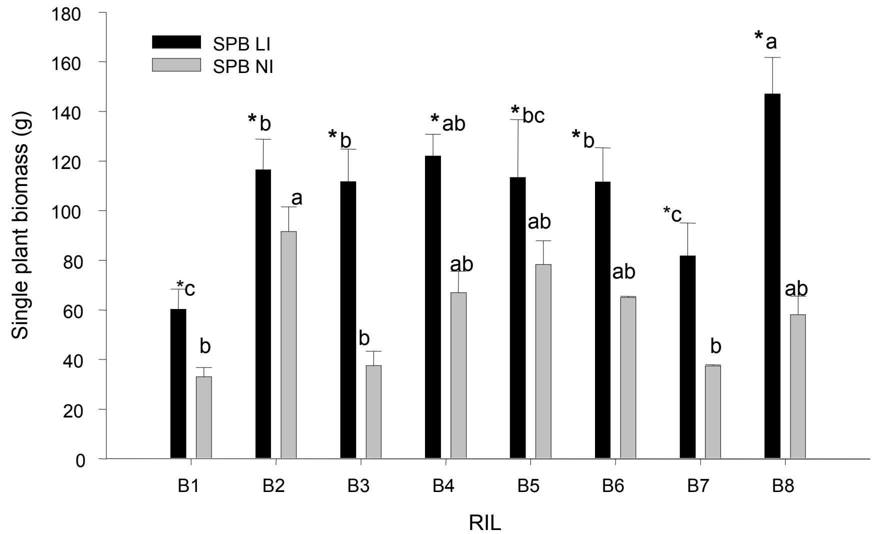

Fig. 2. Effects of an interaction between irrigation treatment and sorghum line on single plant biomass (SPB). Within irrigation treatment, bars followed by a common letter are not significantly different (*P*=0.05). "*" denotes a significant difference for each line between irrigation treatments. Bars indicate 1 standard error

3.1.8 Correlations among morpho-physiological traits and their effect on grain yield

Irrigation treatment was the main effect on all assessed traits following cold temperature germination (Table 3). Phenotypic correlations (Table 8a) of assessed traits under both irrigation treatments revealed direct significant positive correlations for FGP-LAI (0.39**) and FGP-HGT (0.24*) under no irrigationtreatment (numbers in italic in Table 8a). SPY-LAI (0.33*) and SPY-HGT(0.39**) were also positively correlated under no irrigation treatment. Harvest index showed positive correlations with FGP, LAI, HGT, SPB, and SPY under no irrigation treatment. The correlation between CLC and HGT was positive (0.30**) under limited irrigation treatment but negative (-0.25*) under no irrigation treatment.

Phenotypic correlation matrix at the different phenological phases showed positive correlations between FGP-LAI, HGT-SPB, HGT-SPY, and HGT-HI under both irrigation treatments, and a positive correlation between LAI-SPY under no irrigation treatment for phenological phase III (Table 8b). HGT-LAI showed a stronger correlation at phase III (0.52**) compared with phase II (0.40*) under no irrigation treatment. Under limited irrigation treatment, correlations between FGP-LAI, HGT-SPY, CLC-LAI, and HGT-HI were stronger at phase III than phase II, and several of the correlations were absent in phase II. No significant correlations were found under no irrigation treatment in phenological phases I and IV.

