

Seed germination and seedling growth of three sorghum (*Sorghum bicolor* L.) genotypes as affected by low temperatures

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ABSTRACT: Sorghum (*Sorghum bicolor* L.) originated in the semi-arid tropics and generally is sensitive to low-temperature stress. Chilling stresses include low temperature (below 20 °C) and freezing injury (below 0 °C). In this study seed germination and seedling growth of three sorghum genotypes (KFS1, KFS2 and Speedfeed) were examined under four temperature regimes (35/15, 20/10, 13/10 and 11/8) conditions. The results showed that low temperature reduced germination percentage, rate of 50% germination, vigour index, shoot and root length, as well as, shoot and root dry weight; whereas it increased emergence index and root to shoot length ratio (R/S). So that, the seeds of sorghum genotypes (on average) was germinated 10.5 days later at 11/8 than 25/22 temperature regimes. Increased R/S in low temperatures might be an indication of water deficit stress due to cold stress. Since in most traits, there were no significant difference between 25/22 and 15/10, thus the lower temperature regimes below 15/10 could considered as cold stress. The sorghum genotypes responded differently to low temperatures, which it seems that KFS2 and Speedfeed were the most tolerant and sensitive genotypes to low temperature. Further researches about biochemical responses of these sorghum genotypes to low temperature regimes are needed.

Keywords: cold stress, emergence index, vigour index

Abbreviations: GP: Germination percentage, EI: Emergence Index, RL: Radicle length, SL: Shoot length, R/S: Root to shoot length ratio, RDW: Root dry weight, SDW: Shoot dry weight, G50%: Rate of 50% germination, VI: Vigour index

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is a warm-season cereal widely grown in semiarid, sub-humid, and humid tropical and subtropical regions of the world, where minimum mean temperatures during the growing season generally stay above 18 °C (Singh, 1985). However, a substantial amount of sorghum has been grown for centuries in other cooler highland areas. Sorghum suffers chilling injury, where it was subjected to non-freezing temperatures below 10–15 °C (Peacock, 1982).

Stresses at any stage of crop growth can cause an irreversible loss in yield potential (Pirasteh-Anosheh et al., 2011; Hamidi and Pirasteh-Anosheh, 2013). Low-temperature stress at planting time usually results in poor seedling establishment of sorghum because of slow emergence rate, reduced emergence percentage, and reduced growth rate after emergence (Pinthus and Rosenblum, 1961; Singh, 1985). Rapid and uniform field emergence is an essential prerequisite to reach the yield potential, quality, and ultimately profit in annual crops (Parera and Cantliffe, 1994; Pirasteh-Anosheh and Hamidi, 2013). Greater and better synchronized germination is

crucial for achieving an optimal seedling establishment and better productivity; however, environmental constraints such as freezing and low temperature are great impediments (Wahid et al., 2008; Hamidi et al., 2013).

At one century ago, (Shreve, 1913) indicated that the investigation of the control of plant distribution by the various phases of the temperature factor is one of the most important tasks of physiological plant geography, at the same time that it is one of the most backward and difficult. (Singh, 1985) indicated that "cold tolerance" refers to the ability of sorghum genotypes to germinate, grow and produce satisfactory grain yields under conditions of relatively cold (but above-freezing) air and soil temperatures. In his study it was shown that of 380 excessively tall, photoperiod-sensitive, and late-maturing accessions obtained from China, Ethiopia, and Uganda, 39 were cold-tolerant, 757 partially tolerant, and the remainder were susceptible when rated at physiological maturity, and concluded the cold tolerance is differed between genotypes and also appeared to be a dominant trait. (Yu et al., 2004) reported that significant variation of cold tolerance existed in commercial sorghum hybrid seed lots studied under controlled low temperature. Cold-tolerance traits measured under controlled low-temperature conditions were correlated significantly, indicating that simultaneous improvement of these components could be captured in new hybrid cultivars. Significant correlations among traits measured in laboratory and field assays for cold tolerance indicated that a soil-based laboratory assay could be used as an alternative or preliminary screening method for field evaluation.

Early planting, use of minimum tillage, and attempts to expand sorghum into higher elevations and more temperate latitudes necessitate the identification of genotypes that are tolerant to low temperatures during seed germination, seedling emergence, and early plant growth (Singh, 1985; Bacon et al., 1986). In maize (*Zea mays* L.), much effort has been devoted to improving seedling cold tolerance and numerous studies have been reported. Progress made in cold tolerance research partially explained a broader range of adaptation for maize (Yu et al., 2004). Since such parallel improvement of cold tolerance in this crop is needed, and there is low information in this field, thus current study was conducted to investigation the influence of low temperatures on seed germination and seedling growth of sorghum genotypes.

