J. Agr. Sci. Tech. (2013) Vol. 15: 723-736

The Sensitivity of Grain Sorghum (*Sorghum bicolor* L.) Developmental Stages to Salinity Stress: An Integrated Approach

M. Kafi[1*], M. H. Shariat Jafari[1], and A. Moayedi[2]

ABSTRACT

To understand the effects of salinity stress on four different growth stages of sorghum, a greenhouse experiment with 10 treatments, considering all possible combinations of salinity stress and salt- free periods was carried out. The four growth stages for stress application included: emergence until growing point differentiation, growing point differentiation until half bloom, half bloom until soft dough, and soft dough until physiological maturity. Treatments were arranged based on randomized complete block design with 3 replications at the Research Greenhouse of the Ferdowsi University of Mashhad in 2010-2011. Salinity stress during early growth and panicle differentiation declined the plant height and tiller number. The highest biological yield was obtained from the control treatment, but it was the lowest when plants were salinized throughout the growing season. When plants were stress-free at 2-3 early stages and then subjected to salt stress, reductions in total dry matter were remarkably less than those experienced when salinity was imposed in later growth stages, especially if salinity occurred at a late individual stage. Continuation of salt stress from emergence to both blooming and soft dough stages led to remarkably adverse effects on grain yield. The effect of salinity appears to be most effective on yield components that are growing or developing at the time the salt stress is imposed. The critical period of salinity stress for biological yield was more distinct than that of the grain yield. This indicates that sorghum is not sensitive to salinity at seed setting and seed filling periods.

Keywords: Critical period of salinity stress control, Growth stage, Salt stress, Sorghum.

INTRODUCTION

Typical crops require about 6,200 to 9,300 m^3 water ha^{-1} annually. Since irrigation water contains 0.5 to 3.5 mt of salt per 1,000 m^3, 0.3 to 32 mt of salt may be added per hectare of irrigated soil (Wilson *et al.,* 2000). Alterations of various physiological and biochemical processes of plants of different growth stages in the presence of salinity have been documented, however, some studies have shown no variation during the life stages (Francois *et al.,* 1994; Houle *et al.,* 2001; Yan *et al*., 2011). In many plants, seedling growth is more sensitive to salinity than seed germination (Maas *et al.* 1986) but these findings are not supported by other researchers (Houle *et al.,* 2001; Casper *et al.,* 2006).

Yield components are subject to the environmental conditions that prevail during their initiation and growth, hence, analysis of each component could provide a post-harvest assessment of when the environmental stress occurred and how severe it was (Desclaux *et al.,* 2000). Ion absorption, which may lead to ion toxicity or imbalance, is also affected by time of salt stress (Shariat Jafari *et al.,* 2009). Jogeswar *et al.* (2006) subjected seedlings of sorghum varieties to 150 mM NaCl stress for 72 hours and reported that plants failed to exhibit efficient ion exclusion mechanisms

[1] Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Islamic Republic of Iran.
[*] Corresponding author, e-mail: mkafi@um.ac.ir
[2] Khorasan Razavi Agricultural and Natural Resource Research Center, Mashhad, Islamic Republic of Iran.

[DOR: 20.1001.1.16807073.2013.15.4.16.9]

like that of salt tolerant species, but in turn resulted in higher accumulation of Na and Cl ions over a period of 72 hours salt stress. In addition, accumulation of calcium, potassium and proline in seedlings of sorghum varieties was moderate in short-term NaCl stress. But Sabir *et al.* (2011) reported that accumulation of proline and antioxidant enzyme activities were not found to be effective criteria for discriminating the *Panicum miliaceum* accessions for salt tolerance. Mahmood *et al.* (2010) investigated the possible involvement of exogenous salicylic acid in salinity tolerance of sorghum and found that increasing salinity treatments reduced the fresh and dry mass of both root and shoot. Plants treated with salicylic acid showed no recovery from salt induced reduction in biomass production.

Sorghum is well adapted to water limitation and is a moderately salt tolerant crop which often grows in the areas of relatively low rainfall, high temperature and saline soils (Netonda *et al.,* 2004). This crop is more tolerant to salinity at germination than the later stages of growth. This study has been conducted to determine whether sorghum plants of different developmental stages respond differently to salinity stress, regarding growth or reproduction, and to find the most sensitive/tolerant growth period to salt stress. Using the results of this study assists us to identify CPSSC, through which the irrigation program cane be promoted and the integrated environmental stress management (IESM) for sorghum can be improved.

MATERIALS AND METHODS

Plant Growth

A greenhouse experiment was designed to avoid the other environmental stresses and provide a fast transition between stressful and stress- free periods. The plants of line M5 of sorghum (*Sorghum bicolor* L.) were grown in well washed sand in the 60×40×32 cm plastic boxes, in the Research Greenhouse of the Ferdowsi University of Mashhad, Iran, in spring and summer of 2010-2011. Sixteen seeds were planted in each box and were thinned to 8 per box keeping equal distance among the seedlings. Plants were irrigated using an automated dripping system in which the pumps in the drums supplied nutritional solution as well as salinity treatments for any group of 3 boxes (standing for 3 replications of each treatment), and the drainage of each group circulated through its corresponding drum. The sown seeds were irrigated by tap water till emergence, thereafter, Hoagland nutrient solution (Hoagland and Arnon, 1950) was supplied instead of water. The plants were watered daily during the experiment, and were irrigated 15-20 minutes any time to provide at least 50% leaching requirement, in order to avoid accumulation of salts in the root media. The evapotranspired water from the containers was replaced daily, by adding tap water to the desired level. The nutrient solutions were renewed every other week to maintain constant levels of nutrients and salt in the solution. The plants were supplied with natural illumination, average minimum and maximum temperature of 18 and 30°C, respectively and average relative humidity of 60%. The plants were harvested at complete maturation.

The plants were exposed to 150 m*M* NaCl (Jogeswar *et al.,* 2006) according to the following salinity stress treatments. To avoid osmotic shock of salinity, salt treatment was imposed incrementally, by increasing the concentration of 75 *mM* NaCl every second day. For quick transition from salinity to salt free conditions, the boxes were fully washed until the complete depletion of salt.