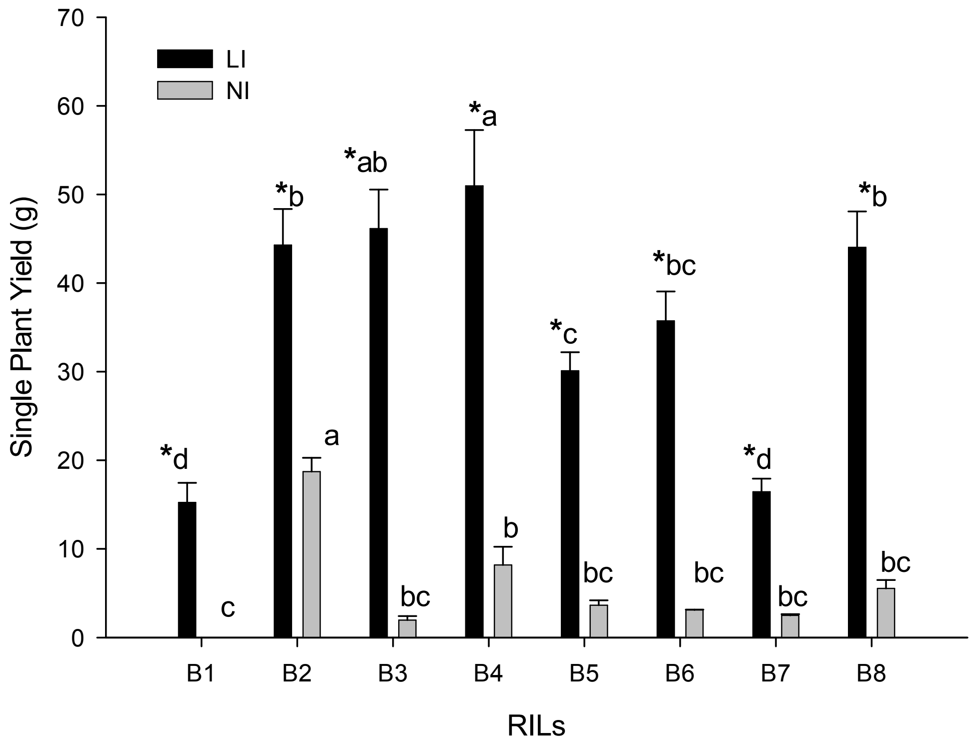

Fig. 3. Effects of an interaction between irrigation treatment and sorghum line on single plant yield (SPY). Within irrigation treatment, bars followed by a common letter are not significantly different (P>0.05). "*" denotes a significant difference for each line between irrigation treatments. Bars indicate 1 standard error

A regression analysis (Table 8c) to assess the contribution of each measured trait to grain yield (SPY), revealed HI (0.753), SPB (0.166), FGP (0.010), and HGT (0.006) as significant predictors for grain yield under limited irrigation treatment. Also, HI (0.910), FGP (0.050), and (HGT, 0.018) were significant predictors for grain yield under no irrigation treatment.

Table 8a. Pearson phenotypic correlations of assessed parameters under limited (LI) and no irrigation (NI, numbers in italics) treatments

	FGP	CLC	LAI	HGT	SPB	SPY	HI
FGP	-	-	_0.39**_	_0.24*_	-	-	_0.24*_
CLC	-	-	-	_-0.25*_	-	-	-
LAI	0.58**	-	-	_0.41*_	-	_0.33*_	_0.34*_
HGT	-	0.30**	-	-	_0.31*_	_0.39**_	_0.32**_
SPB	-	-	-	0.24*	-	_0.83**_	_0.82**_
SPY	-	-	-	0.25*	0.81**	-	_0.96**_
HI	-	-0.25*	-	-	0.30**	0.57**	-

* and ** indicates significant correlations at P=0.05 and P=0.01, respectively

Table 8b. Pearson phenotypic correlations among assessed morpho-physiological traits at various phenological phases, under limited and no irrigation treatments

	I		II		III		IV	
	LI	**NI**	**LI**	**NI**	**LI**	**NI**	**LI**	**NI**
FGP-LAI			0.40*	0.36*	079**	0.42*		
CLC-HGT	0.41*						-0.59**	
CLC-LAI					-0.42*			
HGT-LAI				0.40*		0.52**		
HGT-SPB			0.41*	0.46*	0.50*	0.49*	0.55*	
HGT-SPY				0.59**	0.54**	061**	0.69**	
HGT-HI			0.42*	0.49*	0.59**	0.48*	0.57**	
LAI-SPY						0.46*		

** and ** indicates significant correlations at P=0.05 and P=0.01, respectively*

Table 8c. Linear regression analysis of assessed morpho-physiological traits as predictors to grain yield under limited and no irrigation treatment

		LAI	**CLC**	**FGP**	**HGT**	**SPB**	**HI**
	R Squared			0.011	0.008	0.164	0.758
	Adj. R squared			0.010*	0.006*	0.166*	0.753*
LI	Standard error			4.182	4.499	4.674	8.163
	# observations			43	44	45	46
	Coefficients			0.112	-0.141	0.224	136.776
Constant estimate = -29.473							
	R Squared			0.060	0.019		0.912
	Adj. R squared			0.050*	0.018*		0.910*
NI	Standard error			1.692	1.759		1.961
	# observations			44	45		46
	Coefficients			-0.035	0.061		78.994
Constant estimate = -3.874							