MATERIALS AND METHODS

This study was conducted as a laboratory factorial experiment based on completely randomized design with four replicates in Shiraz University during 2013. In this study, influence of four temperature regimes (25/20 as control, 15/10, 13/10 and 11/8 °C) on seed germination and seedling growth of three sorghum (Speedfeed, KFS1 and KFS2) genotypes was examined under 13/11 hours day/night length cycle. These temperature regimes were selected based on finding of (Yu et al., 2004).

were surface sterilized with 5% NaOCl (sodium hypochlorite) for 5 min to avoid fungal invasion, followed by washing with distilled water (Hamidi et al., 2013). One hundred seeds ((in four petri dishes as a replicate) were sown in 9 cm petri dishes on two layers of filter paper (Whatman No.1), then the petri dishes were placed in growth chamber for inducing temperature regimes.

Seed germination was recorded daily up to 8 and 15 days after sowing for 25/20 and other regimes, respectively; when no seed germinated. A seed was considered germinated when radical emerged by about 2 mm in length (Pirasteh-Anosheh et al., 2011). In each recording, ten seedlings were randomly selected from each petri dish, and their averages were considered as sample data. The measured traits included germination percentage equation 1 (Pirasteh-Anosheh and Hamidi, 2013), emergence index equation 2 (Smith and Millet, 1964), radicle and shoot length, radicle to shoot length ratio, radicle and shoot weight, rate of 50% germination equation 3 (Soman and Peacock, 1985) and vigour index equation 4 (Dhindwal et al., 1991).

$$\text{Equation 1: } GP = \frac{n}{N}$$

In this equation, GP is Germination percentage, n is number of seeds germinated, and N is total number of seeds planted.

$$\text{Equation 2: } EI = \frac{\sum(E_j \times D_j)}{E}$$

where EI and E_j are emergence index and the emergence on day j, respectively; D_j the days after planting, and E the final stand.

$$\text{Equation 3: } R_{50} = 1/D_{50}$$

$$\text{Equation 4: VI} = (\text{RL} + \text{SL}) \times \text{GP}$$

In these two equations R50 and D50 is rate of 50% germination and the time to 50% of seeds germinated, respectively; VI, RL, SL, and Ger% are vigour index, radicle length, shoot length and germination percentage, respectively.

Statistical analysis was performed for each measured traits using SAS v. 9.1 software. Means were compared by LSD Test (Least Significant Difference) at $P < 0.05$.

RESULTS AND DISCUSSION

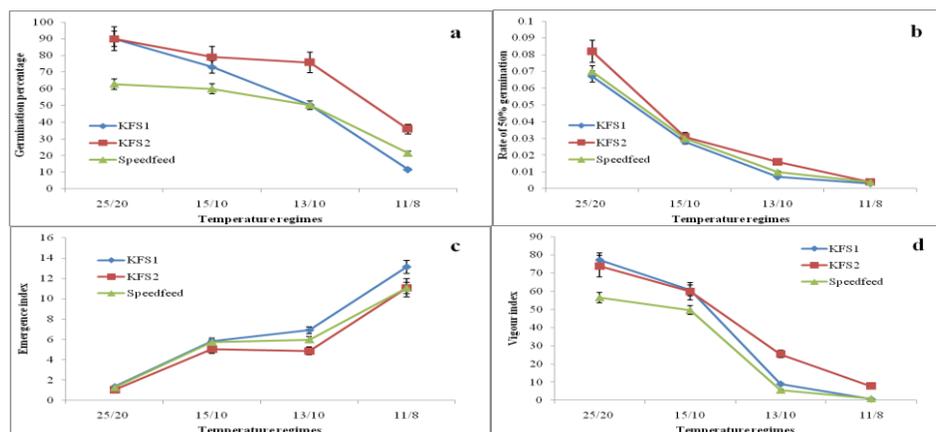
Temperature, genotype and their interaction had significant effect on germination percentage (GP) at 1% probability level, respectively (Table 1). Low temperature decreased GP in all three sorghum genotypes; however, response of these genotypes was different. Change in temperature regime from 25/22 to 11/8 caused 87.1, 60.2 and 65.9% reduction in GP of KFS1, KFS2 and Speedfeed, respectively. In all conditions, the highest GP was observed in KFS2 genotype (Fig. 1a). (Adams, 1970) reported that soil with low temperature in few weeks after sowing reduces seed germination, emergence rate and seedling establishment.

