MITIGATION OF ADVERSE EFFECTS OF WATER DEFICIENCY IN WHEAT WITH SODIUM NITROPRUSSIDE (SNP)

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CERTIFICATE

*This is to certify that the thesis entitled **"MITIGATION OF ADVERSE EFFECTS OF WATER DEFICIENCY IN WHEAT WITH SODIUM NITROPRUSSIDE (SNP)"** submitted to the Department of Agricultural Botany, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in AGRICULTURAL BOTANY**, embodies the results of a piece of bonafide research work carried out by **AFIFA SULTANA SHOSHY,** Registration No. **10-03813** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed during the course of this investigation has been duly acknowledged and style of this thesis has been approved and recommended for submission.

Dated: June, 2016
Place: Dhaka, Bangladesh

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DEDICATED

TO

MY

BELOVED PARENTS

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ABSTRACT

A pot experiment was conducted in the field laboratory of Sher-e-Bangla Agricultural University, Dhaka, during *rabi* season, November 2015 to March 2016 to assess the role of sodium nitroprusside (SNP) as nitric oxide (NO) donor on mitigation of the adverse effects of water deficiency in wheat. In this experiment, the treatments consisted of four different stages of wheat plant when water deficiency condition were made *viz.* D_0 = control, D_c= water deficiency provided at crown root initiation stage, D_b= water deficiency provided at booting stage, D_a= water deficiency provided at after anthesis stage and three different concentrations of SNP *viz*. S_0= 0 mM, S_1= 0.1 mM and S_2= 0.2 mM of SNP. The experiment was laid out in two factors Completely Randomized Design (CRD) with four replications. The total treatment combinations were 12 (4x3). The morphology, yield contributing characters and yield of wheat were significantly affected by water deficiency at different stages including crown root initiation, booting and after anthesis stage. The lowest value of number of leaves plant^{-1}, tillers plant^{-1} and effective tillers plant^{-1} were found from water deficiency at crown root initiation stage. Besides, the maximum reduction of plant height, spike length, number of spikelets spike^{-1}, number of grains spike^{-1}, grain yield were found from water deficiency at booting stage while the lowest 100 grains weight was found from water deficiency at after anthesis stage and indicates that booting stage is very sensitive to water deficiency. Exogenous foliar application of SNP significantly improved the morphology, yield contributing characters and yield of wheat. The SNP increased plant height, number of leaves plant^{-1}, tillers plant^{-1}, effective tillers plant^{-1}, spike length, spikelets spike^{-1}, grains spike^{-1}, 100 grains weight and grain yield of wheat at both water deficit and non-deficit conditions. The interaction between water deficiency at different stages in wheat and SNP influenced the morphological and yield contributing characters and grain yield of wheat. The highest grain yield (12.77 g pot^{-1}) was recorded from D_0S_1 (No water deficiency + 0.1 mM SNP) which was statistically identical with D_0S_2 (No water deficiency + 0.2 mM SNP) treatment combination. The SNP (0.1 mM) increased grain yield from 5.60 to 6.96 g pot^{-1}, 8.65 to 9.79 g pot^{-1} and 10.80 to 11.10 g pot^{-1} under water deficiency at booting, after anthesis and crown root initiation stage, respectively. Therefore, this experimental results suggest that exogenous foliar application of SNP increased grain yield by mitigating the adverse effect of water deficiency in wheat at crown root initiation, booting and after anthesis stages.

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont'd)

TABLE OF CONTENTS (Cont'd)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Cont'd)

LIST OF APPENDICES

ABBREVIATIONS AND ACRONYMS

AEZ	:Agro-Ecological Zone
ANOVA	:Analysis of Variance
BARI	:Bangladesh Agricultural Research Institute
BBS	:Bangladesh Bureau of Statistics
CV%	:Percentage of Coefficient of Variation
cv.	:Cultivars
Df	:Degrees of freedom
FAO	:Food and Agriculture Organization
FAOSTAT	:Food and Agriculture Organization Statistics
F.C.	:Field capacity
IAA	:Indole -3 Acetic Acid
NO	: Nitric oxide
pH	:Potential hydrogen
ROS	:Reactive Oxygen Species
RuBisCO	:Ribulose-1,5-bisphosphate carboxylase or oxygenase
RWC	:Relative Water Content
SAU	:Sher-e-Bangla Agricultural University
SNP	:Sodium nitroprusside

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a cereal grain crop under Poaceae family and is the staple food in more than 40 countries and over 35% of the global population (Matsuoka, 2011). It is one of the major cultivated crops with respect to human nutrition. Wheat provides nearly 55% of the carbohydrates and 20% of the food calories consumed globally (Breiman and Graur, 1995). Due to its wider adaptability it can be grown under diverse agro-ecological conditions ranging from temperate to subtropical climates. In 2016, world production of wheat was 749 million metric tons making it the second most-produced cereal after maize (1.03 billion metric tons), with more than rice (499 million metric tons) (FAOSTAT, 2016). In the year 2014-15, total production of wheat has been estimated 13.5 lac metric tons and average yield rate of wheat has been estimated 3.086 metric tons per hectare in Bangladesh (BBS, 2016).

There are numerous both abiotic and biotic components of the environment which influence the growth and development of crop plants including wheat. Different abiotic stresses known as drought, heat, heavy metals contamination, salinity etc. are considered to be the main source of yield reductions of crops. Water deficiency is one of the most common environmental stresses that affects growth and development of plants. It has been found to be one of the major environmental factors which limits crop yield.

Water deficiency is a serious environmental threat which restricts nutrient absorption by roots and hampers wheat production. The shortage of irrigation water at crown root initiation, booting and early grain fill period results in significant yield losses of wheat. Ismail *et al.* (1996) reported that water stress during tillering, stem elongation, heading, ripening and entire growth stages of wheat were found to significantly reduce grain yield and biomass production. Baser *et al*. (2004) studied the effect of water deficiency on the yield and yield components of winter wheat and found a decrease of about 40% in yield under water stressed conditions as compared to control. Shi *et al*. (2010) expressed that drought can affect vegetative and reproductive stages of wheat. The number of leaves per plant, leaf size and leaf longevity can be affected by water stress (Shao *et al*., 2008). Drought is becoming a serious threat to crop production worldwide resulting in 67 and 82% reduction in K^+ uptake under mild and severe water stress conditions respectively (Baque *et al.*, 2006). Li *et al*. (2000) reported that water deficiency decreased the number of grains per spike and grain weight of wheat. However, to my knowledge limited studies have been conducted about the effect of water deficiency at different stages of wheat.

It has been reported that many physiological processes in plants are impaired by drought stress including photosynthesis, enzyme activity, membrane stability, pollen viability and ultimately growth. Rucker *et al.* (1995) reported that drought can reduce leaf area which can consequently lessen photosynthesis. A common effect of drought stress is the disturbance between the generation and quenching of reactive oxygen species (ROS). ROS such as superoxide, hydrogenperoxide, hydroxyl radicals and singlet oxygen are enhanced during drought stress through the disruption of electron transport system and oxidizing metabolic activities occurring in chloroplast, mitochondria and membrane (Sofo *et al*., 2005). During severe drought conditions the production of ROS exceeds the capacity of the antioxidative systems to remove them, causing oxidative stress (Sofo *et al*., 2005). ROS damage plants by lipid peroxidation, protein degradation and breakage of DNA (Beligni and Lamattina, 2000). Therefore, it suggests that water deficiency changes normal physiological functions in plants.

As ROS, nitric oxide (NO) is a uniquely volatile signaling molecule that has been implicated in the activation of plant defenses (Khan *et al*., 2017). NO is a bioactive free radical which plays important roles in many physiological processes in plants, such as growth, development, senescence and adaptive responses to multiple stresses (He *et al.*, 2012). It has been reported that NO, along with ROS, is involved in plants' responses to a multitude of environmental stimuli such as salinity, drought, high light intensity and mechanical wounding (Corpas *et al.*, 2008).

It has been reported that NO in the form of Sodium nitroprusside (SNP) has been found to be very effective for the improvement of the relative water content, photosynthesis, gas exchange characteristics and redox status in wheat under abiotic stress (Tian and Lei, 2007). Application of SNP addition to wheat seedlings reduced the chances of membrane injury under drought stress conditions (Wang *et al.*, 2011).

Tian and Lei (2006) investigated that 0.2 mM SNP enhanced wheat seedling growth under drought stress. Tu *et al*. (2003) found that 0.1 mM SNP delayed the senescence of wheat leaves by inhibition of the degradation of chlorophyll and soluble proteins, especially RuBisCo. Therefore, it is suggesting that NO has the capacity to cope the plants under adverse environment.

Devastating and regular water deficiency caused by a lack or a late/early arrival of rainfall are common in many parts of Bangladesh, mostly in north-western Bangladesh. Drought is one of the main problems for many nations and the severity of such issue goes big when it comes as obstacle to ensure an optimum agricultural production for a country like Bangladesh. Mitigation of drought stress in wheat is a major objective in arid and semiarid regions of the world due to inadequate precipitation, shortage of irrigation water and high water demand for crop evapotranspiration. However, to my knowledge limited information is available about the roles of SNP as NO donor on improvement of morpho-physiology and yield of wheat under water deficit conditions. Therefore, the present study was conducted to assess whether the exogenous application of SNP could ameliorate the adverse effects of water deficiency on wheat plants.

OBJECTIVES:

Considering the facts described above, the present research work was undertaken to achieve the following objectives-

i. To investigate the independent effects of water deficiency at different stages and SNP or NO on morpho-physiology and yield of wheat.

ii. To investigate the interaction effect between water deficiency at different stages and SNP or NO on morpho-physiology and yield of wheat.

CHAPTER II

REVIEW OF LITERATURE

Water deficiency not only affects plant growth and development but ultimately productivity in almost all the cereals, thus it is one of the most serious threats to world agriculture. In wheat, a major cereal, water stress is one of the major constraints for production and yield stability. Wheat production is severely threatened by water shortages in Bangladesh. The researchers of Bangladesh are conducting different experiments to adopt different crops including wheat in water deficit condition. An attempt has been made to find out the response of wheat at different stages of water deficiency as well as to find out the possible ways of alleviation of water stress by using sodium nitroprusside (SNP). To facilitate the research work diverse literatures have been reviewed in this chapter under the following headlines.

2.1 Effect of water deficiency on morphological characters of plant

Abdel-Motagally and El-Zohri (2016) conducted two field experiments to determine the effect of water stress on yield of wheat plant grown in calcareous soil during 2013-2014 and 2014-2015 seasons. They reported the highest mean values of plant height spike length number of spikelets, spikelets m^2, grain yield $plant^{-1}$, 1000-grain weight and grain yield by adding the normal irrigation level (100% field capacity) whereas the lowest mean values were recorded for the plants that received the lowest irrigation level (50% field capacity) in both the first and second seasons.

Jayalalitha *et al*. (2015) studied twelve *Bt* cotton hybrids to evaluate under rainfed condition (moisture stress) for morpho–physiological characters and seed cotton yield in a field experiment conducted during Kharif 2009 -10 and 2010-11. Significant differences were observed between main treatments viz., no stress (irrigation treatment) and stress (rainfed treatment) and sub treatments (12 *Bt* cotton hybrids). Cotton hybrids under rainfed condition (moisture stress) recorded 15 and 18 percent reduction in plant height and 31.6 and 32.9 per cent reduction in mean number of sympodia per plant at harvest compared to irrigated condition (no stress) in both the years respectively.

Raza *et al*. (2014) carried out an outdoor pot experiment and two field experiments with two wheat varieties under water stress (Auqab-2000 and Lasani-2008) and found that plant height decreased in response to imposed water stress.

Yasmeen *et al.* (2013) conducted an experiment to evaluate the efficacy of foliar applied growth enhancers under water stress in wheat cultivar Sehar-2006. Three irrigation levels were maintained at 100, 75 and 50% field capacity designated as well watered, moderate and severe drought stressed. They found that leaf area was gradually decreased with increasing drought stress and the minimum leaf area was obtained under extreme drought conditions or at 50% field capacity moisture level.

Bunnag and Pongthai (2013) carried out an experiment to examine the effect of water stress on seven rice cultivars. Under mild drought stress conditions the plants showed a slight reduction in the growth rate of the stems and the growth rate reduction became more dramatic under severe stress. The plants subjected to drought stress showed a decrease in tillering rates compared with the well-watered plants. Under mild drought stress, the plants showed a slight reduction in tillering rates and the rates became more dramatic when the plants were subject to severe drought stress.

Hajibabaee *et al*. (2012) carried out an experiment in order to study the effect of drought stress on yield and some morphological and physiological traits in 14 new corn (*Zea mays* L.) hybrids. The main plots were three levels of irrigation; normal, mild stress and severe stress (irrigation after 70, 100 and 130 mm cumulative evaporation respectively) and subplots included 14 hybrid corn. Mean comparison of different irrigation treatments demonstrated that plants height was at the highest (178.54 cm) at normal irrigation conditions and under drought stress condition plant height was at the lowest with a mean height of 121.56 cm.

Rad and Zandi (2012) carried out an experiment in order to evaluate spring rapeseed (*Brassica napus* L.) cultivars subjected to drought stress. They found that water stress caused reduction in plant height, branch plant^{-1}, siliqua plant^{-1} and seed siliqua^{-1} and the highest plant height (122 cm) was that of normal irrigation treatment while applying water deficit stress caused the plant height to decrease by 9.4%. Besides, decreased soil moisture resulted in the reduced number of branches per plant from 5.6 in the control treatment to 4.5 in the water deficit treatment.

Khakwani *et al.* (2012) conducted an experiment where plants of 6 bread wheat varieties (Damani, Hashim-8, Gomal-8, DN-73, Zam-04 and Dera-98) were subjected to 2 treatments i.e. control treatment (100% field capacity) and stressed treatment (20 days water stress was given during booting stage and 20 days water stress after anthesis). The findings revealed highly significant differences among means of wheat varieties in all physiological and yield traits. Leaf area was decreased when 20 days water stress was given during booting stage and 20 days water stress after anthesis in all varieties. Similarly, plant height was also decreased significantly in all varieties when 20 days water stress was given during booting stage and 20 days water stress after anthesis.

Anđelković *et al*. (2012) conducted an experiment to evaluate tolerance of maize seedlings to water stress. The maize genotypes were exposed to 4% polyethylene glycol (PEG) for 24 h and 48 h. Then nine day old seedlings were analyzed and results showed reduction in all parameters under stress. Reduction in length ranged from 9.92% up to 40.65% and from 13.11% to 45.31% in root and shoot respectively. Reduction in root fresh weight varied from 11.25% up to 62.14% and from 22.9% to 63.93% in shoot with increase of osmotic stress as well the dry weight of root and shoot decreased with increased stress compared to control.

Ali *et al.* (2011) conducted a net house study to compare the efficacy of synthetic cytokinins, benzyl amino purine (BAP) with a natural source of cytokinins i.e., leaf extract of *Moringa olifera* as foliar application on maize seedlings subjected to different levels of drought as 75% & 50% of field capacity. They reported that severe drought stress highly reduced the leaf area which is due to accelerated leaf senescence caused by drought stress and also increase in drought intensity resulted in reduced plant height. Moreover, the plants grown at 75% field capacity showed maximum root density which was statistically at par with root density of normal irrigated plants while under severe drought stress the root growth was severely reduced due to reduced root penetration into dry soil and physical damage to young root tips.

Jatoi *et al.* (2011) studied twelve wheat cultivars of diverse characters and origin under water stress and found that plant height was affected by water stress and on an average was reduced by 4.73% compared to non-stressed condition.

Shahbaz *et al*. (2011) carried out an experiment to explore the effectiveness of exogenously applied Glycinebetaine as foliar spray in mitigating the harmful effects of drought on wheat crop using five wheat cultivars (SARC-I, Inqlab-91, MH-97, Bhakkar and S-24) under well-watered and water-stressed (60% of field capacity) conditions. They found that drought stress (60% of field capacity) markedly reduced shoot fresh and dry weights as well root fresh and dry weights of all wheat cultivars. Besides, shoot length and total leaf area per plant of all wheat cultivars decreased significantly under drought regimes.

Bilibio *et al*. (2011) assessed the effects of different levels of water deficit applied in rapeseed crop development in greenhouse with three levels of water deficit (0, 30, and 60% of evapotranspiration) in three treatments and 20 replicates. The treatment with 30% of deficit showed total green matter reduction (27%) as the most sensitive to water deficit followed by number of pods (22%), grain yield (19%), total dry matter (18%), number of leaves (14%), grain oil content (8%), number of branches (4%) and stem diameter (2%). The treatment with 60% of water deficit showed decrease mainly in grain yield, followed by total green matter (47%), number of branches (43%), total dry matter (39%), number of leaves (27%), stem diameter (18%) and grain oil content (11%), respectively.

Ribaut *et al.* (2009) reported that maize was most susceptible to water deficiency during flowering and water stress during this period induced stomatal closure and prolonged periods of water stress caused wilting and rolling, premature senescence of leaves and hence a reduction in the leaf area index and a fall in photosynthetic activity.