** indicates significant correlations at P=0.05*

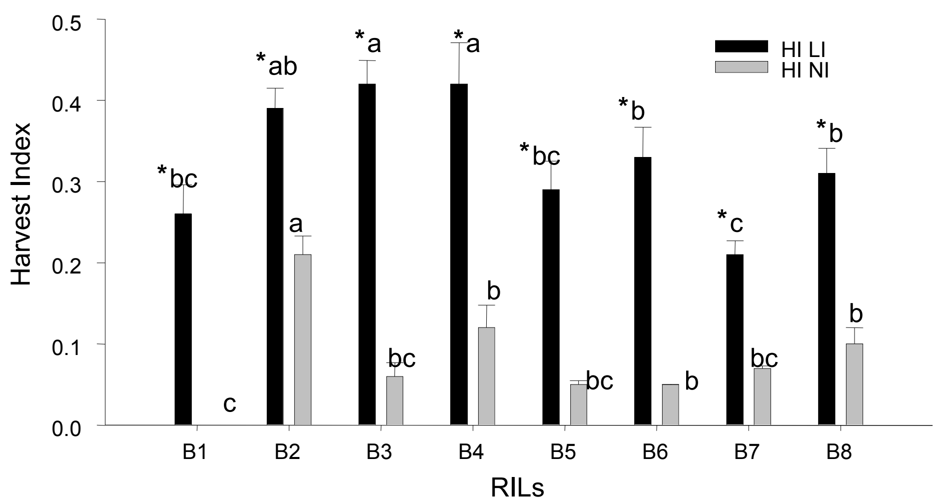

Fig. 4. Effects of an interaction between irrigation treatment and sorghum line on harvest index (HI). Within irrigation treatment, bars followed by a common letter are not significantly different (P=0.05). "*" denotes a significant difference for each line between irrigation treatments. Bars indicate 1 standard error

3.2 Discussion

Numerous physiological and molecular changes occur during cold tolerance, revealing that cold tolerance like drought tolerance is complex, and involves more than just one pathway and cell compartment. Also, like drought stress, there is a wide range of cold stress in temperate areas differing in timing, intensity, and duration [21]. Effects on grain yield are more severe when cold and drought stress occur during the reproductive growth stage. Cold and drought tolerance involves increased chlorophyll accumulation, reduced sensitivity to carbon exchange rates, improved germination, pollen fertility, and seed set under unfavorable growth condition. Imbibition, germination, emergence, and seedling growth have been shown to be independent from each other in their sensitivities to cold temperatures [10]. The germination and seedling establishment phase of sorghum growth is especially sensitive to cold temperatures; with poor germination and seedling loss resulting to reduced plant population and grain yield.

The results presented here suggest that cold tolerance involves improved germination under suboptimal temperatures in the early growing season. The sorghum lines differed in their germination under cold temperatures, with FGP ranging from 30 to 80%. Genetic variation in cold tolerance at germination and seedling stage in sorghum has been documented [22]. The mean cold germination of 71% in this study is similar to 73% reported by Burow *et al.* [23] for similar sorghum lines. FGP was a significant predictor for grain yield under both irrigation treatments, suggesting that grain sorghum lines with high early-season cold temperature germination will have better grain yield under pre-flowering drought stress, than those with low early-season cold temperature germination.

Grain sorghum growth and development is divided into three main stages: 1) from planting to panicle initiation, 2) panicle initiation to flowering, and 3) flowering to physiological maturity. Studies have indicated that drought at any of these stages can result in a substantial yield loss [24,25], affecting both morpho-physiological traits (earliness, leaf area, leaf rolling, wax content, root system, photosynthetic capacity, growth and development, and water use efficiency) and biochemical traits (proline, polyamine, and trehalose content, and nitrate reductase activity) [26]. Likewise, yield losses are most severe when cold stress occurs during the reproductive stage [27]. Lines B1 and B2 ceased growth following pre-flowering drought stress, a mechanism also observed when sorghum is subjected to cold stress at seedling and flowering stages [28]. Results by Sankarapandian et al. [29] on 25 F4/F5 derivatives of elite and drought tolerant sorghum lines showed that average plant height was reduced by 11% when water stress was imposed at the panicle initiation to flowering stage. The current study showed average plant height reductions of 13 and 14% from booting to heading and heading to flowering respectively caused by pre-flowering drought stress. Tuinstra et al. [24] reported that drought at this growth stage delayed flowering, caused poor panicle emergence, floret abortion, and panicle blasting, resulting in poor yields. Similar responses were observed in our study for line B1 which did not produce grain as a result of pre-flowering drought stress.