Table 1. Variance analysis for low temperature and cultivar effect on seed germination and seedling growth of sorghum

Source Of Variance	Degree of Freedom	Mean Squares								
		GP	EI	G50%	VI	RL	SL	R/S	RDW	SDW
Temperature (T)	3	7382.47**	94.07**	11.61**	13602.8**	4753.93**	5083.96**	18.02**	10.73**	13.77**
Cultivar (C)	2	3473.71**	6.88*	7.92**	1307.32*	498.14**	91.45**	3.27**	2.02*	3.47*
TxC	6	2718.89**	1.26**	0.489*	917.6*	51.39*	11.35*	0.89ns	2.132*	2.70*
Error	36	48.23	0.16	0.02	129.22	12.53	2.34	0.86	0.046	0.89
CV (%)		11.75	6.71	4.74	11.83	13.90	7.32	18.10	16.72	14.02

GP: Germination percentage, EI: Emergence Index, RL: Radicle length, SL: Shoot length, R/S: Root to shoot length ratio, RDW: Root dry weight, SDW: Shoot dry weight, G50%: Rate of 50% germination, VI: Vigour index
ns: non-significant; * and ** significant at 5 and 1% probability level, respectively

Rate of 50% germination (G50%) was affected by temperature, genotype and their interaction at 1, 1 and 5% probability level, respectively (Table 1). Low temperature significantly decreased G50% in all three genotypes. There was significant difference in response of genotypes to low temperature; however, difference of G50% of three genotypes was no significant under 11/8 temperature regimes. Reduction in G50% due to the lowest temperature regimes was 95.2, 95.1 and 94.2% in KFS1, KFS2 and Speedfeed, respectively. KFS2 had the highest G50% in all temperature regimes, which had no significant difference with other genotypes under 11/8 treatment (Fig. 1b). It has reported that growth activities of all studied sorghum genotypes were inhibited under temperatures below 10 °C (Anda and Pinter, 1994).



Figurer 1. Influence of different temperature regimes on germination percentage (a), rate of 50% germination (b), emergence index (c) and vigour index (d) of three sorghum genotypes

The effects of temperature, genotype and their interaction were significant on emergence index (EI) and vigour index (VI) at 1, 5 and 1% probability level, respectively (Table 1). Decrease in temperature was associated with increase in EI for all three genotypes. There were significant differences between the genotypes in two last temperature regimes. Reduce temperature regimes to 11/8 led to 8.6, 9.5 and 7.6 time increasing in EI of KFS1, KFS2 and Speedfeed, respectively. The lowest EI was found in KFS2 under all temperature conditions (Fig. 1c). Although low temperatures decreased VI in all three sorghum genotypes; however, these genotypes responded differently to low temperature regimes. There were no difference between VI of KFS1 and KFS2 in control and first temperature regimes, while KFS2 had the higher VI at two last temperature regimes (Fig. 1d). Reduction in VI of KFS1, KFS2 and Speedfeed at 11/8 than 25/22 temperature regimes was 99.0, 89.4 and 98.1%, respectively.

(Soman and Peacock, 1985) reported that sorghum emergence was significantly affected by interaction of low temperatures and genotypes. Seeds of cold-tolerant genotypes tend to emerge faster than susceptible ones and have higher final stands, rapid emergence also would allow plants to initiate seedling growth earlier and accumulate more dry matter (Yu et al., 2004).

As shown in Table 1; temperature ($P<0.01$), genotype ($P<0.01$) and their interaction ($P<0.05$) had significant effect on shoot (SL) and radicle length (RL). The lower temperatures than 15/10 decreased SL and RL in all three genotypes; however, responses of genotypes were different; so that there were no significant differences between 25/22 and 15/10 temperature regimes in terms of SL and RL for all sorghum genotypes (Fig. 2a and 2b). Speedfeed genotype had the highest SL in 25/22 and 15/10 temperature regimes; while experienced considerable reduction in the lower temperatures and had the lowest SL in 13/10 and 11/8 treatments. The reductions due to change temperature regime from 25/22 to 11/8 were 97.3, 82.1 and 97.7%, respectively (Fig. 2a). In all temperature regimes, Speedfeed had the lowest RL, while the highest RL was found in KFS1 at 15/11 and in KFS2 in 13/10 and 11/8 temperature regimes. Radicle length was lower in 11/8 than 25/20 by 88.9, 66.6, and 90.6% in KFS1, KFS2 and Speedfeed, respectively (Fig. 2b). Our results agree with finding of (Yu et al., 2004), who indicated that seedling height, and shoot dry weights decreased, whereas emergence index increased as temperature decreased.

Radicle to shoot length ratio (R/S) was affected significantly by temperature and genotype at 1% probability level (Table 1). Although there was no significant difference between R/S of sorghum at 25/22 with 15/10; however the lower temperatures caused significant increasing in R/S (Fig. 3). These increasing amounts were 1.4 and 2.3 times at 13/10 and 11/8 compared to 25/22, respectively. Comparison of main effects also showed that R/S was not significantly differed between KFS2 and Speedfeed; whereas R/S in these two genotypes significantly was lower than KFS1 (Fig. 3). It has been known that the sensitive plant to low temperatures show water deficit (Aroca et al., 2001). Enhanced root to shoot ratio is a sign of water deficit to water capture increasing (Michele et al., 2009).