Treatments

To understand how tolerant any sorghum growth period is and which periods are the most critical to salinity, four major developmental periods of growth were selected (described by Vandrelip, 1993). These periods were: (i) from emergence until growing point differentiation, i.e. stages 1- 3; (ii) from growing point differentiation until half bloom, i.e. stages 3-6; (iii) from half bloom until soft dough, i.e. stages 6- 7; and (iv) from soft dough until physiological maturity, i.e. stages 7- 9. Considering the above- mentioned stages, and to find all possible combinations of stressful periods, we combined three sets of salt stress as following: (a) salinized set, in which the plants were exposed to salt stress from emergence until the growing point differentiation (G6), half bloom (G7), soft dough (G8), and physiological maturity stages (G1), respectively, and then after each stage, the stress was relieved; (b) salinity-free set, in which salinity was relieved until the growing point differentiation (G2), half bloom (G3), soft dough (G4), and physiological maturity stages (G5), respectively; and then after each stage, the plants were exposed to salinity stress; and (c) individual stages of salinity stress set, in which the plants experienced salt stress at growing point differentiation up to half bloom (G9), and at half bloom up to flowering (G10), and the plants were stress free before and after these growth stages. Combination of these sets resulted in 10 salt stress treatments (Figure 1).

Measurement of Plant Growth

Plant height and the number of leaves, as well as phenological stages (Vardrelip 1993) were measured in two individuals in each box during the growing season. Two plants were removed from the sand surface at the beginning of each above- mentioned period and were subsequently separated into leaf blades, sheaths, stems and paniclespanicle precisely. After the measurement of leaf blades area (by Leaf Area Meter; Model LI-31 DOC, LI-COR), the samples were oven dried at 75°C for 48 hours and then weighed. Two plants were harvested at physiological maturity, were divided into leaf blades, sheaths, stems and panicles, oven dried as mentioned and weighed. Yield and yield components were determined as well. The number and the fresh weight of tillers were

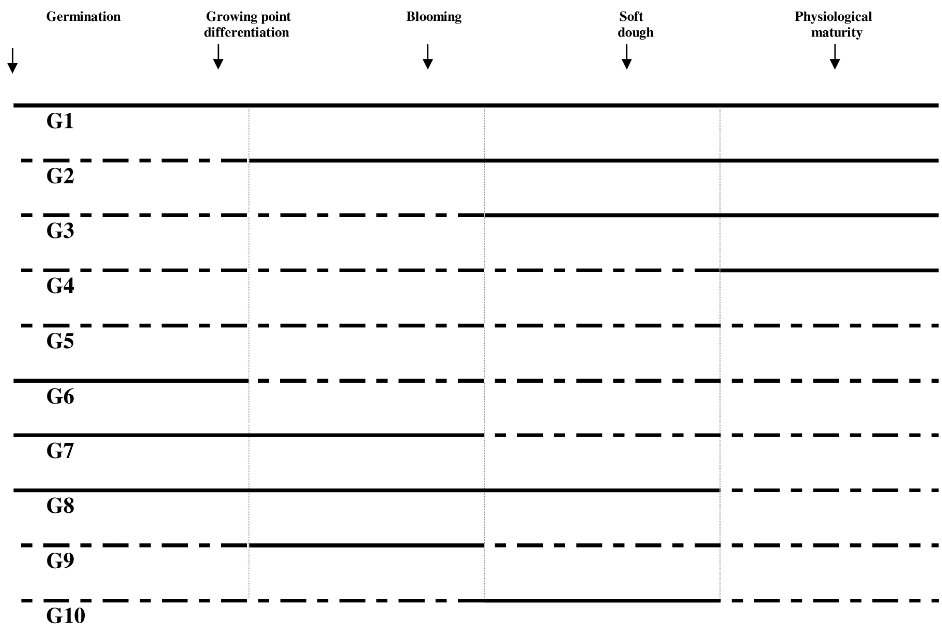

Figure 1. The imposition of salinity according to developmental stages from germination to physiological maturity of sorghum. The treatments included salt stress (-) and stress free periods (---). The length of solid line shows salinization and the length of dashed line shows salt free period.

[DOR: 20.1001.1.16807073.2013.15.4.16.9]

also measured.

Photosynthesis

Gas exchange and relative parameters were measured by using a portable ADC leaf chamber analyzer (model: LCA4), at *PPFD*> 950 µmol m^{-2} s^{-1}, atmosphere pressure 910 mbar, leaf chamber temperature at 33±2°C, and between 11 am to 1 pm. All measurements were carried out on the intact youngest fully developed leaf at the soft dough stage, keeping the chamber constantly vertical to solar radiation, until stabilizing gas exchange in the leaf chamber. More than 10 records were obtained for each leaf after reaching the steady state. All measurements of gas exchange parameters were carried out at blooming and soft dough stages, G3 and G4, respectively.

Statistical Analysis

Experiment was designed and analyzed in a randomized complete block design with tree replications, with salinity treatment at 10 levels with 3 replications. Statistical significance, where indicated, is at least at 5% level as determined by analysis of variance using Minitab 14 and Fisher's LSD test.

RESULTS

Morphological Traits

Salinity imposed at different plant developmental stages, led to various changes in morphological traits. However, those salinity treatments that were imposed at vegetative phase caused adverse effects on plants heights and tillering (Table 1). The plants heights and number of tillers of treatments G3, G4 and G10 in which sorghum plants were salinized after blooming were slightly decreased, whilst salinity stress during early growth and panicle differentiation substantially affected this parameter, especially for G1, G7 and G8 in which salt stress continued up to panicle differentiation. The final heights at maturity stage were

Table 1.The effects of salt stress imposed at various growth stages of sorghum on the plant height, leaf number, tiller number, and leaf area per plant.