Seghatoleslami *et al.* (2008) carried out an experiment in order to study drought tolerance of three important species of millets, proso millet (*Panicum miliaseum*), foxtail millet (*Setaria italica*) and pearl millet (*Pennisetum americanum*).The crops were planted in a split-plot design with two irrigation treatments (well watered and

50% of irrigation requirement) . It was found that water stress caused reduction in the number of tillers, ears, peduncles, ear length and plant height.

Abdulai (2005) conducted a pot experiment in a greenhouse to evaluate the morphological and physiological responses of sorghum to different drought patterns. Pots were subjected to either constant drought or to progressive drought. It was found that constant drought and progressive drought conditions reduced growth and biomass accumulation as well as the gas exchange components.

Desclaux *et al*. (2000) reported that drought stress reduced the number of nodes which is a result due to the reduction of main stem height and the decreased node emergence rate.

2.2 Effect of water deficiency on physiological attributes of plant

Abdel-Motagally and El-Zohri (2016) conducted two field experiments to determine the effect of water stress on yield of wheat plant grown in calcareous soil during 2013-2014 and 2014-2015 seasons. T hey found that the mean values of chlorophyll a and chlorophyll b were decreased but the mean values of carotenoids, proline in leaves and roots, H_2O_2 in leaves and roots were increased under the condition of water stress in both the seasons.

Allahverdiyev *et al.* (2015) conducted an experiment to study the effect of soil water deficit on gas exchange parameters, photosynthetic pigments content, relative water content, area, dry weight, leaf specific mass of flag leaves from durum and bread wheat genotypes. They found that drought caused reduction in photosynthesis rate, stomatal conductance, transpiration rate, mesophyll conductance, pigments content, area, dry weight as well relative water content of flag leaves of wheat.

Mohammadi *et al*. (2015) carried out a pot experiment in greenhouse under well watered and terminal drought stress with two wheat cultivars Pishtaz and Karaj3. Terminal drought was induced by withholding water at anthesis stage and it was reported that terminal drought stress significantly reduced the chlorophyll fluorescence or the ratio between variable and maximal fluorescence.

Cechin *et al.* (2015) found that stomatal conductance was reduced by drought and this reduction was accompanied by a significant reduction in intercellular CO_2 concentration and photosynthesis in sunflower plants. Drought increased the level of malondialdehyde and proline and reduced pirogalol peroxidase activity, but did not affect the activity of superoxide dismutase. After four days of stress imposition, leaf and shoot dry weight and relative water content were reduced by about 23%, 22% and 13% respectively but stem dry weight accumulation was not affected.

Baranyiova *et al.* (2014) conducted an experiment to evaluate the effect of different growth regulators in winter wheat under water stress in 2013-2014. The results indicated a negative effect of drought on the CO_2 assimilation rate and stomatal conductance as in the untreated control these two reduced by almost 50%. The effect of drought was also reflected in the decrease of chlorophyll content in individual leaves within vertical profile.

Sepanlo *et al.* (2014) studied three different soybean genotypes at three different irrigation regimes in order to evaluate the morphological and physiological responses of soybean genotypes to water deficit. Plants were grown either under optimum condition (irrigated), drought stress implemented before the flowering (pre-anthesis) and pod-filling stage (post-anthesis). It was found that leaf relative water content (RWC) was significantly decreased in all genotypes by water deficit at both growing stages as well as progressive fall in chemical osmolytes and chlorophyll content. Interactive effects of genotypes and irrigation regimes for RWC and leaf biochemical attributes showed less decrease in early flowering stress, while when plants subjected to water stress at pod filling stage these characters significantly decreased in response to drought stress.

Akram *et al*. (2013) carried out an experiment to determine the effect of drought stress on some physiological and agronomic parameters of three rice cultivars naming Basmati-Super, Shaheen-Basmati and Basmati-385. It was observed that stomatal conductance was significantly impaired by water deficit at all growth stages in all the genotypes under study and the maximum reduction in conductance of 48.19% was observed under moisture stress at grain filling stage which was followed by anthesis and panicle initiation with decrease in conductance of 38.11% and 16.53 %, respectively. Besides, transpiration rate was adversely affected by water stress at all growth stages and in all the cultivars under study.

Ali *et al.* (2013) studied the physiological responses in twelve wheat genotypes under drought stress to identify drought tolerant genotypes. Stress was imposed by growing the genotypes under four irrigation treatments with each fifteen days interval. The results revealed that electrolytes leakage was increased and other physiological characteristics such as turgidity, relative leaf water contents and plant yield were decreased due to the increase in drought stress.

Yasmeen *et al.* (2013) conducted an experiment to evaluate the efficacy of foliar applied growth enhancers under water stress in wheat cultivar Sehar-2006. Three irrigation levels were maintained at 100, 75 and 50% field capacity designated as well watered, moderate and severe drought stressed. They reported that the increasing water stress decreased leaf chlorophyll *a* contents and minimum chlorophyll *a* and *b* contents were noted under extreme water stress. Moreover, increase in drought stress decreased the total soluble proteins in wheat leaves.

Ghiabi *et al.* (2013) conducted an experiment to assess the differential morpho physiological response to stimulated water deficit and to determine the relationship between some of these morphological and physiological traits and yield components of ten chickpea genotypes grown in field under irrigated and rain-fed conditions. They found that the relative water content, total chlorophyll content, Na^+ and K^+ uptake were decreased in water stress environments compare to irrigated environments as well in most of the genotypes proline content was accumulated higher in water-stress environment.

Khakwani *et al.* (2012) conducted an experiment where plants of 6 bread wheat varieties (Damani, Hashim-8, Gomal-8, DN-73, Zam-04 and Dera-98) were subjected to 2 treatments i.e. control treatment (100% field capacity) and stressed treatment (20 days water stress was given during booting stage and 20 days water stress after anthesis). The findings revealed highly significant differences among means of wheat varieties in all physiological traits. They found that six wheat varieties grown under water stressed condition decreased remarkably leaf area and relative water content as compared to the control plants.

Hajibabaee *et al.* (2012) carried out an experiment in order to study the effect of drought stress on yield and some morphological and physiological traits in 14 new corn (*Zea mays* L.) hybrids. The main plots were three levels of irrigation; normal, mild stress and severe stress (irrigation after 70, 100 and 130 mm cumulative evaporation respectively) and subplots included 14 hybrid corn. Due to means comparison, increase in drought stress decreased leaf area index. Mean comparison of different irrigation treatments showed that from irrigation after 70 to 100 mm of evaporation significantly decreased leaf chlorophyll, while although irrigation after 100 mm to 130 mm evaporation also reduced leaf chlorophyll but was not significant. Effect of different irrigation treatments on dry shoot weight was significant. This was because of the reduction in shoot dry weight in plants treated with irrigation after 130 mm evaporation. Due to higher decrement of dry stem weight (46%) in comparison to leaf dry weight (28%) in the treatment of irrigation after 130 mm evaporation leaf to stem ratio (dry) increased under stress conditions.

Nahar and Ullah (2012) carried out a pot experiment in Bangladesh to evaluate the effect of water stress on some morphological and physiological parameters of tomato plants. Two tomato cultivars, namely BARI Tomato-4 and BARI Tomato-5 were used and three treatments were imposed viz, 100%, 70%, and 40% of the field capacity (F.C.). It was found that the relative water content decreased significantly with increasing stress and the highest value was obtained from 100% F.C. (80.41) followed by 70%F.C. (78.72) and 40% F.C. (75.89) of the soil.

Almeselmani *et al*. (2011) studied tolerant and susceptible durum wheat varieties grown under rainfed conditions where plants were suffered from terminal drought stress. Significant reductions in Membrane Stability Index (MSI) in all varieties were recorded. The results from electrolyte leakage measurements showed that membrane integrity was conserved for tolerant compared to susceptible varieties. Significant difference in relative water content was observed in drought susceptible varieties as well reduction in chlorophyll fluorescence value was also observed in all drought susceptible varieties compared to tolerant varieties.

Ali *et al.* (2011) conducted a net house study to compare the efficacy of synthetic cytokinins, benzyl amino purine (BAP) with a natural source of cytokinins i.e., leaf extract of *Moringa olifera* as foliar application on maize seedlings subjected to different levels of drought as 75% & 50% of field capacity. They recorded least root fresh and dry weight in severely stressed plants. Besides, drought stress reduced the shoot fresh and dry weight significantly where maximum shoot fresh weight was observed at 75% field capacity and dry weight at 100% field capacity while severe drought stress produced the minimum shoot fresh and dry weights.

Akhkha *et al.* (2011*)* carried out a pot experiment to evaluate the effects of water stress on four cultivars of wheat as Al-gaimi, Sindy-1, Sindy-2 and Hab-ahmar. The plants were subjected to three water regimes well-waterd plants at 80% of the field capacity of soil (F.C.) and two levels of water stress; moderate stress at 50% F.C. and severe stress at 30% F.C. The water stress reduced the photosynthesis rates in Hab-ahmar and Sindy-2. The dark respiration was increased as a result of water stress in all cultivars, except Hab-ahmar. Chlorophyll content was reduced in all cultivars, except Al-gaimi. Both free proline and ABA showed generally an increase under water stress conditions.

Shahbaz *et al.* (2011) carried out an experiment to explore the effectiveness of exogenously applied Glycinebetaine as foliar spray in mitigating the harmful effects of drought on wheat crop using five wheat cultivars (SARC-I, Inqlab-91, MH-97, Bhakkar and S-24) under well-watered and water-stressed (60% of field capacity) conditions. They reported that stomatal conductance in all wheat cultivars was markedly higher under normal watering as compared to that under drought stress conditions. It was found that drought stress significantly reduced the photosynthetic rate of all five wheat cultivars.

Jatoi *et al.* (2011) studied twelve wheat cultivars of diverse characters and origin under water stress *and they found a* considerable reduction of 53.83% in relative water content (RWC %) due to water stress. In non-stress, the RWC % varied from 79.67 to 90.00% whereas the range was 34.34 to 45.56% in water stress conditions. Stomatal conductance was much higher in non-stress treatment than in water stress conditions which suggested that cultivars were resisting loss of water through transpiration in drought conditions. On an average, there was 51.14% reduction in stomatal conductance due to water stress at anthesis as well substantial decline of 40.40% was found in leaf area due to water stress.

Bogale *et al.* (2011) conducted an experiment to assess the different morpho-physiological response to stimulated water deficit and to determine the relationship between some of the morphological and physiological traits and yield components of eighteen durum wheat genotypes grown in pots. Water deficit significantly reduced the net photosynthesis rate, transpiration rate and stomatal conductance measured both at anthesis and grain-filling stages. Similarly, the value of initial fluorescence was increased while variable fluorescence, maximum fluorescence and optimum quantum yield fluorescence were decreased under water deficit. Besides, relative water content of the leaves was decreased by 36.7% due to moisture stress relative to the well-watered control.

El-Tayeb and Ahmed (2010) conducted an experiment to evaluate the effects of drought stress on some physiological and biochemical parameters of wheat cultivars (*Triticum aestivum*. cv. Giza 164) and (*Triticum aestivum*. cv. Gemaza 1). They reported that drought stress caused reductions of dry weight of shoot and root in wheat plants of both cultivars. Moreover, drought stress increased proline and sugar content while reduced K, Mg and Ca in shoots and roots of both cultivars.

Efeoğlu *et al.* (2009) conducted an experiment to investigate the physiological responses of maize (Zea mays L.) cultivars to drought conditions. Drought stress was imposed on the plants 12 days after sowing by withholding irrigation for 12 days and then rewatering for 6 days. It was reported that relative water content, fresh and dry biomass, minimum fluorescence, chlorophyll a, chlorophyll b, total chlorophyll (a+b) and carotenoid contents of all maize cultivars were significantly reduced under drought.

Pirdashti *et al.* (2009) conducted an experiment in order to investigate the physiological aspects of different rice cultivars under drought stress conditions. Drought stress in four levels (continuous irrigation or no water stress as a control, drought stress in vegetative, flowering and grain filling stages) and cultivars in four levels (Tarom, Khazar, Fajr and Nemat) were the treatments. They observed that drought stress in vegetative growth stage increased days to flowering and leaf rolling in different cultivars as well drought stress decreased chlorophyll content and relative water content (RWC) in different cultivars.

Al-Tabbal *et al.* (2006) carried out a greenhouse pot experiment during 2000-2001 growing season with two selected wheat cultivars, Hourani and Petra. They found that shoot dry weight for Hourani and Petra decreased up to 70% and 42% of their original weight respectively while the reduction in root dry weight was 52% and 42% respectively under water stressed conditions.

2.3 Effect of water deficiency on yield and yield contributing characters of plants

Jayalalitha *et al*. (2015) studied twelve *Bt* cotton hybrids to evaluate under rainfed condition (moisture stress) for morpho–physiological characters and seed cotton yield in a field experiment conducted during Kharif 2009 -10 and 2010-11. Significant differences were observed between main treatments viz., no stress (irrigation treatment) and stress (rainfed treatment) and sub treatments (12 *Bt* cotton hybrids). It was reported that seed cotton yield was decreased by 39.3 per cent (1634.44 kg ha -1 during 2009-10) and 25.6 per cent (1849.01 kg ha -1 during 2010-11) in rainfed plots compared to irrigated plots (2693.52 kg ha -1 during 2009-10 and 2486.10 kg ha -1 during 2010-11).

Raza *et al.* (2014) carried out an outdoor pot experiment and two field experiments with two wheat varieties under water stress (Auqab-2000 and Lasani-2008). They observed a significant decrease in grains per spike when stress was imposed on the plants. Drought stress at grain filling affected the crop growth and development significantly by affecting all the yield components as spike length, number of spikelets per spike and number of grain per spike and physiological parameters causing decline in the final grain yield of both the wheat varieties.

Singh *et al*. (2014) carried out an experiment to study the morpho-physiological attributes of Indian wheat cultivars of 10 genotypes. To evaluate the genotypes under drought stress seeds of 10 varieties were treated with polyethylene glycol for inducing drought condition. Significant reduction in yield components like seeds per spike, number of filled and unfilled seeds per spike and final grain yield was observed when drought was imposed at seed stage.

Mahpara *et al.* (2014) reported that in wheat crop, although drought stress affects all the growth stages of crop but yield is significantly reduced if drought stress occurs at various critical stages e.g. tillering, booting, anthesis, grain formation and grain filling stage.

Sepanlo *et al.* (2014) studied three different soybean genotypes at three different irrigation regimes in order to evaluate the morphological and physiological responses of soybean genotypes to water deficit. Plants were grown either under optimum condition (irrigated), drought stress implemented before the flowering (pre-anthesis) and pod-filling stage (post-anthesis). It was demonstrated that seed yield and measured morphological characters decreased from normal irrigation regime to water deficit stress in both flowering and pod filling stages.

Mirzaei *et al.* (2013) conducted an experiment to study the effect of drought stress at different growth stages on qualitative and quantitative traits and some agronomic traits of four canola cultivars in western Iran during 2008-2009 and 2009-2010 growing season. Main plots included four drought stress levels (Full irrigation, stress at flowering, pod developing and seed forming stages) and subplots included four cultivars (Hyola401, Hyola308, Zarfam and PF). It was demonstrated that drought stress treatments had significant effect on seed yield, number of seeds pod^{-1}, number of pod per plant, number of branches per plant, 1000-seed weight, plant height and oil content. Stress at flowering stage had the most effect on pod number per plant, number of seeds per pod, number of branches per plant and plant height. Seed forming stage was the most sensitive stage, so that 1000-seed weight and oil content were decreased in this stage. The highest (3151.25 kg.ha-1) and lowest (2377.08 kg.ha-1) seed yield belonged to full irrigation and water stress at flowering stage, respectively.

Akram *et al.* (2013) carried out an experiment to determine the effect of drought stress on some physiological and agronomic parameters of three rice cultivars naming Basmati-Super, Shaheen-Basmati and Basmati-385. They demonstrated that water stress drastically reduced 1000-grain weight in all the three rice cultivars at all growth stages under study. Decrease in 1000-grain weight was maximum (9.78 %) due to water stress at grain filling, it was followed by anthesis and panicle initiation with decrease of 7.69 and 4.87 %, respectively. Paddy yield per hectare was severely affected by water deficit. Moisture stress at panicle initiation was more drastic as regards per hectare paddy yield as reduction was maximum (39.79 %) which was followed by stress at anthesis and grain filling with decrease in per hectare paddy yield of 24.11 and 11.72 %, respectively.

Yasmeen *et al.* (2013) conducted an experiment to evaluate the efficacy of foliar applied growth enhancers under water stress in wheat cultivar Sehar-2006. Three irrigation levels were maintained at 100, 75 and 50% field capacity designated as well watered, moderate and severe drought stressed. They reported that increasing drought stress significantly reduced plant growth and yield.

Hajibabaee *et al.* (2012) carried out an experiment in order to study the effect of drought stress on yield and some morphological and physiological traits in 14 new corn (*Zea mays* L.) hybrids. The main plots were three levels of irrigation; normal, mild stress and severe stress (irrigation after 70, 100 and 130 mm cumulative evaporation respectively) and subplots included 14 hybrid corn. They demonstrated that mean comparison of different irrigation treatments significantly decreased ear dry weight by the increment of intervals between irrigation.