This study showed harvest index as the most predictive and constitutive trait for grain yield following pre-flowering drought stress in early-season cold tolerant sorghum breeding lines. Harvest index explained 75%, and 91% variability in grain yield for limited and no irrigation treatments respectively. Harvest index was positively correlated with early-season cold germination, leaf area index, plant height, and plant biomass, suggesting harvest index to be a reliable trait in selecting sorghum materials for early-season cold germination and pre-flowering drought tolerance in our study. In wheat (*Triticum aestivum)*, most of the increase in grain yield has been achieved through increasing harvest index. Genetic improvement in harvest index is highly researched on although, in intensively bred crops, the upper limit has been approached [30]. The increase in harvest index may come from a reduction in the metabolic investment for production of leaves and other vegetative structures. This might compromise total biomass and thus require an increased rate or duration of photosynthesis to compensate for the reduced photosynthetic area. In maize, harvest index is considered to be already at its maximum and any further increase may be counter-productive as it may reduce plant biomass. Therefore other traits that will increase plant biomass and thereby crop photosynthesis such as chlorophyll content and leaf area index may be of interest to yield increase and sustainability under abiotic stress.

Chlorophyll concentration, delayed leaf senescence, and the stay-green trait are all interconnected [18]. The stay-green trait has been related to maintenance of a more favorable water status as related to deeper roots [31] and closely correlated with the rate of carbon exchange [32] and improved yields and transpiration under limited water availability in sorghum, maize, and wheat [33]. Borrell et al. [34] also showed that the rate of leaf senescence in sorghum (a measure of the stay-green trait) was negatively correlated with grain yield under conditions of late (pre-and post-flowering) water deficit. No such relationship was observed in our study, though chlorophyll content was the only trait impacted by an interaction between irrigation level and phenological phase (Table 3). The negative correlations between chlorophyll content and harvest index (Table 8a), and chlorophyll content and plant height at the flowering to anthesis phase (Table 8b) under limited irrigation treatment, suggest that chlorophyll content may be more useful in assessing post-flowering drought tolerance in cold tolerant sorghum lines. Numerous researches have accredited the stay-green trait (measured by chlorophyll content) as a beneficial trait for grain sorghum yields under post-flowering drought stress. Chlorophyll content in our study ranged from 37.32 to 56.90µmolm^{-2}, similar to values (30.4 to 50.1µmolm^{-2}) reported at comparable growth stages by Sankarapandian et al. [29]. Guo et al. [35] also concluded that chlorophyll content in drought tolerant barley genotypes was significantly higher than in drought sensitive genotypes under drought stress.

Drought stress may reduce canopy apparent photosynthesis and thus yields by either reducing photosynthetic activity per unit leaf area or by reducing the amount of leaf area available for photosynthesis (i.e. reducing the leaf area index). Total photosynthesis has increases as a result of an increase in leaf area and daily duration of photosynthesis or leaf area duration [30]. Good correlations exist for crop yield and leaf area index particularly during the post-flowering stage (anthesis to grain filling) where higher photosynthetic capacity is needed to provide assimilates for the more demanding seed sinks. Due to low seeding rate (2-3 seeds/foot of row), and broader row spacing (1.0m) in this study, observed LAI (1.87-2.07) deviates from values (2-3.5) for seeding rates (3-4 seeds/foot of row) in sorghum planted under optimal irrigation. Leaf area index showed positive correlations to cold germination under both irrigation treatments (Table 8a and 8b) and similar correlation to single plant grain yield under no irrigation treatment in the heading to flowering phenological phase (Tables 8a and 8b respectively). Thus, high leaf area index can be used as a constitutive trait to predict early-season cold tolerance, and as an adaptive trait to predict pre-flowering drought stress tolerance in cold tolerant grain sorghum lines. This prediction agrees with results by Gupta et al. [36] who showed 2 out of 22 triticale (*Triticosecale*) x bread wheat derivatives to be both drought-and cold- tolerant, with high leaf area index, grain yield, spike per plants and spikelet per spike.