Growth stage imposed to salinity[a]	Morphological Traits			
	Height (cm)	Leaf Number	Tiller Number	Leaf area (cm^2 $plant^{-1}$)
G 1	57.0	11.0	0.7	887
G 2	94.2	11.8	0.3	1437
G 3	99.2	10.8	2.3	1773
G 4	103.7	11.0	2.5	1730
G 5	104.5	11.5	3.0	1679
G 6	104.5	12.1	4.5	1543
G 7	65.5	11.7	0.3	889
G 8	83.0	11.7	0.8	971
G 9	101.0	11.3	2.7	1602
G 10	102.5	11.7	2.8	1709
LSD (0.05)	9.8	1.8	1.7	386

[a] G1 to G10 refer to treatments in which the plants either were exposed to salinity from germination to physiological maturity (G1), growing point differentiation (G6), half bloom (G7), and soft dough (G8), respectively, and after each stage, the stress was relieved; or were out of salt stress from germination to growing point differentiation (G2), half bloom (G3), soft dough (G4), and physiological maturity stages (G5), respectively; and then after each stage, were exposed to salinity stress; and individual stages of salinity stress from growing point differentiation to blooming (G9), and from blooming to soft dough (G10) stages.

[DOR: 20.1001.1.16807073.2013.15.4.16.9]

significantly decreased for the treatments G1, G7, G8 and G2 in which salinity stress occurred at early growth stage (Table 1). Tiller numbers per plant in G1, G2, G7, and G8 were significantly lower than other treatments (Table 1). The results indicated that salt stress at early growth stage of sorghum plants, especially from growing point differentiation to blooming stages, had an adverse effect on the number of tillers.

Salinization occurring at various growth stages did not affect the final leaf number of main stem significantly (Table 1). Although we measured leaf area at different growth stages, only the data referring to blooming stage, which nearly overlaps canopy closure, is shown here (Table 1). The leaf areas of treatments G1, G7, and G8 in which salt stress continued from emergence to blooming were highly declined, while the others showed no significant alterations (Table 1).

Yield and Yield Components

Yield characteristics, including both vegetative and reproductive growth of sorghum plants, were drastically affected by salinity when occurred at different growth stages (Table 2, Figure 2). The highest total dry weight was 80.28 g plant^{-1} for the control (G5) whereas the lowest total dry weight was 22.3 g plant^{-1} for G1, in which the plants were salinized throughout the growing season (Table 2). The treatments G7 and G8 in which salt stress continued from emergence to blooming and soft dough stages produced 25.86 and 27.58 g plant^{-1} dry weight, respectively, which showed no significant difference with G1. Treatment G6, in which the plants were under salt stress only at early growth stages up to panicle differentiation, also produced 37.61 g plant^{-1} total dry weight, whilst for G2, G3, G4, G9 and G10 in which the sorghum plants were out of salt stress during early growth, those amounts were 47.36, 49.29, 58.92 and 49.23 g plant^{-1}, respectively which are not significantly different (Table 2).

Grain yield responded differently to salinity. The greatest significant decrease occurred in G1, G8 and G7 compared to the

Table 2.The effects of salt stress imposed at various growth stages of sorghum on yield components per plant, including grain weight (g), grain number, 1,000 grain weight (g), stover weight (g), total dry weight (g), and harvest index.

	Yield components (per plant)					
Growth stage[a]	Grain weight (g)	Grain number	1000 Grains weight (g)	Stover weight (g)	Total dry weight (g)	Harvest index
G 1	7.98	201.5	39.60	14.38	22.36	0.33
G 2	13.20	373.2	35.37	34.16	47.36	0.28
G 3	12.88	412.8	31.21	36.41	49.29	0.27
G 4	22.98	576.7	39.84	36.89	59.87	0.38
G 5	22.09	592.0	37.31	58.20	80.28	0.28
G 6	18.11	462.2	39.19	19.05	37.16	0.48
G 7	8.25	189.3	43.58	17.61	25.86	0.32
G 8	6.19	143.8	43.04	21.39	27.58	0.23
G 9	16.23	511.5	31.73	42.69	58.92	0.28
G 10	14.76	486.2	30.36	34.47	49.23	0.30
LSD (0.05)	4.1	70.3	5.59	8.35	10.07	0.11

[a] G1 to G10 refer to treatments in which the plants either were exposed to salinity from germination to physiological maturity (G1), growing point differentiation (G6), half bloom (G7), and soft dough (G8), respectively, and after each stage, the stress was relieved; or were out of salt stress from germination to growing point differentiation (G2), half bloom (G3), soft dough (G4), and physiological maturity stages (G5), respectively; and then after each stage, were exposed to salinity stress; and individual stages of salinity stress from growing point differentiation to blooming (G9), and from blooming to soft dough (G10) stages.

[DOR: 20.1001.1.16807073.2013.15.4.16.9]

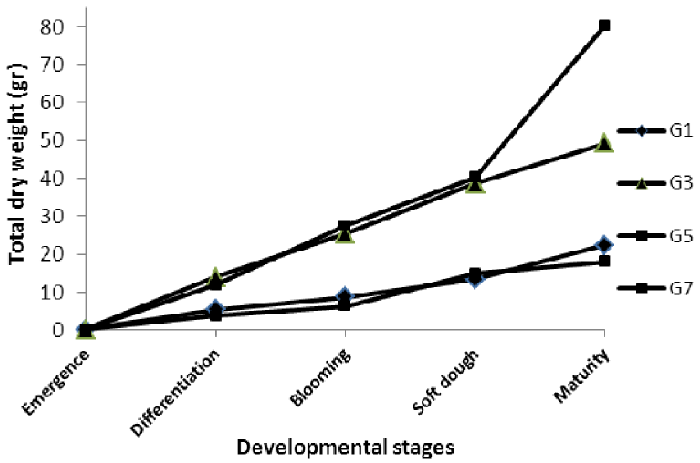

Figure 2. The effects of salt stress imposed at various growth stages of sorghum on accumulation of total dry matter. According to Figure 1. G1 and G7 refer to treatments in which the plants were either exposed to salinity from germination to physiological maturity or half bloom, respectively, and then the stress was relieved. G5 and G3 refer to treatments that the plants were out of salt stress in the whole growth period or up to half bloom, respectively; and then were exposed to salinity stress.

control, when salinization continued from emergence to maturity, soft dough, and blooming stages, with the amount of 7.98, 6.19 and 8.25 g plant^{-1}, respectively. In G4, in which salinity occurred at the last growth phase, produced 22.98 g grain per plant that is the nearest to the control (Table 2).