Rad and Zandi (2012) carried out an experiment in order to evaluate spring rapeseed (*Brassica napus* L.) cultivars subjected to drought stress. They revealed that water deficit stress caused reduction in 1000-seed weight, seed yield, biological yield, oil content, and oil yield. The largest number of siliquae per plant (325) was obtained from the normal irrigation but water deficit stress caused a 37% decrease and the largest number of seeds per siliqua (21.7) was obtained from the normal irrigation and applying water deficit stress caused a 12% reduction. They observed several reductions in the number of siliquae per plant (37%), the number of seeds per siliqua (12%) and total seed weight (9%) due to water deficit stress which tended to decline in the seed yield from 4137.5 kg ha^{-1} to 3184.9 kg ha^{-1}.

Nahar and Ullah (2012) carried out a pot experiment in Bangladesh to evaluate the effect of water stress on some morphological and physiological parameters of tomato plants. Two tomato cultivars, namely BARI Tomato-4 and BARI Tomato-5 were used and three treatments were imposed viz, 100%, 70%, and 40% of the field capacity (F.C.). It was found that flower and fruit characteristics of tomato plants i.e. flowers/cluster, fruits/cluster, clusters/plant, fruit stalk length, fruit length, and diameter and average fruit weight were affected by soil moisture stress.

Khakwani *et al*. (2012) conducted an experiment where plants of 6 bread wheat varieties (Damani, Hashim-8, Gomal-8, DN-73, Zam-04 and Dera-98) were subjected to 2 treatments i.e. control treatment (100% field capacity) and stressed treatment (20 days water stress was given during booting stage and 20 days water stress after anthesis). The findings revealed highly significant differences among means of wheat varieties in all yield traits such as the number of grains per main spike, 1000-grain weight, number of tillers per plant, biological yield per plant and grain yield per plant significantly decreased in all wheat varieties grown under stressed condition as compared to control treatment.

Bilibio *et al*. (2011) assessed the effects of different levels of water deficit applied in rapeseed crop development in greenhouse with three levels of water deficit (0, 30, and 60% of evapotranspiration) in three treatments and 20 replicates. Decrease in crop yield and number of pods were found by decrease in the volume of water applied to treatments, corresponding to evapotranspiration deficit. Maximum grain yield was observed in the treatment with 0% of water deficit (10.74 grams plant^{-1}). Later, there was a decrease of 0.1085 grams plant^{-1} for each unit change in water deficit condition.
Almeselmani *et al*. (2011) studied tolerant and susceptible durum wheat varieties grown under rainfed conditions where plants were suffered from terminal drought stress. They recorded that the number of grains per main spike, 1000-grain weight, number of tillers per plant, biological yield per plant and grain yield per plant were decreased under stressed environment.

Ali *et al.* (2011) reported that water stress decreased grain yield in maize and the reduced yield due to water stress was not only correlated with leaf area but also with chlorophyll *a* and *b.*

Jatoi *et al.* (2011) studied twelve wheat cultivars of diverse characters and origin under water stress and found that in non-stress, the range of seeds per spike in non-stress was 50-68 while in stress were 32-50 seeds per spike. Thus on average, water stress caused 31.09% decline in grains per spike. A sizeable decrease in grain yield per plant was also noticed and the average decline occurred by 27.51% due to water stress in wheat.

Kaur and Behl (2010) studied two varieties of wheat viz. WH730 and UP2565 to study the effects drought where drought led to 8 and 14% decrease in mean grain number/spike, 10 and 14% decrease in mean grain weight and 12 and 17% in mean grain yield per plant, respectively.

Ribaut *et al.* (2009) demonstrated that yield reduction in response to drought was most severe in maize when the stress occurred during three week period following flowering.

Seghatoleslami *et al.* (2008) carried out an experiment in order to study drought tolerance of three important species of millets, proso millet (*Panicum miliaseum*), foxtail millet (*Setaria italica*) and pearl millet (*Pennisetum americanum*).The crops were planted in a split-plot design with two irrigation treatments (well watered and 50% of irrigation requirement) . Deficit irrigation declined yield by reduction of seed number per ear and ear number per plant. Harvest index also reduced in the presence of drought stress due to of both seed per ear and per plant reduction.

2.4 Effect of Sodium nitroprusside (SNP) on plant growth and development under abiotic stress

Bibi *et al.* (2017) conducted two experiments during the year 2012 and 2013 in order to investigate the role of nitric oxide in mitigating chilling induced physiological variations of wheat (*Triticum aestivum* L.) at early seedling development stage. In case of both the experiments Nitric Oxide donor SNP was found to be effective in reducing cold stress.

Yadu *et al.* (2017) conducted an experiment to scrutinize efficacy of SNP to mitigate injury symptoms of saline stress in *Pisum sativum* L. They found that the limiting effects of salinity on radical length and biomass accumulation were considerably released by SNP. Supplemented SNP resulted in a substantial decline in reactive oxygen species accumulation, which later caused reduced accumulations of malondialdehyde, 4-hydroxy-2-nonenal and protein carbonyl. Moreover, SNP had significant inducing effects on activities of superoxide dismutase, catalase, guaiacol peroxidase and ascorbate peroxidase. Additionally, exogenous SNP led to the higher proline, sugar and glycinebetaine contents, than that of the control.

Suryavanshi *et al*. (2016) conducted a field experiment during *rabi* season of 2013-14 and 2014-15 at Ludhiana to evaluate the effect of osmoprotectant on productivity of wheat under terminal heat stress condition. It was revealed that during 2013-14, foliar application of sodium nitroprusside recorded higher plant height, leaf area index and dry matter accumulation at harvest over the unsprayed and water sprayed control. Grain yield also increased to the extent of 18.8% by foliar application of sodium nitroprusside. During 2013- 14, foliar spray of SNP recorded significantly higher grain yield (5.49 tonnes/ha) than water sprayed (4.73 tonnes/ha), unsprayed control (4.62 tonnes/ha) and the increase was 18.8% higher than untreated control.

Habib *et al.* (2016) assessed the effects of exogenously applied SNP on rice growth and yield under stress condition and found that seed priming with SNP was effective in reducing the adverse effects of stress on hundred grain weight and grain yield per plant in rice cultivars and of all SNP levels 0.1mM was more effective in reducing the adverse effects of stress on plant.

Sepehri and Rouhi (2016) studied the effect of sodium nitroprusside on enhancement of aged groundnut (*Arachis hypogaea* L.) seeds under drought stress. Seeds were primed in different solutions containing 0, 50, 100 or 150 µM sodium nitroprusside at 25 °C for 18 h and then subjected to drought stress for 10 days. Priming of aged seeds at different drought levels improved final germination percentage, germination rate, vigor index, seedling length, soluble sugars, total soluble proteins and the activities of antioxidant enzymes (catalase, superoxide dismutase, and ascorbate peroxidase) while mean germination time, electrical conductivity, malondialdehyde and hydrogen peroxide content decreased. Seed priming with 150 µM sodium nitroprusside increased germination rate, seedling length, activity of catalase, superoxide dismutase and ascorbate peroxidase to 38.62%, 66.66%, 39.14%, 14.43%, and 42.51%, respectively.

Esim and Atici (2015) carried out an experiment to examine the effect of pretreatments of nitric oxide (NO) on alleviation of chilling stress in wheat (*Triticum aestivum*). They revealed that application of SNP decreased the Malonedialdehyde level by 21% compared with chilling stress which indicated reduction of oxidative damage under stress.

Hameed *et al.* (2015) reported that SNP priming with 125 μM resulted in maximum increase in levels of protease, α-amylase, and α- and β- naphthyl acetate esterase activities in wheat seeds.

Esim *et al*. (2014) reported that exogenous SNP application as a donor of NO was found to have an important ameliorative effect on cold tolerance of seedling exposed to chilling stress by stimulating antioxidant enzyme activity.

Jamali *et al.* (2014) revealed that earlier application of SNP was more effective for an optimised protection against deleterious influence of salinity stress in strawberry plants because pre-treated plants had a sufficient time to develop an appropriate antioxidant response.

Hayat *et al*. (2014) conducted an experiment where seeds of tomato were soaked in double distilled water (control) or sodium nitroprusside (SNP). Proline content and antioxidant enzyme exhibited an increase in response to SNP. It was demonstrated that nitric oxide acted as a stimulator at low concentrations whereas at higher concentration it acted as a stress inducer.

Kausar *et al*. (2013) carried out an experiment to assess whether foliar-applied nitric oxide (NO) could alleviate the adverse effects of salt stress on wheat (*Triticum aestivum* L.). It was revealed that exogenous application of NO proved to be beneficial in enhancing plant growth parameters like dry weights and lengths of both shoot and root of salt-stressed plants.

Hasanuzzaman and Fujita (2013) studied the possible regulatory role of exogenous nitric oxide (NO) in mitigating oxidative stress in wheat seedlings exposed to arsenic (As). Seedlings were treated with NO donor (0.25 mM sodium nitroprusside, SNP) and As (0.25 and 0.5 mM Na2HAsO4_7H2O) separately and/or in combination and grown for 72 h. It was revealed that the exogenous application of NO rendered the plants more tolerant to As-induced oxidative damage by enhancing their antioxidant defense and glyoxalase system.

Shallan *et al.* (2012) carried out an experiment to investigate the effect of sodium niroprusside (SNP) on alleviation of drought stress in cotton plant. The cotton plants pre-treated with three concentrations of SNP (0.05, 0.1 and 1 mM) and then exposed to drought stress. Pretreatment of cotton plants under drought stress with SNP caused enhancement of growth and yield characters and increasing of pigments content, total soluble sugars, proline content, total free amino acids, total phenols, total soluble proteins, total antioxidant capacity and antioxidant enzyme activities. The optimum concentration of SNP to alleviate the drought stress in cotton plant was 0.05 mM.

Kumari *et al.* (2010) carried out an experiment to investigate the effect of long and short term Cadmium (Cd) stress in chickpea plants and evaluate the protective effect of exogenous nitric oxide (NO) supplementation using sodium nitroprusside (SNP). Sodium nitroprusside treatments decreased ion leakage and lipid peroxidation levels significantly. Nitric oxide showed its positive effect by further increasing the activities of antioxidant enzymes. SNP treatments decreased the Membrane injury (%) by 39 to 16 % and MDA content by 53 to 45 % after 24 and 72 h of application. About 50 % reduction in yield was found with long term Cd stress against only 12% decline under short term Cd stress whereas nitric oxide donor SNP treatments improved the yield and 50 % increase in seed yield was observed with long and short term Cd stress.

Duan *et al.* (2007) studied the effect of SNP, an NO donor, on seed germination of wheat (Triticum aestivum L.) under salt stress. They found that priming of seeds with SNP for 24 h markedly alleviated the decrease of the germination percentage, germination index, vigor index and imbibition rate of wheat seeds under salt stress.

SNP significantly alleviated the decrease of the beta-amylase activity but almost did not affect the alpha-amylase activity of wheat seeds under salt stress.

Beligni and Lamattina (2000) demonstrated that NO promoted seed germination and de-etiolation, and inhibits hypocotyls and internode elongation, processes mediated by light. Wheat seedlings sprayed with SNP and grown in darkness contained 30±40% more chlorophyll than control seedlings. These results implicated NO as a stimulator molecule in plant photomorphogenesis, either dependent on or independent of plant photoreceptors.

CHAPTER III

MATERIALS AND METHODS

The pot experiment was conducted at the field laboratory of Agricultural Botany Department, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from November 2015 to March 2016 to study the mitigation of adverse effect of water deficiency in wheat with sodium nitroprusside (SNP). The materials and methods those were used and followed during the experiment have been presented below:

3.1 Experimental site

This was a pot experiment conducted in the net house at the field laboratory of Agricultural Botany Department, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka. The location of the site is 23º74´N latitude and 90º38´E longitude with an elevation of 8.2 meter from sea level (Anon., 2004).

3.2 Characteristics of the Soil

The soil used in this experiment belongs to the Modhupur Tract under AEZ No. 28 (Anon., 1988). The characteristics of the soil in the experiment were analyzed in the Laboratory of Soil Science Department, Sher-e-Bangla Agricultural University, Dhaka. The details of the soil characteristics have been presented in Appendix III.

3.3 Climatic condition of the experimental site

The experimental site is situated in the subtropical monsoon climatic zone, which is characterized by heavy rainfall during the months from mid March to mid October, *kharif* season and scarcity of rainfall during the rest of the year, mid October to mid March, *rabi* season which is suitable for growing of wheat in Bangladesh.

3.4 Planting material

The variety BARI Gom 24 is known as Prodip was used as a planting material. The seeds of wheat were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. It is high yielding but sensitive to heat. The total crop duration is almost 102-110 days. In addition, this variety is also tolerant to *Bipolaris* leaf blight along with leaf rust (BARI, 2015).

3.5 Treatments of the experiment

The two factors experiment was carried out in Completely Randomized Design (CRD) with four replications having -

Factor (A) Water deficiency at different stages of wheat

i. D_0= Control
ii. D_c=Water deficiency at crown root initiation stage (20-29 DAS)
iii. D_b= Water deficiency at booting stage (45-54 DAS)
iv. D_a= Water deficiency at after anthesis stage (55-64 DAS)

Factor (B) Different concentrations of Sodium nitroprusside (SNP)

i. S_0= 0 mM SNP
ii. S_1=0.1mM SNP
iii. S_2=0.2 mM SNP

Total 12 treatment combinations were:

D_0S_0=No Water deficiency + 0 mM SNP

D_0S_1= No Water deficiency + 0.1 mM SNP

D_0S_2= No Water deficiency + 0.2 mM SNP

D_cS_0= Water deficiency at crown root initiation stage + 0 mM SNP

D_cS_1= Water deficiency at crown root initiation stage + 0.1 mM SNP

D_cS_2= Water deficiency at crown root initiation stage + 0.2 mM SNP

D_bS_0= Water deficiency at booting stage + 0 mM SNP

D_bS_1= Water deficiency at booting stage + 0.1 mM SNP

D_bS_2= Water deficiency at booting stage + 0.2 mM SNP

D_aS_0= Water deficiency at after anthesis stage + 0 mM SNP

D_aS_1= Water deficiency at after anthesis stage + 0.1 mM SNP

D_aS_2= Water deficiency at after anthesis stage + 0.2 mM SNP

3.6 Design and layout of the experiment

The two factors experiment was laid out in Completely Randomized Design (CRD) with four periods of water deficiency and three concentrations of sodium nitroprusside (SNP). Four replications were maintained in this experiment. The total number of unit pots was 48 (12×4). Each earthen pot was 35 cm in diameter and 30 cm in height. The experiment was set in the net house which was made by bamboo with nylon net and each pot was kept on the individual earthen plate to reuse the deposited water properly.

3.7 Fertilizer application and Pot preparation

The fertilizers N, P, K, S, Zn and B in the form of Urea, TSP, MP, Gypsum, Zinc Sulphate and Boric acid were applied into the soil @ 180 kg, 150 kg, 40 kg, 70 kg, 10 kg and 10 kg ha^{-1} respectively. Cowdung was mixed with the soil @ 10 t ha^{-1} 15 days before seed sowing. The entire amount of TSP, MP, Gypsum, Zinc Sulphate and Boric acid, two- third of urea and half of MP were applied in the soil during final preparation (BARI, 2015). Rest of Urea and MP were top dressed at tillering stage. The prepared soil was put into the pot and each pot contained 10 kg soil. All 48 pots were filled on 26 November, 2015. Weeds and stubbles were completely removed from the soil.

3.8 Seed sowing

Seeds were sown on 26th November 2015. Ten (10) seeds were sown in each pot. Seeds were placed in 2 cm depth and then covered with soil properly.

3.9 Application of the treatments

Water deficiency was induced by controlling the application of water to the plants in the pot. The scarcity of water was generated at three different period of wheat from vegetative to reproductive phase. The use of required amount of water was considered as control to analyze the effect of water deficiency in wheat at different period. Therefore the different water deficit conditions were- (i) control, well watered; (ii)water deficiency at crown root initiation stage (20-29 DAS); (iii) water deficiency at booting stage (45-54 DAS) and (iv) water deficiency at after anthesis stage (55-64

DAS). During these water deficit conditions soil moisture of 45%-55% field capacity was maintained which was determined by a soil moisture tester. Throughout these periods polythene sheet was used over the net to avoid rainfall.

As a water stress mitigating agent, foliar application of Sodium nitroprusside (SNP) was done through spraying at 0, 0.1 mM and 0.2 mM concentrations which were maintained by adding 0, 0.03 g and 0.06 g SNP respectively per liter of water and 0.1% of Tween-20 was used as an adhesive material. Two times foliar application of SNP was done through spraying with 5 days interval during each period of water deficit condition to the plants in accordance with the treatments by a hand sprayer at 1.00 -3.00 pm. The SNP in the form of $C_5FeN_6Na_2O.2H_2O$ was collected from the local market.