The ability of grain sorghum lines to cease growth (lines B1 and B2), maintain LAI (lines B1, B2, B4 and B6), and retain high CLC (lines B4 and B8) and high HI (line B2), will be important selection traits to develop sorghum cultivars for temperate and highland regions, with possibility of pre-flowering drought occurrence.

4. CONCLUSION

This study identified harvest index as being the most significant amongst the assessed traits in relating early-season cold germination to pre-flowering drought tolerance. Harvest index was also the most significant predictor of grain yield for sorghum lines subjected to pre-flowering drought stress preceding early-season cold temperature germination. High leaf area index can be used to select for pre-flowering drought tolerance in early-season cold tolerant sorghum lines. Chlorophyll content and plant height are also plausible traits to select for early-season cold germination and pre-flowering drought tolerance in sorghum inbred line. This study identified the heading to flowering phenological phase as the best pre-flowering phenological phase of the reproductive growth stage, to relate early-season cold tolerance to pre-flowering drought tolerance. There is an array of morpho-physiological traits that could be selected for both early-season cold germination and pre-flowering drought tolerance in grain sorghum. It is worth mentioning that such morpho-physiological traits should be constitutive (i.e. also expressed under optimum condition) and stress-responsive (i.e. expressed only under pronounced stress situations). In this respect, emphasis should be given to traits that constitutively enhanced yield *per se* and also enhanced plant survival under cold or drought conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mittler R, Blumwald E. Genetic engineering for modern agriculture: Challenges and perspectives. Annu. Rev. Plant Bio. 2010;61:443-462.
2. Shulaev V, Cortes D, Miller G, Mittler R. Metabolomics for plant stress response. Physiol. Plant. 2008;132:199-208.
3. Quinby JR, Hesketh JD, Voigt RL. Influence of temperature and photoperiod on floral initiation and leaf number in sorghum. Crop Sci. 1973;13:243-246.
4. Singh SP. Use of genetic male sterility for simultaneous conversion and improvement of alien sorghums. Crop Sci. 1977;17:482-484.
5. Emendack YY, Herzog H, Goetz K-P, Malinowski DP. Mid-season water stress on yield and water use of millet (*Panicum miliaceum*) and sorghum (*sorghum bicolor* L. Moench). Aust. J. of Crop Sci. 2011;5(11):1486-1492.
6. Adams, JE. Effect of soil temperature on grain sorghum growth and yield. Agron. J. 1962;54:257-261.
7. Brar GS, Stewart BA. Germination under controlled temperature and field emergence of 13 sorghum cultivars. Crop Sci. 1994;34:1336-1340.
8. Payne WA, Balota M, Rosenow DT. Sorghum diversity for germination and elongation under cool conditions. Int. Sorghum Millet Newsl. 2003;44:76-83.
9. Frank CD, Burow GB, Burke JJ. A comparison of U.S. and Chinese sorghum germplasm for early season cold tolerance. Crop Sci. 2006;46:1371-1376.