Grain number per plant varied among treatments as well. G1, G8 and G7 which were subjected to continuous salt stress from germination to maturity, soft dough and blooming stage, had the lowest numbers of grain with the amounts of 201.5, 143.8 and 189.3 per plant, respectively (Table 2). Salinization after blooming stage imposed little influence on grain number, i.e. G4, G9 and the control (G5) had no significant difference in grain number (Table 2). Although salt stress led to some changes in 1000 grains weight, this yield component was not affected markedly by salinity. The treatments G7 and G8 produced the largest seeds with the amount of 43.58 and 43.04 g for 1,000 seeds, respectively, while G3, G2, G9 and G10 produced the smallest seeds (Table 2).

The treatments G5 and G1 had the highest and the lowest stover yields of 58.20 and 14.38 g/plant, respectively, similar to total dry weight (Table 2). The stover yield for treatments G6, G7 and G8 in which the plants were subjected to salinity from emergence to panicle differentiation, blooming and soft dough stage, respectively, also declined significantly, with the amounts of 19.05, 17.61 and 21.39 g plant-1 (Table 2).

Harvest Index was also affected by salt stress (Table 2). Treatment G8, in which the grain weight showed higher decline as compared with stover weight, had the greatest reduction in harvest index amounting 23%. Treatment G6 had the highest harvest index (48%) which indicates more proportional allocation of assimilates to the grains, rather than the vegetative organs.

Plant Growth

The growth of different plant organs including leaf, stem, panicles and total dry matter accumulation was affected by different salinity treatments during the growing season (Figure 2). The time courses of growth were significantly affected from the beginning of growth cycle by salinity stress. It could be deduced from Figure 2 that exposure of each growth stage to salinity, particularly at vegetative growth up to blooming, would lead to a remarkable decline in growth rate. The slope of trend-line of total dry matter accumulation throughout growing season was 0.506 g $plant^{-1}$ d^{-1} for control, whilst as the plants were under salt stress during all growing cycles, i.e. G1, the slope was 0.137 g $plant^{-1}$ d^{-1}, referring to a drastic decrease in growth rate (Table 3).

Treatments G7 and G8, in which salinization occurred from the beginning of vegetative growth until blooming and soft dough stages also had the lowest growth slopes of 0.118 and 0.157 g $plant^{-1}$ d^{-1}, respectively. This implies that despite cessation of salinity stress after vegetative stages at G6, G7 and G8, the sorghum plants could not restore the growth rate, as compared with the control (Figure 3). The rates of assimilate accumulation in leaves and panicles were also variable among different salinity stress treatments (Figure 3, Table 3). The slope of dry matter accumulation in leaves was the highest for the control (4.238 g $plant^{-1}$ d^{-1}) and lower for treatments G1, G6, G7 and G8 (1.92, 2.305, 1.878 and 2.591 g $plant^{-1}$ d^{-1} respectively; Table 3, Figure 3), which implies that the rate of leaf growth declined dramatically when the plants were imposed to salt stress either throughout the growing season or at the primary vegetative growth until blooming and seed filling stages, and that the sorghum plants could not recover leaf growth rate after salt stress removal.

Similar trends were observed for the rate of panicles growth. The steepest slopes of panicle dry matter accumulation belonged to the control whereas the least was seen for G1, G6, G7 and G8 (Table 3, Figure 3), which indicates that salinization from the primary growth would lead to a drastic decline in reproductive organs growth rate. The stem dry weight showed a conspicuous decline at maturity stage for all treatments, and the difference was the lowest for the control and highest for G1, which was exposed to salinity at all growing stages (Figure 3). These alterations refer to remobilization of assimilates from the stem to reproductive

Table 3.The effects of salt stress imposed at various growth stages of sorghum on the slope of trend-line of dry matter accumulation of leaves, inflorescences, and total dry weight.

Growth stage imposed to salinity[a]	The slope of trend-line of dry matter accumulation (g $plant^{-1}$ day^{-1})		
	Leaf dry matter	Inflorescence dry matter	Total dry matter
G 1	1.92	2.722	0.137
G 2	3.371	7.298	0.301
G 3	3.542	9.68	0.306
G 4	3.973	9.116	0.389
G 5	4.238	13.15	0.506
G 6	2.305	5.143	0.232
G 7	1.878	3.629	0.118
G 8	2.591	2.502	0.157
G 9	3.94	7.936	0.376
G 10	3.878	6.191	0.295

[a] G1 to G10 refer to treatments in which the plants either were exposed to salinity from germination to physiological maturity (G1), growing point differentiation (G6), half bloom (G7), and soft dough (G8), respectively, and after each stage, the stress was relieved; or were out of salt stress from germination to growing point differentiation (G2), half bloom (G3), soft dough (G4), and physiological maturity stages (G5), respectively; and then after each stage, were exposed to salinity stress; and individual stages of salinity stress from growing point differentiation to blooming (G9), and from blooming to soft dough (G10) stages.

[DOR: 20.1001.1.16807073.2013.15.4.16.9]