3.10 Intercultural Operations

After the germination of seeds, various intercultural operations such as watering, weeding, top dressing of fertilizer and plant protection measures were accomplished for better growth and development of the wheat seedlings.

3.10.1 Watering

Required watering was provided with water cane from seed sowing to grain filling period according to treatments. The amount of water was limited up to that quantity which does not leache out through the bottom. The water deposited on the earthen plate was poured into the pot again.

3.10.2 Thinning

Thinning was done as required and finally seven plants were kept in each pot.

3.10.3 Weeding

Weeding was done whenever it was necessary, mostly in vegetative stage.

3.10.4 Top dressing

After basal dose, the remaining dose of urea and MP was top-dressed in tillering stage and was applied on both sides of seedlings rows in the soil.

3.10.5 Plant Protection Measures

Marshal 20 EC was applied @ 2ml/ L water against aphid. Malathion 57 EC was applied @ 2 ml/ L of water against the cutworm when needed.

3.11 Harvesting

Maturity of crop was determined when 90% of the spike became golden yellow in color. Five plants per pot were preselected randomly from which different growth and yield attributes data were collected and then each pot was harvested separately and bundled, properly tagged and then brought to the threshing floor for recording grain yield. The grains were cleaned and sun dried to a moisture content of 12%.

3.12 Recording of Data

Experimental data were recorded from 70 days of sowing and continued up to harvest. The following data were recorded during the experiment.

Morphological characters

1. Plant height (cm)
2. Number of leaves plant^{-1}
3. Number of tillers plant^{-1}
4. Number of effective tillers plant^{-1}

Physiological characters

5. SPAD value

Yield contributing and yield characters

6. Spike length (cm)
7. Number of spikelet spike^{-1}
8. Non-effective spikelets spike^{-1}
9. Number of grains spike^{-1}
10. 100 grains weight (g)
11. Grain yield (g pot^{-1})
12. Mitigation (%)

3.13 Detailed Procedures of Recording Data

A brief outline of the data recording procedure followed during the experiment is given below:

Morphological characters

3.13.1. Plant height (cm)

Plant height was measured at harvest of five plants from each pot. The height of the plant was determined by measuring the distance from the soil surface to the tip of the leaf. Mean values of the collected data were recorded.

3.13.2. Number of leaves plant^{-1}

Leaf number was counted at 70 DAS. The number of leaves plant^{-1} was determined by counting the leaves of five plants from each pot and then averaged.

3.13.3. Number of tillers plant^{-1}

Number of tillers plant^{-1} were counted at 70 DAS of five plants from each pot and then averaged.

3.13.4. Number of effective tillers plant^{-1}

Number of effective tillers plant^{-1} were counted at 70 DAS of five plants from each pot and then averaged.

Physiological characters

3.13.5. SPAD value

Leaf chlorophyll content was measured in SPAD value using a hand-held SPAD meter (CCM-200, Opti-Science, USA) at 70 DAS. At each evaluation the value was measured 3 times from five leaves at different positions plant^{-1} and the average was used for analysis.

Yield contributing and yield characters

3.13.6. Spike length (cm)

Length of spike was measured at harvest of five plants from each pot and then averaged.

3.13.7. Number of spikelet spike^{-1}

The number of spikelet was counted from five spikes from each pot and then averaged.

3.13.8. Non-effective spikelets spike^{-1}

Non effective spikelets were counted from five spikes from each pot and then averaged.

3.13.9. Number of grains spike^{-1}

The number of grains was counted from five spikes from each pot and then averaged.

3.13.10. 100 grains weight (g)

100 grains were counted randomly from the total cleaned harvested grains of each individual treatment and then weighed and recorded which was expressed in grams.

3.13.11. Grain yield (g pot^{-1})

Grain yield pot^{-1} of wheat was determined by weighing total grain in the pot and recorded amount was expressed in gram.

3.13.12. Mitigation (%)

Mitigation percentage was determined by the following formula:

$$\text{Mitigation (\%)} = \frac{\text{Yield value of treatment combination}}{\text{Yield value of control treatment}} \times 100$$

3.14 Statistical Analysis

All the data collected on different parameters were statistically analyzed following the analysis of variance (ANOVA) technique using MSTAT-C computer package program and the mean differences were adjudged by least significant difference (LSD) test at 5% level of significance.

CHAPTER IV

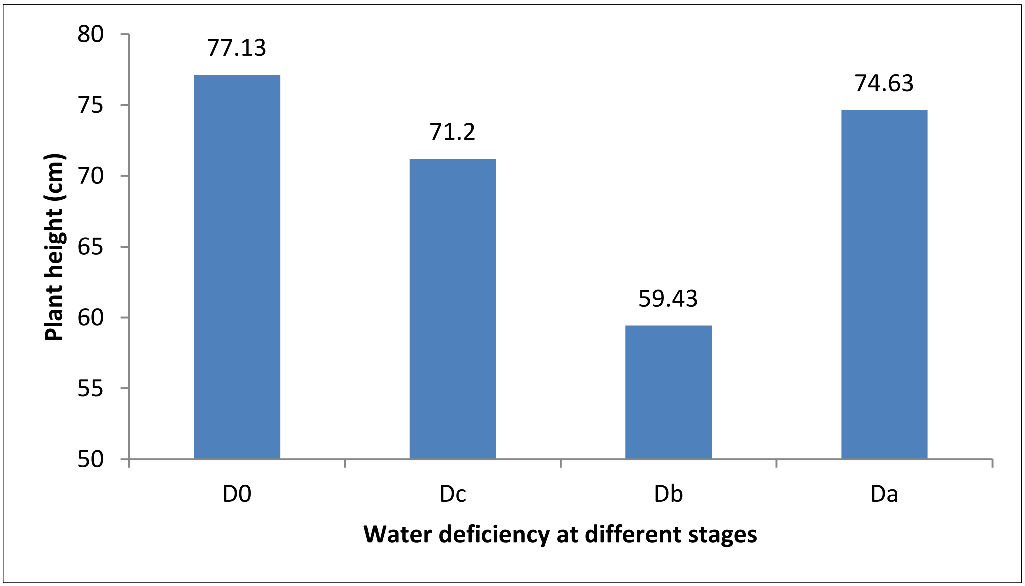
RESULTS AND DISCUSSION

The experiment was conducted to examine the mitigating effect of sodium nitroprusside on water deficiency at different growth stages of wheat. The results obtained with water deficiency at different growth stages (D) and different concentrations of sodium nitroprusside (S) and their combinations are presented and discussed in this chapter. Data on different morpho-physiological parameters and yield contributing characters of wheat have been presented in both tables and figures. Analyses of variance and corresponding degrees of freedom have been shown in appendices.

4.1 Plant height

Effect of water deficiency

Water deficiency is the major environmental threat which limits agricultural productivity worldwide by inducing physiological, morphological and biochemical changes in crop plants. This study was carried out to investigate the protective role of exogenous sodium nitroprusside (SNP) on mitigation of adverse effects of water deficiency in wheat. In this experiment, I imposed water deficiency in wheat at three different stages such as crown root initiation (D_c), booting (D_b) and after anthesis (D_a) stage other than control. Plant height is an important morphological feature which usually assists to analyze growth of crop plant. In this study, I measured plant height at harvest with a measuring scale. The experimental results of plant height showed significant variation to water deficiency at different stages of wheat plant (Figure 1 and Appendix IV). The water deficiency of each stage showed interesting results on changes in plant height. The booting stage (D_b) showed the lowest plant height (59.43 cm) compared to both crown root initiation and after anthesis periods. On the other hand, the highest plant height (77.13 cm) was found from D_0 or control. These findings are showing negative impact of water deficiency on plant height which are also supported by several researchers. Khakwani *et al.* (2012) reported that plant height decreased significantly in wheat varieties when 20 days water stress was given during booting stage and 20 days water stress after anthesis. It was found that water stress at vegetative stage significantly reduced plant height in rice (Pirdashti *et al.*, 2009). Desclaux *et al.* (2000) reported that drought stress reduced the number of nodes which is a result due to the reduction of main stem height and the decreased node emergence rate. It was suggested that the inhibitory effect of water stress on plant growth could be attributed to the low photosynthesis and reduced rate of cell division, expansion and enlargement by increasing stiffness of cell wall (Zhu *et al.*, 2004). All together, the results suggest that water deficiency significantly reduces the plant height and booting stage is the most sensitive compared to other stages in case of reduction of plant height under water deficit condition.

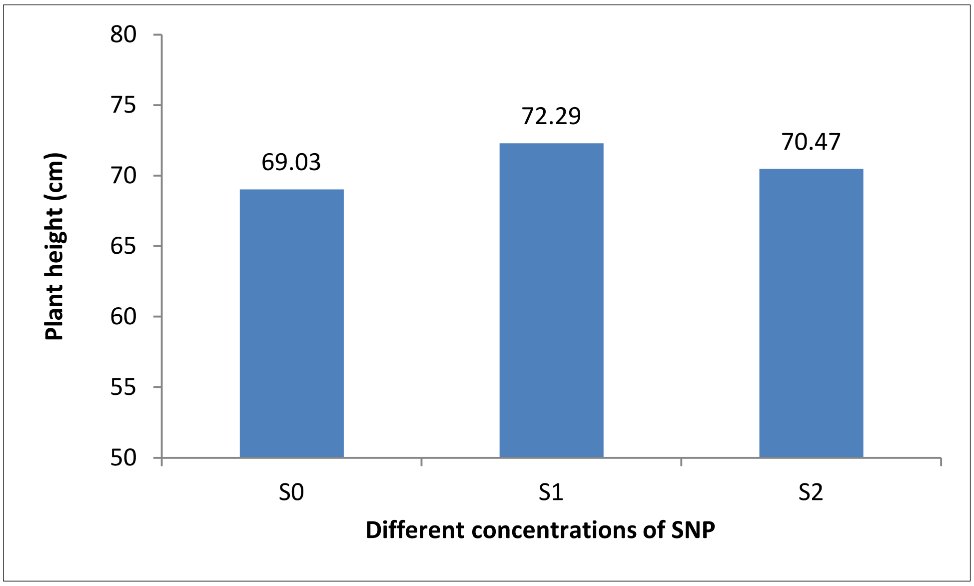


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 1. Effect of water deficiency at different stages on plant height of wheat ($LSD_{0.05}$ = 0.52)

Effect of sodium nitroprusside (SNP)

It has been reported that NO plays a key role in the plant growth and defense. NO is a small molecule which crosses cell membrane and acts as an important signaling messenger (Seabra *et al.*, 2014). However, so far I know no study has been conducted whether the role of NO on changes in plant height of wheat under Bangladesh environmental conditions. In this study, I used sodium nitroprusside (SNP) as a source of NO donor. The experimental results showed that SNP significantly increased plant height of wheat at harvest (Figure 2 and Appendix IV). At harvest, the tallest plant height was 72.29 cm, which was recorded from S_1 or 0.1 mM sodium nitroprusside (SNP) whereas the shortest 69.03 cm was found from S_0 or control. The results showed that the plant height was increased by the application of SNP and the height differed significantly with different concentrations of SNP. The results are consistent with several findings as Siddiqui *et al.* (2017) found that the application of SNP alone, as well as in combination with IAA, under both stressed and non-stressed condition, enhanced all growth characteristics including plant height and additionally, exogenous application of SNP increased fresh weight and dry weight plant^{-1}, and area leaf^{-1} compared with their respective controls under stressed conditions. Plant height at harvest was recorded higher by foliar application of SNP as compared to control (Suryavanshi *et al*., 2016). Although exogenous application of SNP alleviated the deleterious effects of abiotic stress in young rice plants, high concentrations of SNP treatment decreased growth rate (Uchida *et al.*, 2002). All together, the experimental results indicate that 0.1 mM SNP exhibited better result of plant height compared to control and other concentration.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 2. Effect of different concentrations of SNP on plant height of wheat (LSD $_{0.05}$= 0.45)

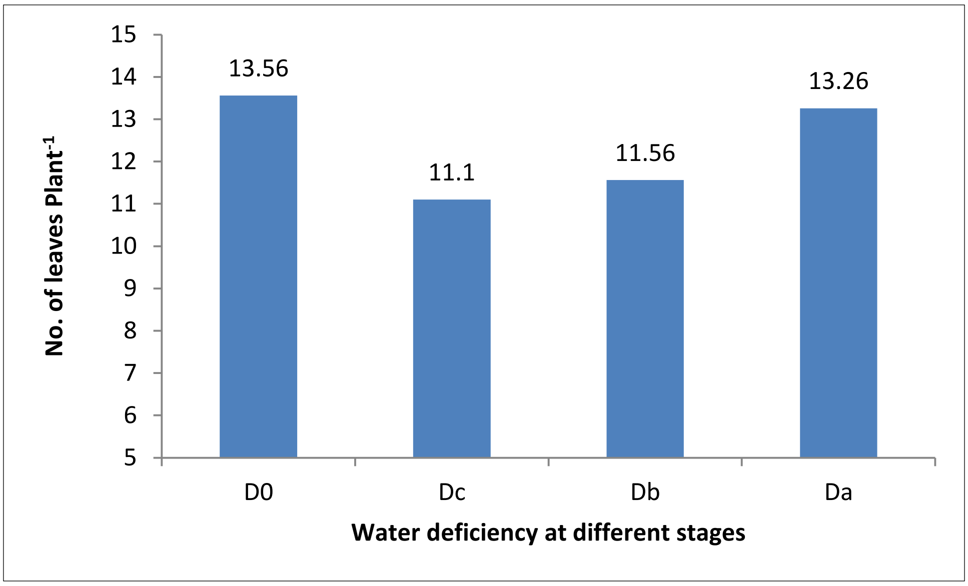
Interaction effect of water deficiency and sodium nitroprusside (SNP)

Interaction of water deficiency and SNP showed significant variation on plant height of wheat at harvest (Table 1 and Appendix IV). At harvest, the highest plant height, 78.40 cm was recorded from D_0S_1 (no water deficiency and 0.1 mM SNP) which showed significant difference statistically from other treatment combinations. On the other hand, the lowest height 56.90 cm was found from D_bS_0 (water deficiency at booting stage and 0 mM SNP) treatment combination which varied significantly from other treatment combinations. SNP is found to regulate auxin-mediated processes enhancing plant growth (Fernández-Marcos *et al.*, 2012). Application of SNP in wheat seedlings has been found to confer water deficit tolerance by maintaining more water than the well watered plants (García-Mata and Lamattina, 2001). Lei *et al*. (2007) found that application of exogenous SNP prevented drought-induced decrease in growth performance by enhancing proline accumulation and activation of antioxidant enzymes accompanied by a decrease in lipid peroxidation and H_2O_2 content under drought stress. Foliar-applied NO has been reported to enhance growth under both non-stressed and stressed conditions (Uchida *et al*., 2002). Overall, these experimental results specify that plant height of wheat was severely affected by water deficit condition at different stages whereas foliar application of SNP was found to be effective in increasing the height under water stress.

4.2 Number of leaves plant^{-1}

Effect of water deficiency

Water deficiency, being the most important environmental stress severely impairs plant growth and development, limits plant production and the performance of crop plants more than any other environmental factor. Water deficiency can reduce the number of leaves per plant which can consequently lessen photosynthesis. In this experiment, water deficit condition was imposed on different stages of wheat and to investigate the effect of water deficiency on wheat morphology, number of leaves plant^{-1} was counted at 70 days after sowing (DAS). It was observed that water deficiency caused significant difference on number of leaves plant^{-1} of wheat at 70 DAS (Figure 3 and Appendix IV). The water deficiency of each stage showed remarkable results on changes in number of leaves plant^{-1}. The crown root initiation (D_c) showed the lowest number of leaves plant^{-1} (11.10) compared to reduced value of both booting and after anthesis periods. The maximum number of leaves plant^{-1}, 13.56 was observed from D_0 or control. It is known that the production of new leaves depends on the availability of water. According to Shao *et al.* (2008), the number of leaves per plant, leaf size, and leaf longevity can be affected by water stress. It has been reported that leaf expansion is most sensitive to water stress during the early vegetative stage (Acevedo *et al*., 2002). But water stress at any growth stage decreases leaf area index (LAI) development due to a reduction in the number of leaves per plant, leaf expansion and leaf size (Zhang *et al.*, 1999). Bilibio *et al.* (2011) found that in rapeseed 60% of water deficiency decreased number of leaves by 27%, when compared to the control plant. All together, these experimental results suggest that water deficiency significantly reduces the number of leaves plant^{-1} and crown root initiation stage of wheat is more affected compared to other stages under water deficit condition.