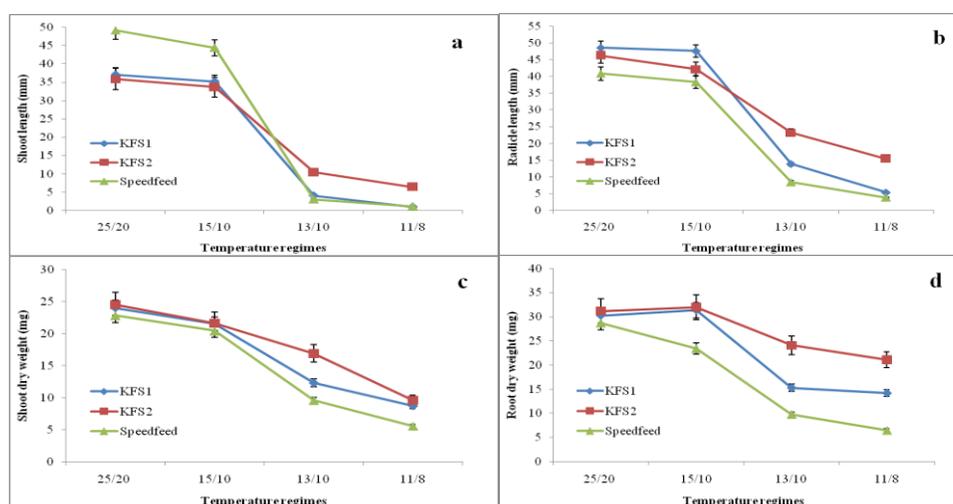
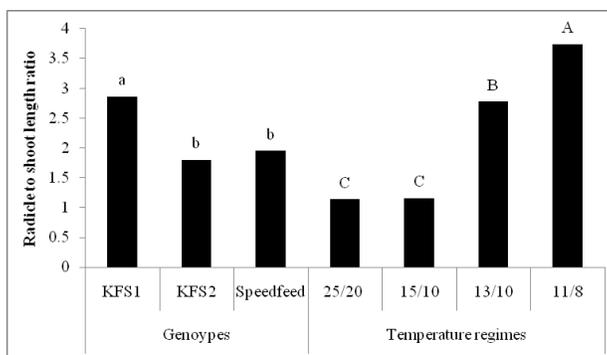


Figure 2. Influence of different temperature regimes on shoot length (a), root length (b), shoot dry weight (c) and root dry weight (d) of three sorghum genotypes (\pm SE)



Figuer 3. Changes of root to shoot length ratio between three sorghum genotypes and four temperature regimes. Column with similar letter are not significantly differed (LSD<0.05)

Analysis of variance (Table 1) showed that the effect of temperature ($P<0.01$), genotype ($P<0.05$) and their interaction ($P<0.05$) was significant on shoot (SDW) and radicle dry weight (RDW). Although low temperatures reduced both SDW and RDW; however, sensitivity of SDW was more than RDW (Fig. 2c and 2d). There was no significant difference between SDW of KFS1 and Speedfeed in all temperature conditions; whereas KFS2 had the higher SDW and it seems to be a less sensitivity genotype to low temperature (Fig 2c). There were no significant difference also between RDW of all three genotypes in 25/22 condition and the highest differences between genotypes was found in 11/8 condition (Fig 2d). Reductions in SDW and RDW due to the lowest temperature were 63.7 and 52.9% for KFS1, 60.8 and 32.3 for KFS2; and 75.5, 77.4 for Speedfeed, respectively. Emergence and seedling growth in sorghum under low-temperature stress were suggested to be under different genetic control (Soujeole, 1985; Steffen et al., 1989) also suggested that different cultivars could be used for achieve the better seedling growth and emergence. Differences in emergence of genotypes could interact with seedling growth rate, thus may partly explain the differences in cumulative dry matter measured at a certain time after planting, particularly plant dry weight (Yu et al., 2004).

CONCLUSION

Overall, low temperature reduced GP, G50%, VI, SL, RL, SWD and RDW; whereas EI and R/S were enhanced under these conditions. Based on our results temperature below 15 °C could be considered as cold stress, which caused considerable reduction in seed germination and seedling growth. On average, cold stress delayed sorghum seed germination by 10.5 days. Regarding differences in genotypes response to low temperature regimes, KFS2 and Speedfeed could be considered as the most tolerant and sensitive genotypes to low temperature. The sorghum genotypes with high cold stress resistance can be introduced for sowing in regions with higher altitude and/or unpredictable freezing, as well as, for breeding programs.

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