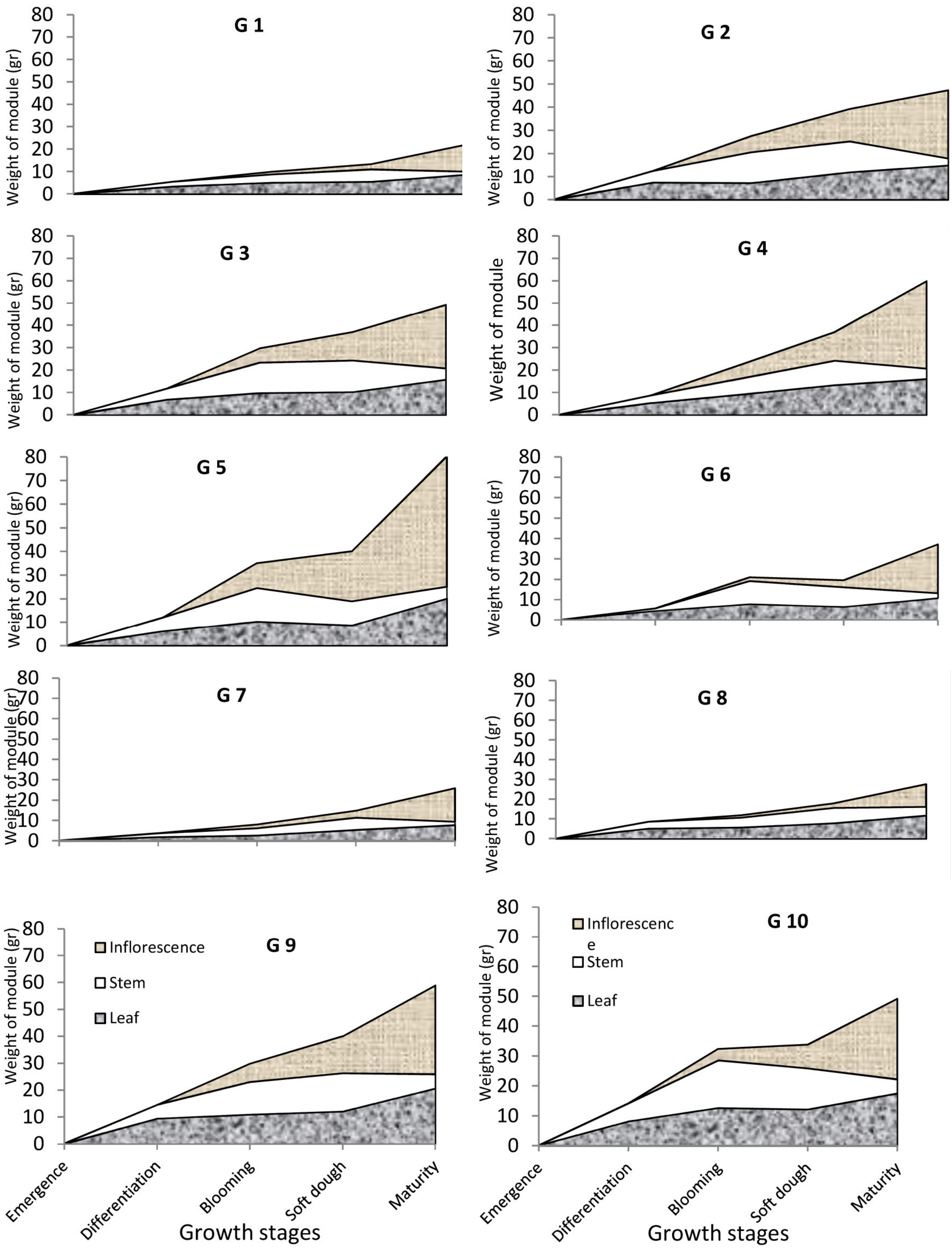

Figure 3. The effects of salt stress imposed at various growth stages of sorghum on dry matter changes of leaf, stem and inflorescence against growth stages. G1 to G10 refer to treatments in which the plants either were exposed to salinity from germination to physiological maturity (G1), growing point differentiation (G6), half bloom (G7), and soft dough (G8), respectively, and then after each stage, the stress was relieved; or were out of salt stress from germination to growing point differentiation (G2), half bloom (G3), soft dough (G4), and physiological maturity stages (G5), respectively; and then after each stage, were exposed to salinity stress; and individual stages of salinity stress from growing point differentiation to blooming (G9), and from blooming to soft dough (G10) stages.

organs, which were accelerated in the presence of salinity stress.

Gas Exchange Characteristics

CO_2 assimilation rate, as measured at blooming and soft dough stages, was partially altered by various salinization phases (Table 4). The lowest rate of CO_2 assimilation was recorded at G4 and G5, which were out of stress until seed filling stage, with 18.2 and 18.4 µmol CO_2 m^{-2} s^{-1} at blooming and 19.4 and 17.8 µmol CO_2 m^{-2} s^{-1} at soft dough stages, respectively. Long term exposure to salt stress in G1, G7 and G8, increased CO_2 assimilation rate to 21.9, 22.3 and 21.9 µmol Co_2 m^{-2} s^{-1}, respectively. When the plants were subjected to short term salinization in other treatments, the photosynthesis rate exhibited little increase. Transpiration rate as measured in the abovementioned stages did not have a particular pattern and did not noticeably vary among treatments. Substomatal CO_2 concentrations were 368.2 and 373.6 at blooming and 361.2 and 366.9 µmol mol^{-1} at soft dough stages, for G4 and G5, respectively, but 394.2, 392.7 and 389.4 at blooming and 389.6, 387.4 and 383.2 µmol mol^{-1} at soft dough stages, for G1, G7 and G8, respectively (Table 4). These rates are in accordance with those of assimilation rate.

Critical Period of Salt Stress Control

Two approaches might be assumed to determine the critical period in which the salt stress could have the most adverse effects on plant growth. The first approach is the critical salinity-free period that is a consequence of salinized stage which is substituted by salinity free stage, gradually, i.e. G1, G2, G3, G4 and G5, respectively, in the current study, also called the minimum time-span of salinity-free period (MTSF). The second one is the critical salinized period that is a consequence of salt-free stages which are substituted by imposing salt stress, gradually, i.e. G5, G6, G7, G8 and G1, respectively, in the current experiment, also called the maximum time-span of salinization (MTS). The time interval between MTSF and MTS would reveal the critical period for salinity stress

Table 4.The effects of salt stress imposed at various growth stages of sorghum on assimilation rate, substomatal CO_2 concentration, and transpiration rate.

Growth stage imposed to salinity[a]	Assimilation (µ mol CO_2 m^{-2} s^{-1})		Substomatal CO_2 (µmol mol^{-1})	
	Blooming	Soft dough	Blooming	Soft dough
G 1	21.9	21.2	394.2	389.6
G 2	20.5	20.2	388.2	373.2
G 3	19.1	19.9	371.5	370.1
G 4	18.2	19.4	368.2	361.2
G 5	18.4	17.8	373.6	366.9
G 6	19.7	17.9	376.4	371.4
G 7	22.3	19.1	392.7	387.4
G 8	21.9	22.3	389.4	383.2
G 9	19.9	17.9	381.2	373.5
G 10	18.7	21.3	373.6	387.7

[a] G1 to G10 refer to treatments in which the plants either were exposed to salinity from germination to physiological maturity (G1), growing point differentiation (G6), half bloom (G7), and soft dough (G8), respectively, and after each stage, the stress was relieved; or were out of salt stress from germination to growing point differentiation (G2), half bloom (G3), soft dough (G4), and physiological maturity stages (G5), respectively; and then after each stage, were exposed to salinity stress; and individual stages of salinity stress from growing point differentiation to blooming (G9), and from blooming to soft dough (G10) stages.