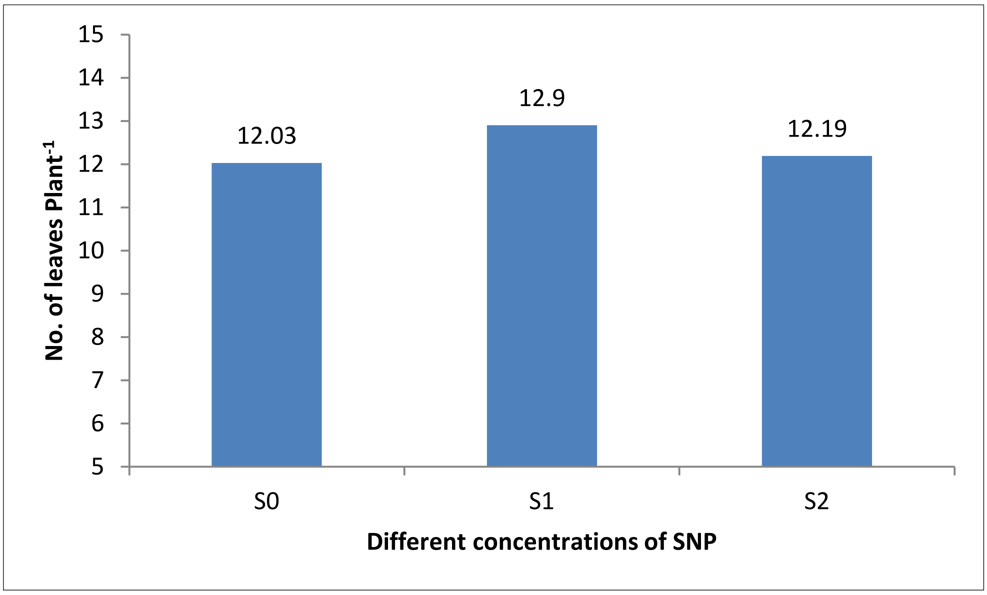


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 3. Effect of water deficiency at different stages on number of leaves plant^{-1} of wheat ($LSD_{0.05}$ = 0.22)

Effect of sodium nitroprusside (SNP)

NO, a small diffusible and ubiquitous molecule, improves the tolerance of plants to abiotic stress by activating a series of cellular signaling pathways. In this experiment, NO was used in the form of SNP to examine the effect of it in wheat plant under water deficit condition. In this study, foliar application of SNP showed significant effect on number of leaves plant^{-1} of wheat observed at 70 DAS (Figure 4 and Appendix IV). The maximum number of leaves plant^{-1}, 12.90 was found from S_1 or 0.1 mM SNP application whereas the minimum number of leaves plant^{-1}, 12.03 was observed from S_0 or control which is found statistically similar with S_2 or 0.2 mM SNP application. The results corroborated with several findings as García-Mata and Lamattina (2001) observed that treatment with SNP increased the survival rate of leaves of wheat and maize seedlings. Pretreatment with NO donor, SNP, protected young rice seedlings, resulting in better plant growth and viability (Uchida *et al.*, 2002). It has been found that leaf total phenolic content also increased significantly due to exogenous SNP treatment under adverse environmental conditions (Buss *et al.*, 2011). Overall, these experimental results reveal that application of SNP increases the number of leaves plant^{-1} of wheat under water deficit condition and of the two concentrations, 0.1 mM SNP is found to be more effective.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 4. Effect of different concentrations of SNP on number of leaves plant^{-1} of wheat (LSD $_{0.05}$= 0.19)

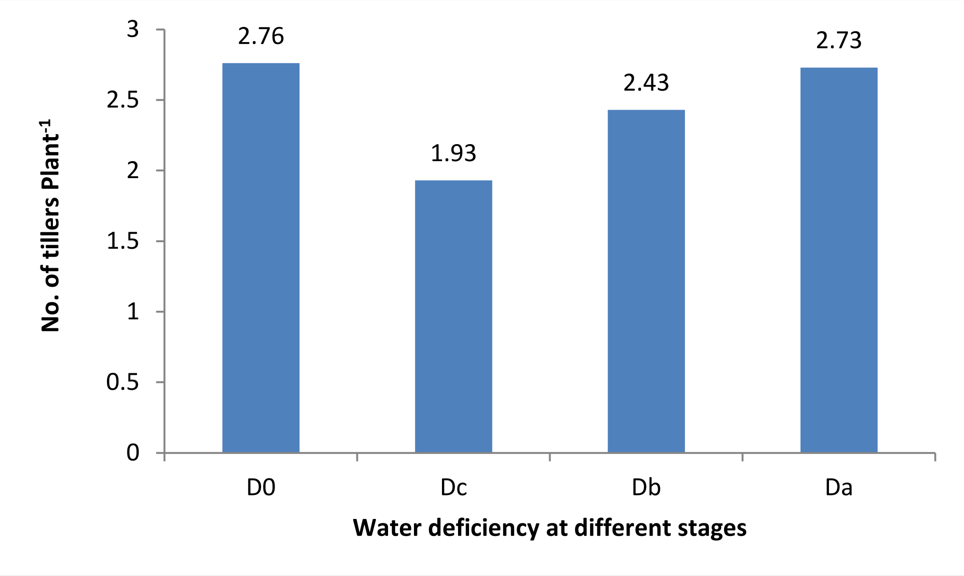
Interaction effect of water deficiency and sodium nitroprusside (SNP)

The combined effect of water deficiency and SNP showed significant variation on number of leaves plant^{-1} of wheat at 70 DAS (Table 1 and Appendix IV). The maximum number of leaves plant^{-1}, 14.60 was found from the combination treatment D_0S_1 (no water deficiency and 0.1 mM SNP) which varied significantly from other treatment combinations. The minimum number of leaves plant^{-1}, 10.80 was recorded from D_cS_0 (water deficiency at crown root initiation stage and 0 mM SNP) which is statistically similar with D_cS_2 (11.00) and D_bS_0 **(**11.10). Leaf growth inhibition is among the earliest responses to drought. It was reported that water stress reduced the number of leaves per plant and individual leaf size, leaf longevity by decreasing the soil's water potential (Rucker *et al.*, 1995). Hao *et al*. (2008) reported that treatment with NO prevented water loss and oxidative damage by enhancing SOD activity in maize leaves under water deficit conditions. Chohan *et al.* (2012) found that due to increase in number of nodes, number of leaves also increased by application of SNP. Taken together, these results suggest that, water deficiency at different stages causes reduction in number of leaves plant^{-1} of wheat under water deficiency while application of SNP can counteract the stress by increasing number of leaves plant^{-1}.

4.3 Number of tillers plant^{-1}

Effect of water deficiency

Water stress is one of the most common environmental stresses that affect growth and development of plants. In this experiment, water deficiency was imposed in wheat plant at three different stages other than control. In response to water stress, number of tillers plant^{-1} of wheat showed significant variation at different stages observed at 70 DAS (Figure 5 and Appendix IV). Variation in reduction of number of tillers plant^{-1} was found in different stages. The lowest number of tillers plant^{-1}, 1.93 was recorded from D_c or water deficiency at crown root initiation stage whereas the highest number of tillers plant^{-1}, 2.76 was recorded from D_0 or control which is found statistically similar with D_a (2.73). These findings are consistent with the findings of number of leaves plant^{-1} (Figure 3). It was reported that wheat crop is mainly sensitive to moisture deficit at tillering stage and number of tillers per unit area were reduced when stress was applied at tillering in wheat (Waheed *et al.*, 1998). Bunnag and Pongthai (2013) found that under mild drought stress rice cultivars showed a slight reduction in tillering rates and the rates became more dramatic when the plants were subject to severe drought stress. Seghatoleslami *et al.* (2008) reported reduction in the number of tillers in three important species of millets, Proso millet (*Panicum miliaseum*), Foxtail millet (*Setaria italica*) and Pearl millet (*Pennisetum americanum*) caused by water stress. All together, these results show that number of tillers plant^{-1} reduces under water deficit condition and crown root initiation stage is more sensitive than other stages in case of reduction of number of tillers plant^{-1} of wheat crop.

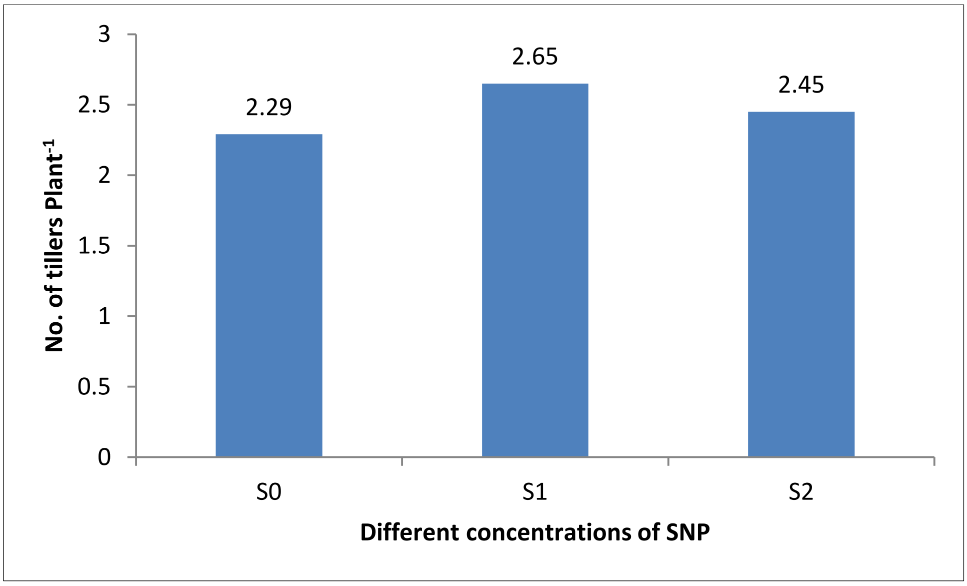


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 5. Effect of water deficiency at different stages on number of tillers plant^{-1} of wheat ($LSD_{0.05}$ = 0.12)

Effect of sodium nitroprusside (SNP)

SNP application mitigates decrease in plant growth caused by abiotic stress through improving antioxidant system and alleviating oxidative damage. In this experiment, SNP was used as a NO donor to alleviate water deficiency in wheat. Application of SNP had significant effect on number of tillers plant^{-1} of wheat observed at 70 DAS (Figure 6 and Appendix IV)). The maximum number of tillers plant^{-1}, 2.65 was found from S_1 or 0.1 mM SNP whereas the minimum number of tillers plant^{-1}, 2.29 was recorded from S_0 or control. It was reported by Huaifu *et al.* (2007**)** that exogenous SNP significantly alleviated seedling's injury caused by abiotic stress and increased seedling growth. Overall, the experimental results suggest that application of SNP increases the number of tillers plant^{-1} of wheat in response to water deficiency.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 6. Effect of different concentrations of SNP on number of tillers plant^{-1} of wheat (LSD $_{0.05}$= 0.10)

Interaction effect of water deficiency and sodium nitroprusside (SNP)

Interaction of water deficiency and SNP showed significant variation on number of tillers plant^{-1} of wheat at 70 DAS (Table 1 and Appendix IV). The maximum number of tillers plant^{-1}, 3.00 was recorded from D_0S_1 (no water deficiency and 0.1 mM SNP) which is statistically similar with D_0S_2 (2.80) and D_aS_1 (2.90). On the contrary, the minimum number of tillers plant^{-1}, 1.80 was recorded from D_cS_0 (water deficiency at crown root initiation stage and 0 mM SNP) which is statistically similar with D_cS_2 (1.90). The results are in agreement with several researchers. According to Musik *et al.* (1980) plant water stress limits leaf area and tiller development during vegetative growth in winter wheat and drought during jointing stage accelerates tiller death (senescence). The reduction of tillers production under lower soil moisture levels might be happened for less amount of water uptake to prepare sufficient food and inhibition of cell division of meristematic tissue (Zubarer *et al.,* 2007). Habib *et al.* (2016) demonstrated a significant ameliorative effect of seed priming with SNP on number of tillers under salt stress in rice cultivars and SNP levels 0.1 and 0.2 mM were found equally effective in this regard. Taken together, the experimental results suggest that water deficiency causes reduction in number of tillers plant^{-1} of wheat and application of SNP can reduce the effect of the stress by increasing number of tillers plant^{-1}.

Table 1. Interaction effect of water deficiency and SNP on plant height, number of leaves plant^{-1}, number of tillers plant^{-1}and number of effective tillers plant^{-1}of wheat

Treatment	Plant height (cm)	Number of leaves plant^{-1}	Number of tillers plant^{-1}	Number of effective tillers plant^{-1}
D_0S_0	75.90 c	13.00 c	2.50ef	2.50 b
D_0S_1	78.40 a	14.60 a	3.00 a	2.70 a
D_0S_2	77.10 b	13.10 c	2.80abc	2.40 b
D_cS_0	69.10 f	10.80 f	1.80 i	1.60 h
D_cS_1	73.70 d	11.50 e	2.10gh	1.90fg
D_cS_2	70.80 e	11.00 f	1.90 hi	1.80 g
D_bS_0	56.90 i	11.10 f	2.30fg	2.00ef
D_bS_1	61.60 g	11.90 d	2.60cde	2.30bc
D_bS_2	59.80 h	11.70 de	2.40ef	2.10 de
D_aS_0	74.23 d	13.23 bc	2.56 de	2.13cde
D_aS_1	75.46 c	13.60 b	2.90ab	2.40 b
D_aS_2	74.20 d	12.96 c	2.73bcd	2.26bcd
LSD $_{(0.05)}$	**0.90**	**0.39**	**0.20**	**0.16**
CV %	**5.81**	**6.41**	**4.24**	**4.39**

In a column, means with same letter (s) are not significantly different by LSD at 5% level of significance.

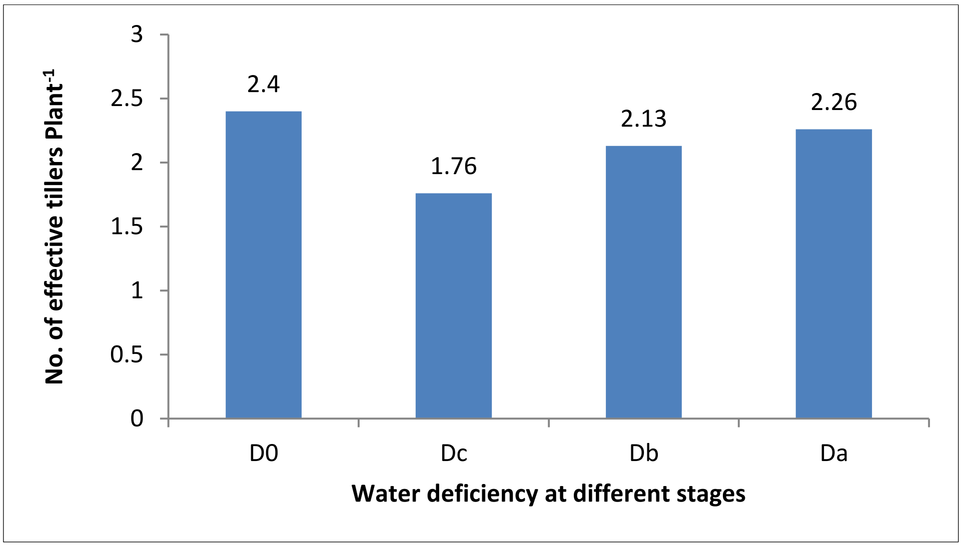
D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage; S_0: 0 mM SNP (control), S_1: 0.1 mM SNP, S_2: 0.2 mM SNP

4.4 Number of effective tillers plant^{-1}

Effect of water deficiency

Shortage of water limits the production of agricultural crops to varying degree throughout the world. In this experiment water deficiency was imposed at three different stages of wheat. The result showed that number of effective tillers plant^{-1}

varied significantly with water deficiency at different stages of wheat observed at 70 DAS (Figure 7 and Appendix IV). Water deficiency caused reduction in number of effective tillers plant^{-1} in each stage under water deficiency. The crown root initiation stage (D_c) showed minimum number of effective tillers plant^{-1}, 1.76 whereas the maximum number of effective tillers plant^{-1}, 2.40 was found from D_0 or control. The results are consistent with the findings of number of leaves plant^{-1} (Figure 3) and number of tillers plant^{-1} (Figure 5). The results are supported by several authors as Singh *et al.* (2010) evaluated that under drought condition number of ear bearing tillers reduced in six crosses of rice. The moisture shortage in the soil affected the stress tolerance by affecting fertile tillers number per plant in wheat (Dadbakhsh *et al.*, 2011). Akram (2011) reported reduction in number of fertile tillers when water stress was imposed at vegetative stage of wheat. All together, the experimental results suggest that crown root initiation stage of wheat is more sensitive to water stress compared to other stages in case of number of effective tillers plant^{-1}.