10. Soujeole AA, Miller FR. Cold tolerance of sorghum during early development stages. Proc. Ann. Corn Sorghum Res. Conf. 1984;39:18-32.
11. Reynold M, Dreccer F, Trethowan R. Drought-adaptive traits derived from wheat wild relatives and landraces. J. Exp. Bot. 2007;58:177-186.
12. Emendack YY. Drought performance of Millet (*Panicum miliaceum*) and Sorghum (*Sorghum bicolor* (L.) Moench); 2007. Ph. D. Dissertation: dissertation.de. ISBN 978-3-86624-231-9.
13. Rebetzke, GJ, Ellis M, Bonnett DG, Richards RA. Molecular mapping of genes for coleoptile growth in bread wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 2007;114:1173-1183.
14. Blum A. Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? Aust. J. Agric. Res. 2005;56:1159-1168.
15. Blum A. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Res. 2009;112:119-123.
16. Tuberosa R, Salvi S, Giuliani S, Sanguinetti MC, Frascaroli E, Contiet S, et al. Genomics of root architecture and functions in maize, in *Root Genomics* eds A. Costa de Oliveira and R. K. Varshney (Dordrecht, The Netherlands: Springer). 2011b;179-204.
17. Monneveux P, Ribaut J-M. Secondary traits for drought tolerance improvement in cereals, in Drought Adaptation in Cereals*,* ed J. -M. Ribaut (Binghamton, NY: The Haworth Press, Inc). 2006;97-143.
18. Shukla AK, Ladha JK, Singh VK, Dwivedi BS, Balasubramanian V, Gupta RK, et al. Calibrating the leaf color chart for nitrogen management in different genotype of rice and wheat in systems perspective. Agron. J. 2004;96:1606-1621.
19. Burke JJ, Chen J, Burow GB, Mechref Y, Rosenow DT, Payton P et al. Leaf Dhurrin content is a quantitative measure of the level of pre-and post flowering drought tolerance in sorghum. Crop Sci. 2013;53(3):1056-1065.
20. Balota M, Payne WA, Veeragoni SK, Stewart BA, Rosenow DT. Respiration and its relationship to germination, emergence, and early growth under cool temperatures in sorghum. Crop Sci. 2010;50:1414-1422.
21. Singh SP. Sources of cold tolerance in grain sorghum. Can. J. Plant Sci. 1985;65:521-257.
22. Tiryaki I, Andrews DJ. Germination and seedling cold tolerance in sorghum: I. Evaluation of rapid screening methods. Agron. J. 2001;93:1386-1391.
23. Burow GB, Burke JJ, Xin Z, Franks CD. Genetic dissection of early season cold tolerance in sorghum (*sorghum bicolor* (L.) Moench).Mol. Breeding. 2011;38:391-402.
24. Tuinstra MR, Grote EM, Goldsbrough PB, Ejeta G. Genetic analysis of post-flowering drought tolerance and components of grain development in *Sorghum bicolor* (L.) Moench.Mol. Breeding. 1997;3:439-448.
25. Hattendorf MJ, Dedelfs MS, Amos B, Stone LR, Given RE. Comparative water use characteristics of six row crops. Agron. J.1988;80:80-85.
26. Mitra J. Genetics and genetic improvement of drought resistance in crop plants. Current Sci. 2001;80:758-763.
27. Shangera GS, Wani SH, Hussain W, Singh NB. Engineering cold tolerance in crop plants. Current Genomics. 2011;12:30-43.
28. Maulana F, Tesso T. Cold temperature episode at seedling and flowering stages reduces growth and yield components in sorghum. Crop Sci. 2013;53:564-574.
29. Sankarapandian P, Audilakshmi S, Sharma V, Ganesamurthy K, Talwar HS, Patil JV. Effect of morpho-physiological traits on grain yield of sorghum grown under stress at different growth stages, and stability analysis. J. Agric. Sci. 2013;151:630-647.

30. Richards RA. Selectable traits to increase crop photosynthesis and yield of grain crops. J. Exp. Bot. 2000;51:447-458.
31. Mace ES, Singh V, Van Oosterom EJ, Hammer GL, Hunt CH, Jordan DR. QTL for nodal root angle in sorghum (*Sorghum bicolor* L. Moench) co-locate with QTL for traits associated with drought adaptation. Theor. Appl. Genet. 2012;124:97-109.
32. Fracheboud Y, Jompuk C, Ribaut J-M, Stamp P, Leipner J. Genetic analysis of cold tolerance of photosynthesis in Maize. Plant Mol. Biol. 2004;56:241-253.
33. Verma V, Foulkes MJ, Worland AJ, Sylvester-Bradley R, Caligari PDS, Snape JW. Mapping quantitative trait loci for flag leaf senescence as a yield determinant in winter wheat under optimal and drought-stressed environments. Euphytica. 2004;135:255-263.
34. Borrell AK, Hammer GL, Henzell RG. Does maintaining green leaf area in sorghum improve yield under drought? II. Dry matter production and yield. Crop Sci. 2000;40:1037-1048.
35. Guo Y-P, Zhou H–F, Zhang L-C. Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species. Sci. Hort. 2006;108:260-267.
36. Gupta D, Mittal RK, Kant A, Singh M. Association studies for agro-physiological and quality traits of triticale X bread wheat derivatives in relation to drought and cold stress. J. Environ. Sci. 2007;28(2):265-269.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=586&id=2&aid=5202