[DOR: 20.1001.1.16807073.2013.15.4.16.9]

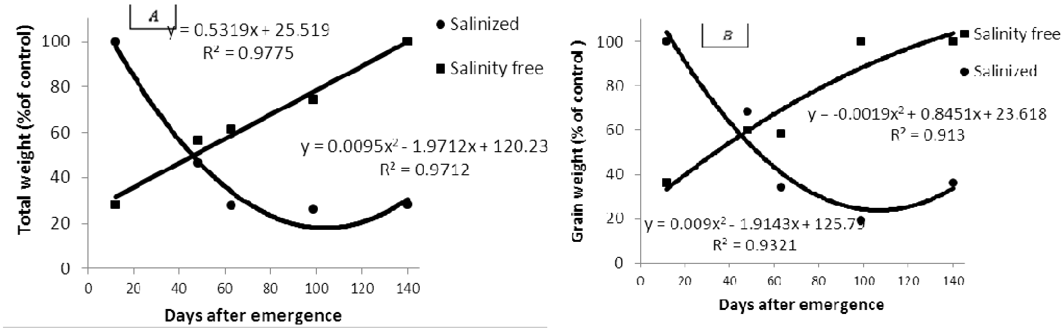

Figure 4. The effects of salinity-free and salinized consequent periods on total dry weight (A), and grain dry weight (B) expressed as the percentage of the control, against time from emergence.

control (CPSC). Moreover, the crossing point of MTSF and MTS, stands for the equality point of salt-free and salt-stress. In fact, this time-point might determine the equality of any increase or decrease in crop production/yield in response to salt stress (Figure 4). The polynomial regressions of critical salinity-free periods and critical salinized period against time from emergence are shown in Figure 4 for biological weight and grain yield, as the percentage of the control. The estimated maximum period of salinity stress and minimum period of salinity-free at 10 and 20% acceptable loss in biological yield as well as grain yield are indicated in Table 5. The maximum duration of salinization for biological yield was 16.6 and 22.7 days after emergence (DAE) at 10 and 20% yield loss, respectively. Therefore, for instance, to prevent more than 20% loss in biological yield, salt stress should be ceased after 22.7 DAE, which coincide with the 5th leaf stage. The minimum duration of salinity–free for biological yield was 121.7 and 102.7 DAE at 10 and 20% acceptable yield loss, respectively, which indicates that sorghum plants need at least a period of 102.7 DAE salinity free to avoid 20% yield loss, that nearly coincided with the soft dough stage. Hence, the critical period of salinity stress for avoiding remarkable grain loss at thresholds of 10 and 20%, were 68.6 and 45.7 days. This implies that in order to prevent more than 20% decrease in grain yield, the sorghum plants should be kept against salinity stress for 45.7 days, almost from the 5th leaf to blooming stage (Figure 4).

DISCUSSION

The growth of sorghum plants subjected to 150 m*M* NaCl salinity stress, especially those of long-term salinization, was considerably reduced. Decline in plant growth might reflect the increase of metabolic energy cost to manage salinity stress and reduced carbon gain. It also

Table 5. Maximum duration of salinity stress, minimum duration of salinity- free, and duration of critical period, at 10% and 20% loss in biological, grain and stover yields of sorghum.

	Biological yield loss		Grain yield loss	
Duration (days)	10%	20%	10%	20%
Maximum duration of salinity stress	16.6	22.7	20.6	27.4
Minimum duration of salinity-free	121.5	102.7	89.2	73.1
Duration of critical period	104.9	80	68.6	45.7

[DOR: 20.1001.1.16807073.2013.15.4.16.9]

reflects salt impacts on tissues and reduction in photosynthetic rates (Ashraf, 2004; Karimi *et al.,* 2005; Yan *et al.,* 2011). Many studies show that the plants performance under salinity is variable and time-dependent (Wilson *et al.,* 2000; Houle *et al*., 2001; Del Amour *et al*., 2001).

The sorghum plants were markedly smaller, when salinity occurred all during the growing season or in the early vegetative stages. This implies that the factors determining plant height including those of internode and panicle height are established during early vegetative growth and panicle differentiation stages. Afterwards, the plant height is almost constant and salinity stress does not change it significantly. Maas *et al.* (1986) found that although salinity significantly decreased plant height during the vegetative stage, the plants recovered considerably during reproductive and maturity stages, however, salinization at the maturity stage had no effect on plant height since the plants were fully grown at that stage. According to our findings, salt stress at early growth stages of sorghum plants, especially from germination to panicle differentiation, had adverse effects on biomass production and tillering potential. Studying the timing of salinity stress of rice (Zeng *et al.,* 2001) also indicated that reduction in tiller number per plant was significant only when plants were salinized for 20 days before panicle initiation.

Biological yield of sorghum plants was dramatically affected by salinization throughout full growing season, if salt stress of 150 m*M* occurred at all growing cycles. Salinity stress from emergence to soft dough and blooming stage also had the same adverse effect on biological yield and plants could not restore the growth, if salinity discontinued later.

For the treatments in which the plants were stress-free at early growth stages and then subjected to salt stress, reductions in total dry matter were less remarkable, especially if salinity occurred at a late individual stage. Our findings showed that sorghum biological yield is more sensitive than grain yield, if imposed to salinity stress from emergence until growing point differentiation and blooming stage, especially for continuous stress, and could not recover growth after stress cessation. This variation in salt tolerance of biological yield at different development stages has been reported previously in wheat and rice (Khatkar and kuhad*,* 2000; Zeng *et al.,* 2001). Khatkar and kuhad (2000) studying short term salinization on two wheat cultivars at different stages found that wheat plants were most sensitive to salinity at crown root initiation stage and the least at soft dough stage. They found that, in wheat, plant resistance to salinity increased with age. Zeng *et al.* (2001) reported that shoot dry weight of rice plants harvested at seed maturity was significantly decreased only when plants were under short-term salt stress before booting. However, Lutts *et al.* (1995), studying changes in the response of rice varieties to NaCl during developmental stages, showed that varietal levels of resistance to salinity at different growth stages were not necessarily independent characteristics and varieties with the greatest variability in salt stress responses during vegetative phase showed the greatest variability during the reproductive development as well.