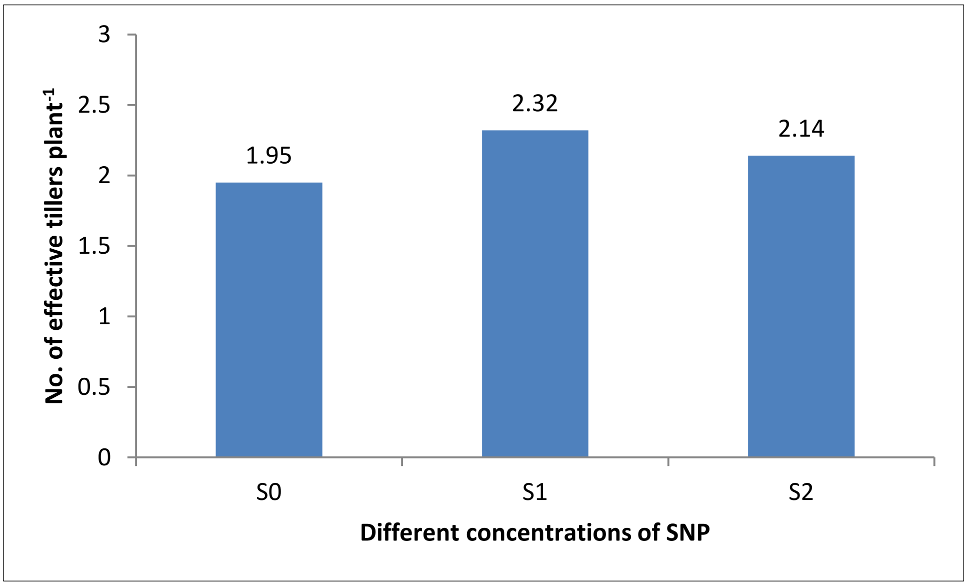


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 7. Effect of water deficiency at different stages on number of effective tillers plant^{-1} of wheat ($LSD_{0.05}$ = 0.09)

Effect of sodium nitroprusside (SNP)

NO is emerging as an important plant growth and stress regulator considerably, despite the current knowledge about its signaling pathway is still limited. NO donor SNP was used in this experiment to evaluate the efficiency of SNP in mitigating water deficiency of wheat. In this study, application of SNP caused significant variation on the number of effective tillers plant^{-1} of wheat observed at 70 DAS (Figure 8 and Appendix IV). The highest number of effective tillers plant^{-1}, 2.32 was found from S_1 or 0.1 mM SNP whereas the lowest number of effective tillers plant^{-1}, 1.95 was found from S_0 or control. Nasrin *et al.* (2012) reported that pretreatment of plants with SNP significantly enhanced the growth of chamomile plants under saline condition. Taken together, it can be said that exogenous SNP effects in increasing the number of effective tillers plant^{-1} in wheat under water deficit condition.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 8. Effect of different concentrations of SNP on number of effective tillers plant^{-1} of wheat (LSD $_{0.05}$= 0.08)

Interaction effect of water deficiency and sodium nitroprusside (SNP)

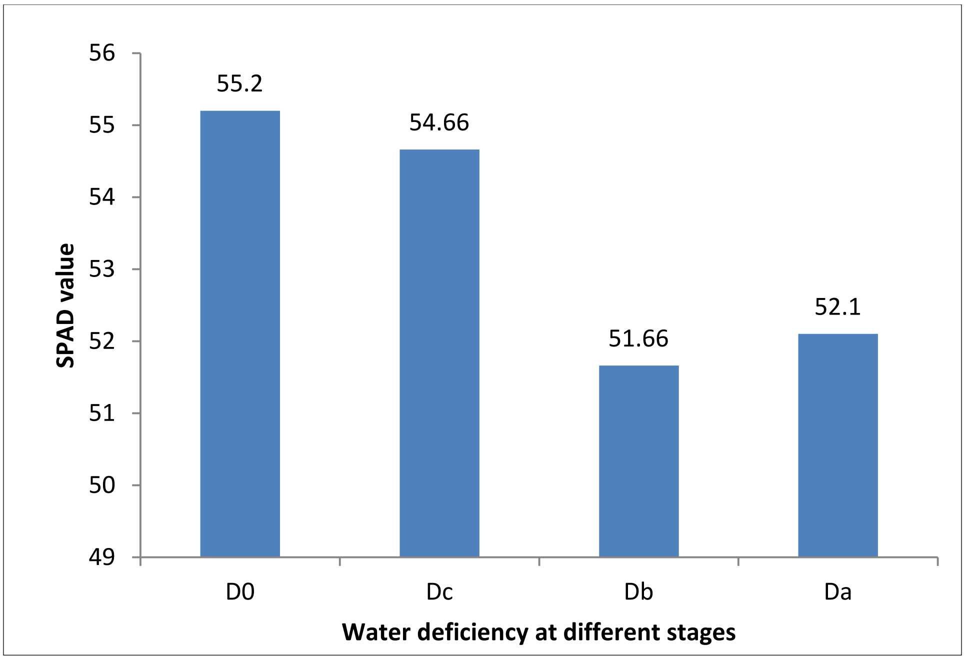
Number of effective tillers plant^{-1} was significantly influenced by interaction of water deficiency and SNP at 70 DAS (Table 1 and Appendix IV). The maximum number of effective tillers plant^{-1}, 2.70 was observed from D_0S_1 (no water deficiency and 0.1 mM SNP) which varied significantly from other treatment combinations whereas the minimum number of effective tillers plant^{-1}, 1.60 was recorded from D_cS_0 (water deficiency at crown root initiation stage and 0 mM SNP) which also showed significant variation from other treatment combinations. The findings are consistent with findings of number of leaves plant^{-1} (Table 1) and number of tillers plant^{-1} (Table

1). It was reported that tillering is very sensitive to water stress, being almost halved if conditions are dry enough (Peterson *et al.*, 1984). Severe water deficit during the vegetative stages results in reduced leaf area and this in turn affects tillering and spike size (Jones and Corlett, 1992*).* The yield reduction under water stress environment is attributed to the decrease in productive tillers per plant (Pal, 1992). Tian and Lei (2006) found that NO donor, SNP exerts a protective effect in wheat seedlings exposed to polyethylene glycol-induced drought stress, observed as enhanced growth and less oxidative damage. Overall, the experimental results suggest that the number of effective tillers plant^{-1} of wheat shows significant reduction under water deficiency while exogenous SNP has positive effect in increasing the number of effective tillers plant^{-1} in response to water deficiency.

4.5 SPAD value

Effect of water deficiency

Water deficiency is one of the most important manifestations of abiotic stress in plants. In this study, SPAD value of wheat crop was measured using a SPAD meter. SPAD value is measured to analyze leaf chlorophyll level of a plant. Chlorophyll is one of the major factors which regulate photosynthetic capacity of a plant. Reduction or no-change in chlorophyll content of plant under drought stress has been observed in different plant species and its intensity depends on stress rate and duration (Rensburg and Kruger, 1994). In this experiment, SPAD value observed at 70 DAS did not show any significant variation statistically with water deficiency at different stages of wheat (Figure 9 and Appendix V). But numerically the highest value 55.20 was recorded from D_0 or control whereas the lowest value, 51.66 was recorded from D_b or water deficiency at booting stage of wheat. In this study, significant effect of water stress was not found in case of SPAD value. The results are corroborated with Shahbaz *et al*. (2011) who observed a nonsignificant effect of saline stress on chlorophyll contents in sunflower. Kulshreshtha *et al.* (1987) found that drought stress did not have effect on chlorophyll concentration. In contrast, Massacci *et al*. (2008) reported a reduction in chlorophyll content in drought stressed cotton. Taken together, the results suggest that SPAD values do not change in wheat under water deficit conditions at different stages.

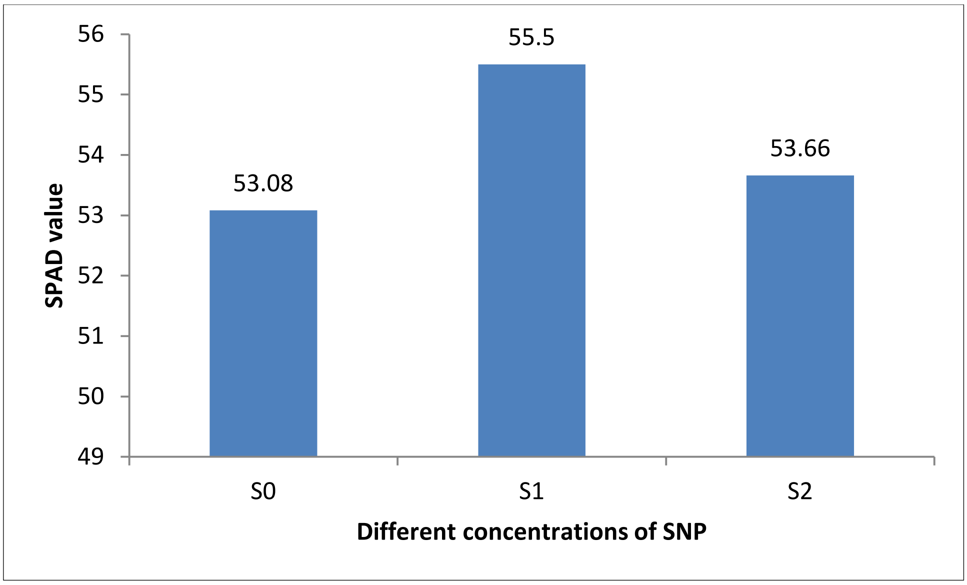


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 9. Effect of water deficiency at different stages on SPAD value of wheat ($LSD_{0.05}$ = 6.62)

Effect of sodium nitroprusside (SNP)

Nitric oxide acts to prevent oxidative damage induced by stressed condition which likely helps to maintain photosynthetic capacity as well as other major metabolic processes. In this study, application of SNP showed insignificant effect on SPAD value of wheat observed at 70 DAS. But numerically the highest SPAD value, 55.50 was found from S_1 or 0.1 mM SNP whereas the lowest value, 53.08 was found from S_0 or control (Figure 10 and Appendix V). Although the result showed insignificant effect but Ruan *et al.* (2004) stated that NO application was found effective in enhancing chlorophyll contents in wheat plants. Nitric oxide-induced high accumulation of chlorophyll was also observed in wheat under heat stress (Hasanuzzaman *et al.*, 2012). This phenomenon was not observed in the present study. The inconsistent findings observed in plant responses to water stress and to application of NO may be related to the different approaches used or to the variation in sensitivity of wheat cultivar. These experimental results showed that SNP application at 0.01 mM concentration increased the SPAD value of wheat leaves although the increase was found statistically insignificant.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 10. Effect of different concentrations of SNP on SPAD value of wheat (LSD $_{0.05}$= 3.41)

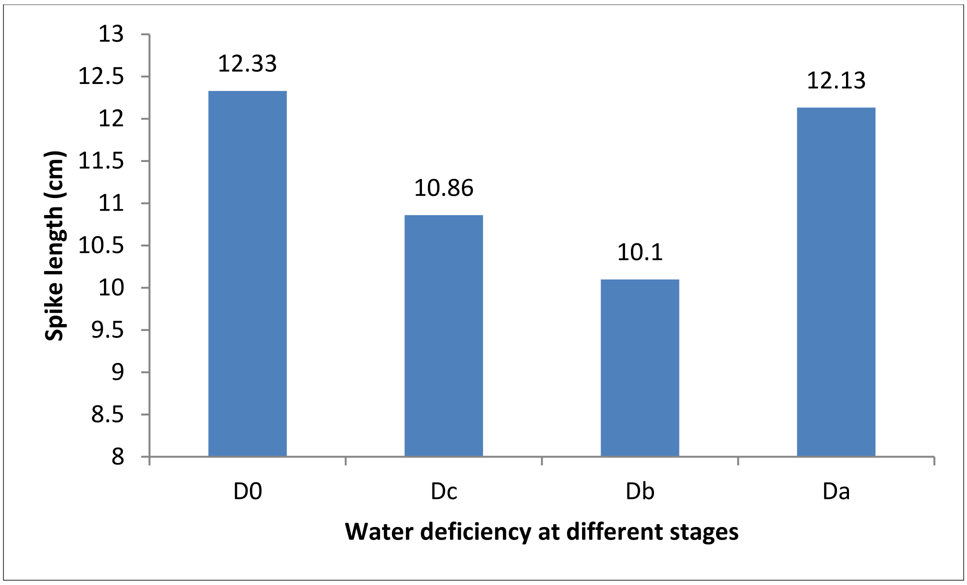
Interaction effect of water deficiency and sodium nitroprusside (SNP)

Interaction of water deficiency and SNP application did not show any significant difference statistically in case of SPAD value of wheat (Table 2 and Appendix V). But numerically slight difference was found as the highest value, 56.30 was recorded from D_0S_2 (no water deficiency and 0.2 mM SNP) and the lowest value, 50.93 was recorded from D_bS_0 (water deficiency at booting stage and 0 mM SNP) treatment combination. Several researchers reported that water deficit showed significant effect in chlorophyll content as Kiani (2008) found that the chlorophyll content decreased to a significant level at higher water deficits in sunflower plants. In contrast, David *et al.* (1998) demonstrated that dehydration increased the chlorophyll content in young leaves and that it was slightly decreased in the older ones. It has been found that NO applications increased leaf chlorophyll of plants under water stress, suggesting that NO treatment protected the photosynthetic apparatus (Fan & Liu, 2012). However, such prominent effect was not observed in our study. This might have been due to many factors, such as the type of wheat cultivar, NO concentration and time of application used. Taken together, it can be suggested that interaction effect of water deficiency and SNP did not show any significant effect in case of SPAD value of wheat.

4.6 Spike length (cm)

Effect of water deficiency

Water deficiency is the most important factor and ever-growing problem limiting wheat productivity worldwide. In this experiment, spike length was significantly influenced by water deficiency at different stages of wheat at harvest. Water deficiency caused significant reduction in spike length when water stress was imposed at crown root initiation and booting stages of wheat (Figure 11 and Appendix V). The lowest spike length, 10.10 cm was recorded from D_b or water deficiency at booting stage which showed significant statistical variation from others. On the contrary, the highest spike length, 12.33 cm was found from D_0 or control which is statistically similar with D_a or water deficiency at after anthesis stage. The results are consistent with the plant height described earlier (Figure 1). The reduction in spike length under water deficit condition is confirmed by several researchers. Singh *et al. (*2010) evaluated six crosses of rice in which panicle length reduced from 24.31 cm to 21.36 cm under drought condition. Raza *et al.* (2014) found that drought stress at reproductive stage affected the crop growth and development significantly by affecting all the yield components including spike length in wheat. In contrast, it was observed by Yadav *et al.* (2004) that under environmental stress conditions the spike length remains stable. Although spike length shows some stability under different conditions, Moayedi *et al*. (2010) demonstrated that it decreased significantly under water deficit during the floral initiation to anthesis period. All together, the experimental results indicate that booting stage of wheat was more affected under water deficiency compared to other stages.

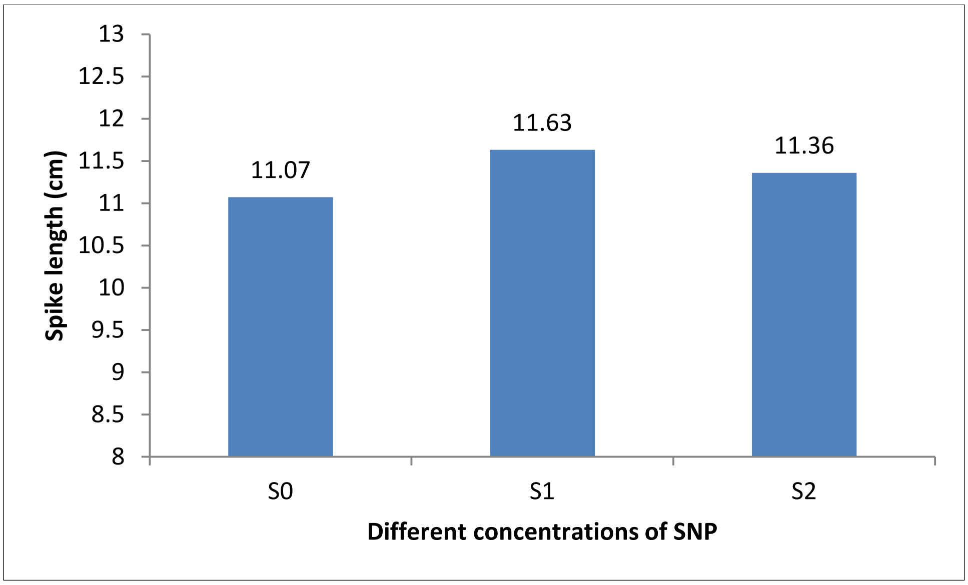


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 11. Effect of water deficiency at different stages on spike length of wheat ($LSD_{0.05}$ = 0.25)

Effect of sodium nitroprusside (SNP)

Nitric oxide (NO) acts as one critical component in several plant acclimation responses to both biotic and abiotic stress conditions. In this study, SNP was used as a NO donor to alleviate water deficit affects. Spike length of wheat was significantly influenced by different concentration of SNP which was observed at harvest (Figure 12 and Appendix V). Spike length significantly varied where the highest length, 11.63 cm was recorded from S_1 or 0.1 mM SNP application. On the other hand, the lowest spike length, 11.07 was found from S_0 or control which showed significant statistical variation from others. The experimental results suggest that SNP is an effective agent in increasing the spike length of wheat under water deficiency.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 12. Effect of different concentrations of SNP on spike length of wheat (LSD $_{0.05}$= 0.21)