Continuation of salt stress from emergence to both blooming and soft dough stages as well as throughout the growing season led to remarkably adverse effects on grain yield. The grain number per plant also showed a similar pattern; however, salinization after blooming stage had no significant effect on grain number per plant. In general, the effect of salinity appears to be most pronounced on yield components that are growing or developing at the time the salt stress is imposed, which is in conformity with some other studies (Francois *et al.,* 1994; Zeng *et al.,* 2001). Francois *et al.* (1994) studied the effect of time of salt stress on growth and yield components of irrigated wheat. They reported that salinity imposed prior to terminal spikelet differentiation (TSD) reduced the number of spikelet per spike and

[DOR: 20.1001.1.16807073.2013.15.4.16.9]

the number of tiller per plant, whereas salinity imposed after TSD significantly reduced only kernel number and weight. The sorghum plants that were salinized during early vegetative stage had the lowest grain yield and the highest kernel weight (Maas *et al.,* 1986). Zeng *et al.* (2001) also concluded that when rice plants were subjected to salinity at different growth stages, the reduction in spikelet per panicle and seed weight per panicle were most pronounced if plants were stressed between the 3rd leaf stage and panicle initiation (PI) stage or between PI and booting stages.

Net CO_2 assimilation of sorghum plants exposed to salt stress did not significantly decrease at 150 mM of salinity. Yan *et al.* (2011) reported photosynthetic rate (Pn) reduction of sorghum by salt treatment at 150 mm NaCl, which could be ascribed to stomatal limitation. They reported that salt stress had no effect on photosystem II (PSII) activity. As for this pattern of photosynthetic response, sorghum seems to be a reliable crop species in the face of global warming and increasing salinity of agricultural land (Kafi, 2009).

Studying the critical period of salt stress control (CPSSC) resulted in an interesting concept. Our findings showed that the critical period of salinity stress for biological yield is much more distinct than that of the grain yield. The maximum duration of salinization, at both thresholds of 10 and 20% yield loss was more for grain yield than biological yield. In the same manner, the minimum duration of salinity-free at both above-mentioned thresholds, was lower for grain yield than that for biological yield. Hence, for 10% acceptable yield loss, the critical period of salinity stress for biological yield was 104.6 days, nearly between the 3rd leaf stage and hard dough stage, and for grain yield it was 68.6 days, nearly between the 4th leaf and soft dough stages. It should be kept in mind that these values are gained through calculation and may differ depending on experimental conditions. In addition, developmental stages of plant are not exactly distinguished and may overlap each other. Moreover, these results are reliable at greenhouse conditions with 150 mM NaCl, and should be tested for other levels of salinity.

In general, according to our findings, depending on the developmental stage at which the plant is exposed to salinity, sorghum plant might be placed in different tolerance groups. Regarding biological and vegetative growth, sorghum is more sensitive during early growth stages and more tolerant at the late stages of growth, while considering grain production it is more tolerant after the soft dough stage. Knowledge of the salt-sensitive and tolerant stages of sorghum crop would make it feasible to use brackish or salt water for growing sorghum with minimum yield loss. A broader application of the concept of CPSSC is to apply low or high salinity water in synchrony with salt-sensitive or salt-tolerant growth stages, which will assist us with better usage of saline water resources for sorghum production.

ACKNOWLEDGEMENTS

We would like to thank Vice President of Research and Technology, Ferdoawsi University of Mashhad for the financial support of this project. We also thank Research Department (Dr. Shahriari and Mrs. Abeddan) and green house personnel (Dr. Goldani and Mr. Gorji) of Faculty of Agriculture for their help during data collection.

REFERENCES

1. Ashraf, M. 2004. Some Important Physiological Selection Criteria for Salt Tolerance in Plants. *Flora,* 199: 361-376.
2. Casper, B. B., Forseth, I. N. and Walt, D. A. 2006. A Stage Base Study of Drought Response in *Cryptantha flava* (Boraginacea): Gas Exchange, Water Use Efficiency, and Whole Plant Performance. *Am. J. Bot.*, 93(7): 977- 987.