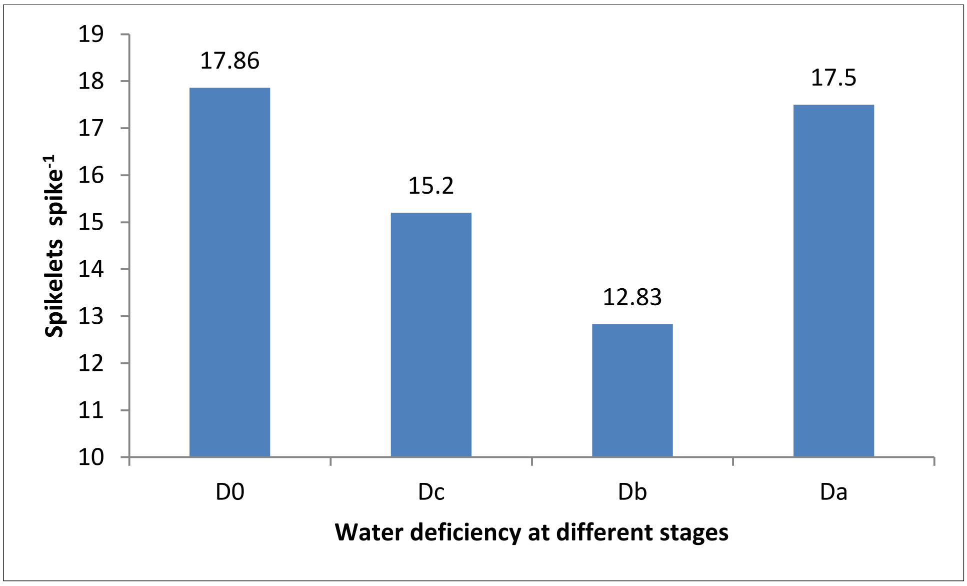
Interaction effect of water deficiency and sodium nitroprusside (SNP)

Interaction of water deficiency and SNP showed significant effect on the spike length of wheat observed at harvest (Table 2 and Appendix V). The highest spike length, 12.80 cm was recorded from D_0S_1 (no water deficiency and 0.1 mM SNP) which is found statistically similar with D_aS_1 (12.53 cm) and D_0S_2 (12.40 cm). The lowest length was found as 10.10 cm from three treatment combinations D_bS_0 (water deficiency at booting stage and 0 mM SNP), D_bS_1 (water deficiency at booting stage and 0.1 mM SNP) and D_bS_2 (water deficiency at booting stage and 0.2 mM SNP) which is found to be statistically similar with D_cS_0 (10.50). The result showed that spike length in booting stage was greatly affected by water stress which might be due to the lesser availability of carbohydrate during spike growth. Giunta *et al.* (1993) reported that the spike length was adversely affected by water deficit between stem elongation and ear formation stage. Abdel-Motagally and El-Zohri (2016) recorded the lowest mean value of spike length from the plants that received the lowest irrigation level or suffered water stress. The effect of sodium nitroprusside (SNP) treatment on wheat plant under drought stress was investigated by Wang *et al.* (2011) who found that the exogenous 0.2 mmol L^{-1} SNP treatment could significantly alleviate the stress injury and accelerate the progress of recovery. Taken together, the experimental results indicate that spike length of wheat was affected by water deficiency and application of SNP could ameliorate the negative effect of water deficiency at different stages though it did not show any major effect among the treatments of booting stage to increase the spike length.

4.7 Number of spikelets spike^{-1}

Effect of water deficiency

Water stress is one of the most adverse factors of plant growth and productivity and considered as a severe threat for sustainable crop production in the conditions on changing climate. In this experiment, water deficiency was imposed at three different stages of wheat. Number of spikelets spike^{-1} was counted which was found significantly influenced by water deficiency at different stages of wheat. Under water deficit condition each stage showed reduction in the number of spikelets spike^{-1} (Figure 13 and Appendix V).The minimum number of spikelets spike^{-1}, 12.83 was recorded from D_b or water deficiency at booting stage whereas the maximum number of spikelets spike^{-1}, 17.86 was recorded from D_0 or control. It was found that water deficiency caused significant reduction in case of number of spikelets spike^{-1}. In this study, the decrease in number of spikelets per spike at booting stage was highest. It was reported that water stress during jointing to anthesis stage decreases the spikelets per spike of fertile tillers (Moustafa *et al.*, 1996) and causes death of the distal and basal florets of the spikes (Oosterhuis and Cartwright, 1983). Floret abortion starts in the boot stage and finishes at anthesis. Under extreme environmental stress, all of the florets in the spikelets at the top and bottom of the head may abort prior to flowering. Floret death is probably, at least partially, due to competition for carbohydrates at this stage (Kirby, 1988). Carbon and nitrogen availability for spike growth are critical at this stage of development; both are decreased by water stress **(**Acevedo *et al.*, 2002). Number of spikelets spike^{-1} is more sensitive to drought stress in different cultivars of wheat (Dencic *et al*., 2000). Abdel-Motagally and El-Zohri (2016) demonstrated that spikelets spike^{-1} reduced drastically of wheat plant grown under water stress. Moayedi *et al.* (2010) demonstrated that the most susceptible stage to drought stress was during floral initiation to anthesis. All together, the experimental results suggest that water deficiency causes reduction in number of spikelets spike^{-1} and booting stage is more sensitive compared to other stages in case of reduced number of spikelets spike^{-1} under water stress.

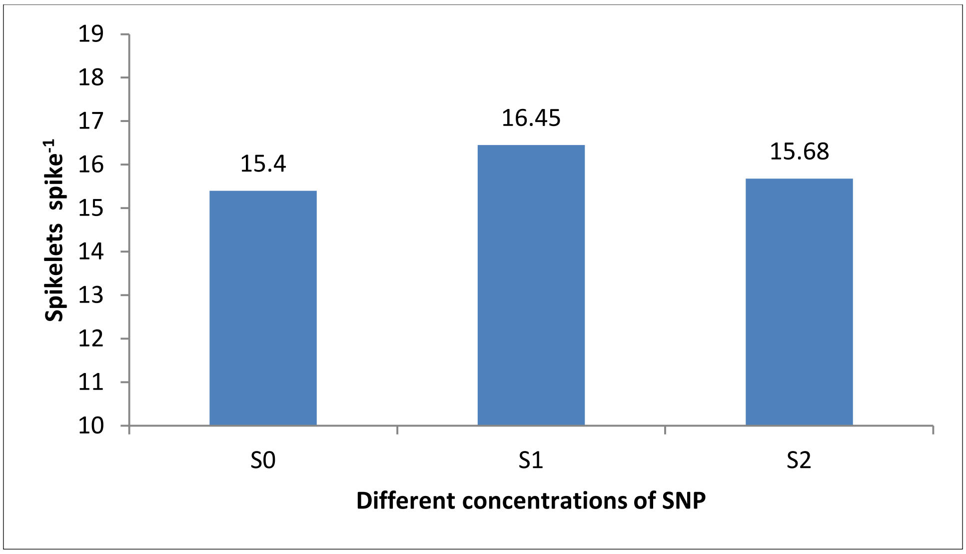


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 13. Effect of water deficiency at different stages on number of spikelets spike^{-1} of wheat ($LSD_{0.05}$ = 0.20)

Effect of sodium nitroprusside (SNP)

Application of SNP significantly increased the number of spikelets spike^{-1} of wheat (Figure 14 and Appendix V). Significant variation was caused by SNP application where the maximum number of spikelets spike^{-1}, 16.45 was found from S_1 or 0.1 mM SNP application and the minimum number of spikelets spike^{-1}, 15.40 was found from S_0 or control. Overall, the experimental results suggest that application of SNP acts in increasing number of spikelets spike^{-1} of wheat plant under water deficiency.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 14. Effect of different concentrations of SNP on number of spikelets spike^{-1} of wheat (LSD $_{0.05}$= 0.18)

Interaction effect of water deficiency and sodium nitroprusside (SNP)

Number of spikelets spike^{-1} was significantly varied with the combination treatments of water deficiency and SNP application (Table 2 and Appendix V). The maximum number of spikelets spike^{-1}, 18.60 was recorded from D_0S_1 (no water deficiency and 0.1 mM SNP) which differed significantly from other treatment combinations whereas the minimum number of spikelets spike^{-1}, 12.60 was recorded from D_bS_0 (water deficiency at booting stage and 0 mM SNP) which is statistically similar with the treatment combination D_bS_2 (12.70). Booting stage was found most affected in case of spikelets spike^{-1}. It might be due to reduced root growth about the time of spike formation that resulted in reduced nutrient uptake. Taiz and Zeiger (1991) reported that reduced number of spikelets per ear may be due to limited photosynthetic activity before spike emergence because spikelets per spike are determined before spike emergence. Raza *et al*. (2014) found a significant decrease in spikelets per spike recorded when stress was imposed on the plants. According to Frank *et al. (*1987), water stressed spring wheat got shorter spikelet development stages, resulting in fewer spikelets per spike. It was reported that water deficit between the terminal spikelets and the boot stage decreased the spikelet number per spike remarkably (Blum and Pnuel, 1990). Kausar *et al*. (2013) reported that exogenous application of 0.1 mM proved to be beneficial in enhancing plant growth parameters and yield contributing characters in wheat under stressed condition. Taken together, these experimental results suggest that water deficiency reduces the number of spikelets spike^{-1} which can counteract by application of SNP.

Table 2. Interaction effect of water deficiency and SNP on SPAD value, spike length, spikelets spike^{-1}, non-effective spikelets spike^{-1}of wheat

Treatment	SPAD value	Spike length (cm)	Spikelets Spike^{-1}	Non effective spikelets Spike^{-1}
D_0S_0	56.10	11.80 c	17.10 c	1.60ef
D_0S_1	52.20	12.80 a	18.60 a	1.50 f
D_0S_2	56.30	12.40ab	17.90 b	1.50 f
D_cS_0	53.90	10.50 e	14.60 e	1.70 de
D_cS_1	55.30	11.10 d	16.20 d	1.50 f
D_cS_2	54.80	11.00 d	14.80 e	1.60ef
D_bS_0	50.93	10.10 e	12.60 g	3.60 a
D_bS_1	52.26	10.10 e	13.20 f	3.40 b
D_bS_2	51.80	10.10 e	12.70 g	3.50ab
D_aS_0	52.30	11.90 c	17.33 c	1.80 cd
D_aS_1	52.23	12.53 a	17.83 b	1.66 e
D_aS_2	51.76	11.96bc	17.33 c	1.83 c
LSD $_{(0.05)}$	**8.13**	**0.43**	**0.36**	**0.11**
CV %	**4.24**	**5.22**	**6.31**	**6.16**

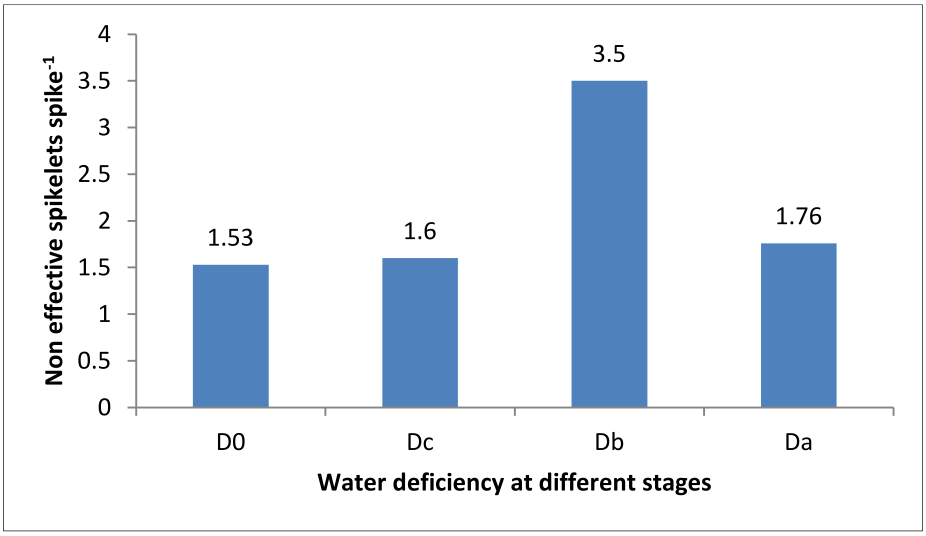
In a column, means with same letter (s) are not significantly different by LSD at 5% level of significance.

D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage; S_0: 0 mM SNP (control), S_1: 0.1 mM SNP, S_2: 0.2 mM SNP

4.8 Number of non-effective spikelets spike^{-1}

Effect of water deficiency

Water is a major limiting factor affecting plant growth, development and yield mainly in arid and semiarid regions where plants are often exposed to periods of water deficit stress also known as drought stress. In this experiment, water deficiency was imposed at three different stages of wheat. It was observed that the number of non-effective spikelets spike^{-1} was dramatically influenced by water deficiency imposed at different stages of wheat. The highest number of non-effective spikelets spike^{-1}, 3.50 was recorded from D_b or water deficiency at booting stage whereas the lowest number of non-effective spikelets spike^{-1}, 1.53 was found from D_0 or control (Figure 15 and Appendix V). The result showed that the number of non-effective spikelets spike^{-1} varied with different stages of wheat. Akram *et al.* (2004) reported that drought tension increased spikelet unproductivity in wheat. Taken together, these experimental results suggest that water deficiency causes increase in non-effective spikelets spike^{-1} and booting stage shows more sensitivity to water stress compared to other stages by increasing the number of non-effective spikelets spike^{-1}.



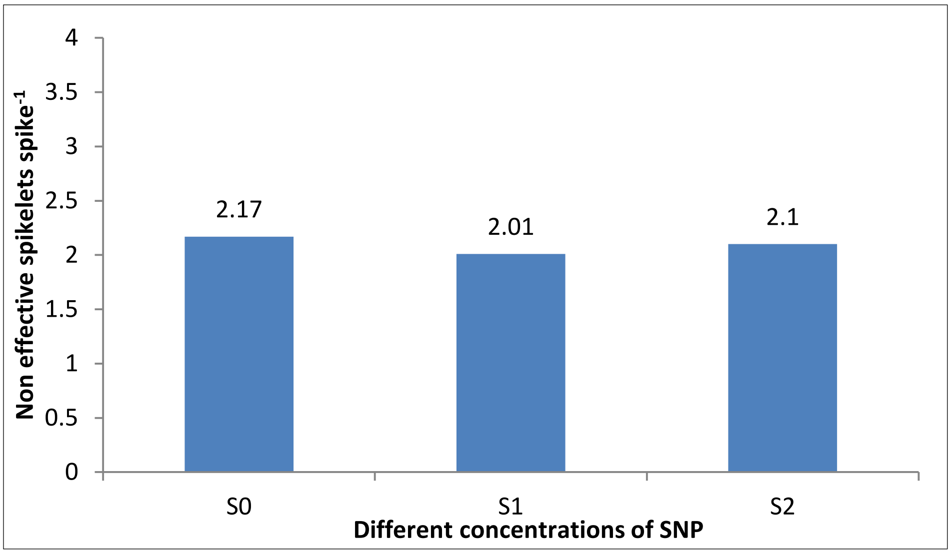
D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 15. Effect of water deficiency at different stages on number of non-effective spikelets spike^{-1} of wheat ($LSD_{0.05}$ = 0.06)

Effect of sodium nitroprusside (SNP)

NO stimulates defensive mechanisms in plants in order to reduce oxidative damage by improving the antioxidant system and maintaining ROS balance (Khan *et al.*, 2017). In this experiment, application of different concentration of SNP significantly decreased the number of non-effective spikelets spike^{-1} (Figure 16 and Appendix V). The highest number of non-effective spikelets spike^{-1}, 2.17 was recorded from S_0

whereas the lowest number of non-effective spikelets spike^{-1}, 2.01 was found from S_1 or 0.1 mM SNP. All together, the experimental results indicate that SNP application decreased the number of non-effective spikelets spike^{-1} under water deficit condition.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 16. Effect of different concentrations of SNP on number of non-effective spikelets spike^{-1} of wheat (LSD $_{0.05}$= 0.05)

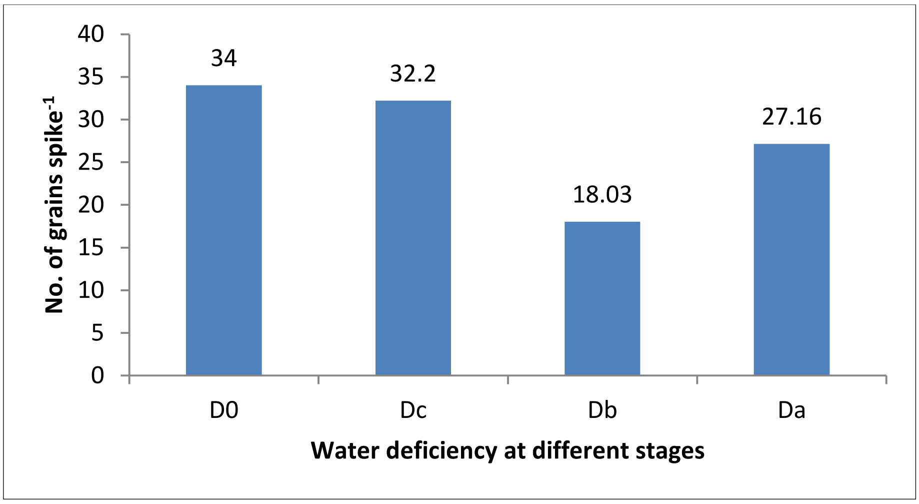
Interaction effect of water deficiency and sodium nitroprusside (SNP)

Number of non-effective spikelets spike^{-1} negatively influences the grain yield of crops. Combination of water deficiency and SNP was found to provide significant variation on the number of non-effective spikelets spike^{-1} (Table 2 and Appendix V). The highest number of non-effective spikelets spike^{-1}, 3.60 was recorded from D_bS_0 (water deficiency at booting stage and 0 mM SNP) which is statistically similar with D_bS_2 (3.50) and lowest number of non-effective spikelets spike^{-1}, 1.50 was found from three treatments D_0S_1 (no water deficiency and 0.1 mM SNP), D_0S_2 (no water deficiency and 0.2 mM SNP) and D_cS_1 (water deficiency at crown root initiation stage and 0.1 mM SNP) which is statistically similar with D_0S_0 (1.60) and D_cS_2 (1.60). Booting stage was found to be most affected by water deficiency. The scarce of carbohydrate caused by water stress affected spike growth and aborted florets during booting stage which might be the reason behind the increase in number of non-effective spikelets spike^{-1} at this stage. Ji *et al.* (2010) indicated that water stress at the reproductive stage of wheat leads to spikelet sterility. The result showed that SNP application ameliorated the negative effect of water stress on spike growth which corroborated with Shi *et al.* (2007) who demonstrated that the exogenous NO mitigated decrease in plant growth caused by stress through increasing antioxidant system and alleviating oxidative damage. Taken together, these experimental results suggest that water deficiency increases the number of non-effective spikelets spike^{-1} which can counteract by application of SNP.