3. Del Amour, F. M., Martinez, V. and Cerd, A. 2001. Salt Tolerance of Tomato Plants as Affected by Stage of Plant Development. *Hort. Sci*., 36: 1187- 1193.
4. Desclaux, D., Huynh, T. T. and Roumet, P. 2000. Identification of Sorghum Plant Characteristics that Indicate the Timing of Drought Stress. *Crop Sci*., 40: 716-722.
5. Francois. L. E., Grieve, C. M., Maas, E. V. and Lesch, S. M. 1994. Time of Salt Stress Affects Growth and Yield Components of Irrigated Wheat. *Agron. J.,* 86: 100-107.
6. Hamzei, J., Mohammady Nasab, A. D., Rahimzade Khoie, F., Javanshir, A. and Moghaddam, M. 2007. Critical Period of Weed Control in Three Winter Oilseed Rape (*Brassica napus* L.). *Turk. J. Agric. Forest*., 31: 83-90.
7. Hoagland, D. R. and Arnon, D. I. 1950. The Water Culture Method for Growing Plants without Soil. *Calif. Agric. Exp. Sta. Circ*., **347**: 313-320.
8. Houle, G., Morel, L., Reynolds, C. E. and Siegel, J. 2001. The Effect of Salinity on Different Developmental Stages of an Endemic Annual Plant, *Aster laurentianus* (Asteracea). *Am. J. Bot*., **88(1):** 62- 67.
9. Jogeswar, G., Pallela, R., Jakka, N. M., Reddy, P. S., Venkateswara Rao, J., Sreenivasulu, N. and Kavi Kishor, P. B. 2006. Antioxidative Response in Different Sorghum Species under Short-term Salinity Stress. *Acta Physiol. Plant.,* **28(5):** 465-475.
10. Kafi, M. 2009. The Effects of Salinity and Light on Photosynthesis, Respiration and Chlorophyll Fluorescence in Salt-tolerant and Salt-sensitive Wheat (*Triticum aestivum* L.) Cultivars. *J. Agric. Sci. Tech.,* **11**: 535-547
11. Karimi, J., Ghorbanli, M., Heidari, H., Khavari Nejad, R. A. and Assareh, M. H. 2005. The Effects of NaCl on Growth, Water Relations, Osmolytes, and Ion Content in *Kochia prostrata. Biol. Planta,* **49**: 301- 304.
12. Khatkar, D., and Kuhad, M. S. 2000. Short-term Salinity Induced Changes in Two Wheat Cultivars at Different Growth Stages. *Biol. Planta*., 43(4): 629-632.
13. Lutts, S., Kinet, J. M. and Bouharmont, J. 1995. Changes in Plant Responses to NaCl during Development of Rice (*Oryza sativa* L.) Varieties Differing in Salinity Resistance. *J. Exp. Bot*., 46(12): 1843- 1852.
14. Maas, E. V., Poss, J. A. and Hoffman, G. J. 1986. Salinity Sensitivity of Sorghum at Three Growth Stages. *Irrig. Sci*., 7: 1- 11.
15. Mahmood, T., Iqbal,N., Raza, H., Qasim, M. and Yasin Ashraf M. 2010. Growth Modulation and Ion Partitioning in Salt Stressed Sorghum (*Sorghum bicolor* L.) by Exogenous Supply of Salicylic Acid. *Pak. J. Bot*., **42(5):** 3047-3054.
16. Netonda, G. W., Onyango, J. C. and Beck, E. 2004. Sorghum and Salinity. I. Responses of Growth, Water Relations, and Ion Accumulation to NaCl Salinity. *Crop Sci*., **44**: 797- 805.
17. Sabir, P., Ashraf M. and Akram N. A. 2011. Accession Variation for Salt Tolerance in Proso Millet (*Panicum miliaceum* L.) Using Leaf Proline Content and Activities of Some Key Antioxidant Enzymes. *J. Agron. Crop Sci.,* **197:** 340–347.
18. Shariat Jafari, M. H., Kafi, M. and Astaraie A. 2009. Interactive Effects of NaCl Induced Salinity, Calcium and Potassium on Physiomorphological Traits of Sorghum (*Sorghum bicolor* L.). *Pak. J. Bot*., 41(6): 3053- 3063.
19. Vandrelip, R. L. 1993. How a Sorghum Plant Develops. URL: http://www.oznet.ksu.edu/library/crps12/samplers/s3.asp; Retrieved 3 December, 2008.
20. Wilson, C., Lesch, S. M. and Grieve, C. M. 2000. Growth Stage Modulates Salinity Tolerance of New Zealand Spinach (*Tetragonia teragonioides* Pall.) and Red Orach (*Atriplex hortensis* L.). *Ann. Bot*., 85: 501- 509.
21. Yan, K., Chen, P., Shao, H., Zhao, S., Zhang, L., Zhang, L., Xu, G. and Sun, J. 2012: Responses of Photosynthesis and Photosystem II to Higher Temperature and Salt Stress in Sorghum. *J. Agron. Crop Sci.,* **198(3):** 218-225.
22. Zeng, L., Shannon, M. and Lesch. S. M. 2001. Timing of Salinity Stress Affects Rice Growth and Yield Components. *Agric. Water Manag.,* 48(3): 191- 206.

حساسیت مراحل مختلف نمو سورگوم دانه ای به تنش شوری: یک رهیافت تلفیقی

م. کافی، م. ح. شریعت جعفری، ع. مویدی

چکیده

برای درک اثرات تنش شوری در چهار مرحله نموی سورگوم شامل ۱- از جوانه زنی تا تمایز نقطه رشد ۲- از تمایز نقطه رشد تا ۵۰٪ گلدهی ۳- از ۵۰٪ گلدهی تا خمیری نرم و ۴- از خمیری نرم تا رسیدگی فیزیولوژیکی، یک آزمایش گلخانه‌ای شامل ۱۰ تیمار مختلف متشکل از تمام ترکیبات مختلف شوری و عاری از شوری در چهار مرحله فوق در سال ۱۳۸۹-۱۳۹۰ در گلخانه تحقیقاتی دانشگاه فردوسی مشهد اجرا شد. اعمال تنش شوری در مراحل مختلف رشد باعث ایجاد تغییرات مختلف در خصوصیات رشدی سورگوم شد. تنش در مراحل اولیه رشد و تمایز پانیکول باعث کاهش ارتفاع گیاه و تعداد پنجه‌ها شد. بیشینه عملکرد بیولوژیکی در تیمار شاهد بدون شوری و کمینه آن در تیمار تنش شوری در کل فصل رشد مشاهده گردید. در گیاهانی که در ۲ تا ۳ مرحله اولیه رشد بدون تنش رشد کردند و سپس در معرض تنش قرار گرفتند کاهش در کل زیست توده بسیار کمتر از تیمار هایی بود که در آنها در مراحل اولیه رشد گیاهان در معرض تنش و در مراحل انتهایی رشد عاری از تنش بودند. هر چه اعمال تنش به آخرین مرحله نمو نزدیکتر باشد عملکرد کمتر تحت تاثیر قرار می گیرد. تداوم تنش شوری از جوانه زنی تا ۵۰٪ گلدهی و تا خمیری نرم همانند کل فصل رشد باعث کاهش شدید عملکرد دانه شد. اثرات شوری در اجزای عملکردی که در زمان اعمال تنش شوری در حال آغازش و رشد بودند بیشتر بود. مرحله بحرانی تنش شوری برای عملکرد بیولوژیکی بسیار محدود تر از این دوره، در عملکرد دانه بود. این نتیجه بیانگر این است که در دوره تشکیل و پر شدن دانه حساسیت به شوری سورگوم پایین است.

[DOR: 20.1001.1.16807073.2013.15.4.16.9]