4.9 Number of grains spike^{-1}

Effect of water deficiency

Water deficiency is a limiting factor in agriculture production by preventing a crop from reaching the genetically determined theoretical maximum yield. In this experiment, significant variation in the number of grains spike^{-1} was recorded when water deficiency was imposed at different stages of wheat. Each stage of wheat under water deficiency showed reduction in the number of grains spike^{-1} (Figure 17 and Appendix VI). The minimum number of grains spike^{-1}, 18.03 was recorded from D_b or water deficiency at booting stage whereas the maximum number of grains spike^{-1}, 34.00 was found from D_0 or control. These results are corroborated with several findings as Almeselmani *et al*. (2011) found that number of grains per main spike was decreased under stressed environment. Water stress imposed during later stages of wheat might cause a reduction in number of kernels/ear and kernel weight (Gupta *et al.*, 2001). Abayomi and Wright (1999) found that water stress at reproductive stage impairs the number of ears and number of kernels per ear, which ultimately leads to a reduction in yield compared to stress at other stages of wheat. All together, the results show that water deficiency caused significant reduction on the number of grains spike^{-1} and booting stage was most affected under water deficiency in case of the number of grains spike^{-1} of wheat.

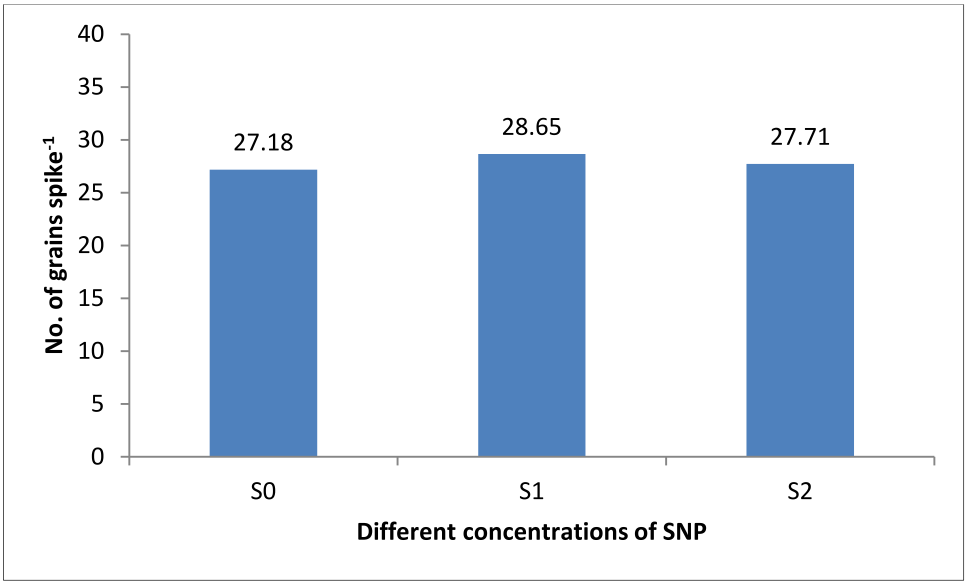


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 17. Effect of water deficiency at different stages on number of grains spike^{-1} of wheat ($LSD_{0.05}$ = 0.52)

Effect of sodium nitroprusside (SNP)

NO acts as a signaling molecule leading to alterations of anti-oxidative gene expression and thus protects plant cells from oxidative damage under abiotic stress (Arasimowicz and Wieczorek, 2007). In this study, the number of grain spike^{-1} was significantly increased by different concentrations of SNP application (Figure 18 and Appendix VI). The maximum number of grains spike^{-1}, 28.65 was found S_1 or 0.1 mM SNP whereas the minimum number of grains spike^{-1}, 27.18 was recorded from S_0 or control. In consistent with this finding Kausar *et al.* (2013) reported that exogenous application of NO as foliar spray markedly increased number of seeds per plant of wheat cultivar under stressed conditions. Besides, maximum number of seeds pod^{-1} was recorded with 150µM SNP treatment in chilling stress in chickpea plants (Chohan *et al.*, 2012). Altogether, the experimental results indicate that SNP application acts in increasing the number of grains spike^{-1} of wheat under water deficit condition.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 18. Effect of different concentrations of SNP on number of grains spike^{-1} of wheat (LSD $_{0.05}$= 0.45)

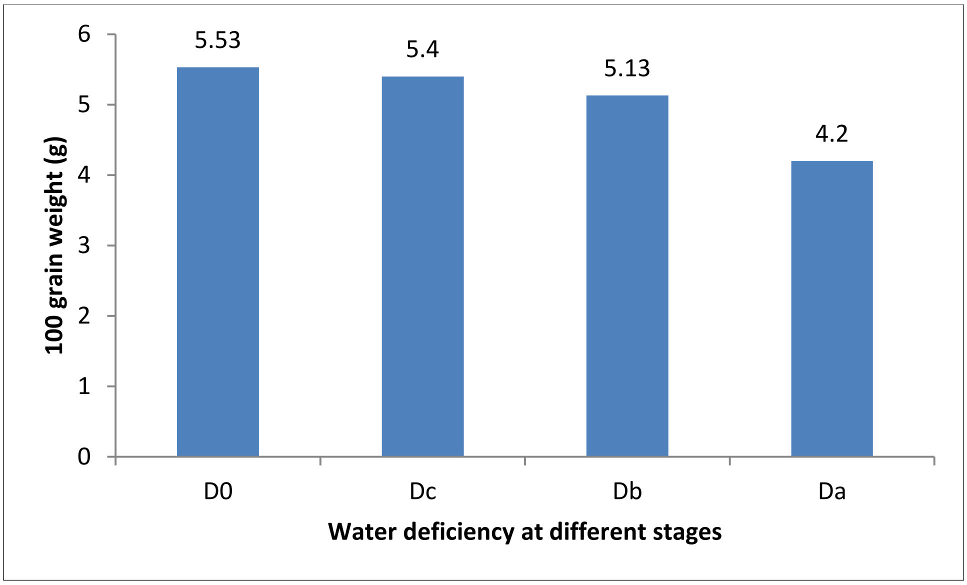
Interaction effect of water deficiency and sodium nitroprusside (SNP)

Combination of water deficiency and SNP showed significant variation on the number of grains spike^{-1} of wheat (Table 3 and Appendix VI). The maximum number of grains spike^{-1}, 34.60 was recorded from D_0S_1 (no water deficiency and 0.1 mM SNP) which is statistically identical with D_0S_2 (34.00) and the minimum number of grains spike^{-1}, 17.30 was recorded from D_bS_0 (water deficiency at booting stage and 0 mM SNP) which differed significantly from other treatment combinations. The result shows that number of grains spike^{-1} reduced drastically under water deficit condition. The findings supported by several researchers as Raza *et al*. (2014) reported a significant decrease in grains per spike when drought stress was imposed on wheat. Grain number decreases sharply when water stress occurs during the spike growth period (Hochman, 1982). Mirzaei *et al.* (2011) found that water stress at the reproductive stage of wheat decreased the number of kernels per spike. The yield components as grain number and grain size were decreased under pre-anthesis drought stress treatment in wheat (Edward *et al.*, 2008). The effect of SNP in increasing grains per spike corroborated with the findings of Suryavanshi *et al.* (2016) who observed that foliar application of SNP produced significantly higher number of grains/spike than untreated control in wheat under stressed condition. Taken together, these experimental results suggest that water deficiency reduces the number of grains spike^{-1} while application of SNP acts in increasing the number of grains spike^{-1} of wheat under water deficit condition.

4.10. 100 grains weight (g)

Effect of water deficiency

Water deficiency is an ever-growing problem that harshly limits the crop production and result in important agricultural losses especially in arid and semiarid areas. Grain weight (g) is positively related with grain yield of crop plant. In this study, significant variation on the 100 grains weight was found due to the imposed water deficiency and it varied with each stages of wheat (Figure 19 and Appendix VI). The lowest 100 grains weight, 4.20 g was recorded from D_a or water deficiency at after anthesis stage whereas the highest 100 grains weight, 5.53 g was recorded from D_0 or control. The results are supported by several researchers as Anjum *et al.*, (2011) reported that drought reduced plant yield components especially grain weight. Drought at reproductive stage decreased number of fertile tillers per meter square, number of grains per spike and 1000-grain weight which ultimately reduce the final grain yield of crop (Qadir *et al.*, 1999). Moreover, Jones *et al.* (1985) showed that water deficiency after anthesis reduces grain size and weight. Taken together, the results indicate that water deficiency caused significant reduction on the 100 grains weight and after anthesis stage was most affected under water deficiency in case of the 100 grains weight of wheat.

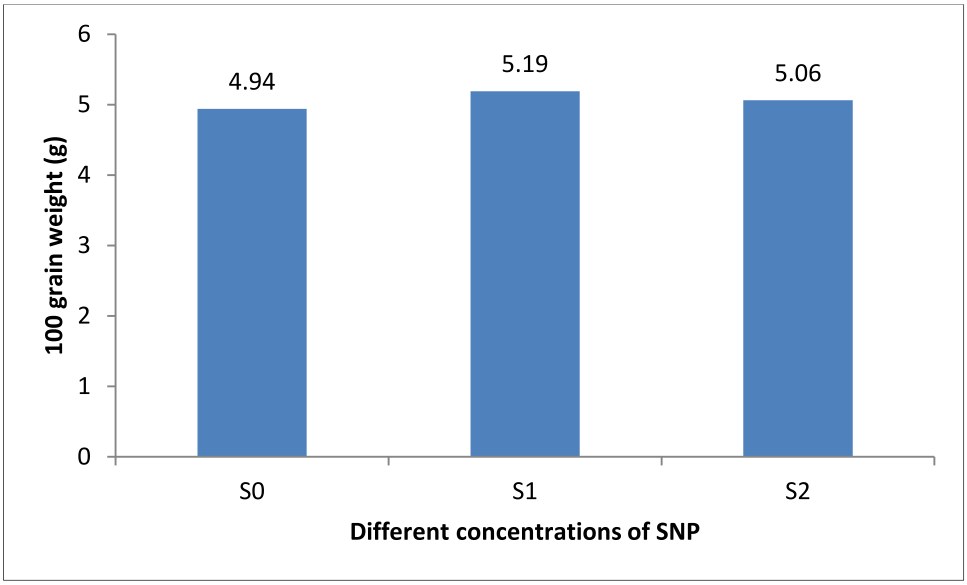


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 19. Effect of water deficiency at different stages on 100 grains weight (g) of wheat ($LSD_{0.05}$ = 0.04)

Effect of sodium nitroprusside (SNP)

NO in the form of SNP protects plant cells from oxidative damage under abiotic stress condition. In this experiment, grains weight was significantly influenced by different concentrations of SNP (Figure 20 and Appendix VI). It was found that 100 grains weight (g) of wheat was significantly increased by SNP application. The highest 100 grains weight, 5.19 g was recorded from S_1 or 0.1 mM SNP and the lowest 100 grains weight, 4.94 g was recorded from S0 or control. The results are supported by Kausar *et al.* (2013) who found that exogenous application of NO as foliar spray markedly increased 100-seed weight of wheat cultivar under stressed conditions. Overall, the experimental results indicate that SNP application acts in increasing the 100 grains weight of wheat under water deficit condition.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 20. Effect of different concentrations of SNP on 100 grains weight (g) of wheat (LSD $_{0.05}$= 0.03)

Interaction effect of water deficiency and sodium nitroprusside (SNP)

100 grains weight (g) of wheat varied significantly due to the interaction between water deficiency and SNP application (Table 3 and Appendix VI). The highest 100 grains weight, 5.70 g was found from D_0S_1 (no water deficiency and 0.1 mM SNP) which differed significantly from other treatment combinations. On the other hand, the lowest 100 grains weight, 4.16 g was found from two treatment combinations D_aS_0 (water deficiency at after anthesis stage and 0 mM SNP) and D_aS_2 (water deficiency at after anthesis stage and 0.2 mM SNP) which showed significant variation from other treatment combinations. The results showed that 0.1 mM SNP application influenced positively to increase the 100 grains weight (g) of wheat. Several researchers *as* Kumar *et al. (*2006) and Davatgar *et al.* (2012) observed that the percentage of unfilled grains were significantly higher in plants that were affected by drought at reproductive stage which ultimately affected grain weight. More reduced 1000-grain weight was observed in wheat when water deficit occurred at anthesis stage than at vegetative stage (Sinaki *et al*., 2007). The effective influence of SNP was confirmed by Habib *et al.* (2016) who assessed the effects of exogenously applied sodium nitroprusside (SNP) on rice growth and yield under stress condition and found that seed priming with only lower level (0.1 mM) of SNP was effective in reducing the adverse effects of stress on hundred grain weight in all rice cultivars.

Taken together, these experimental results suggest that water deficiency reduces the 100 grain weight in different stages while application of SNP acts in increasing the 100 grain weight of wheat under water deficit condition.

Table 3. Interaction effect of water deficiency and SNP on number of grains spike^{-1}, 100 grains weight (g), grain yield (g pot^{-1}) and mitigation (%) of wheat

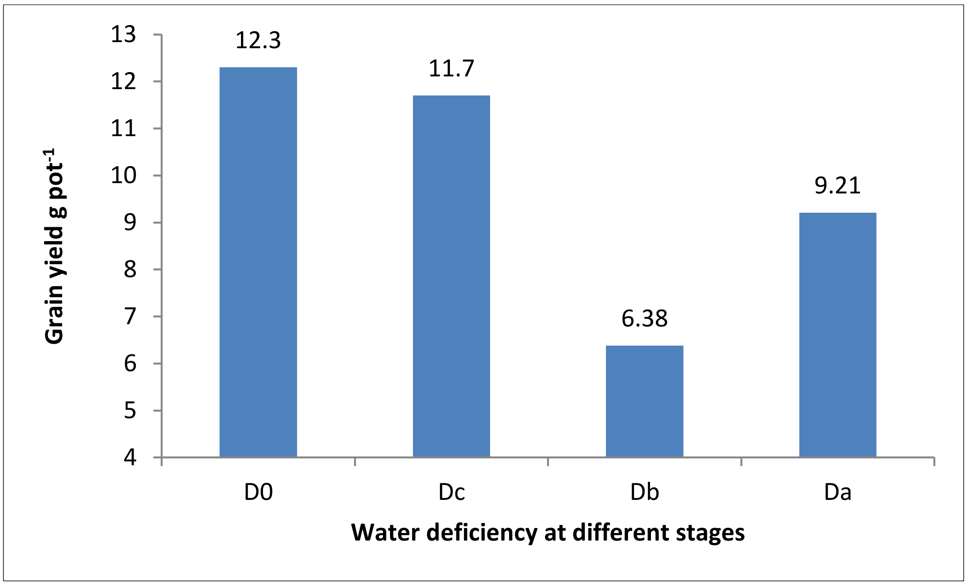
Treatment	Number of grains spike^{-1}	100 grains weight (g)	Grain yield (gpot^{-1})	Mitigation (%)
D_0S_0	33.40 b	5.51 b	11.52 b	100.00 b
D_0S_1	34.60 a	5.70 a	12.77 a	110.85 a
D_0S_2	34.00ab	5.50 b	12.61 a	109.46 a
D_cS_0	31.20 c	5.30 d	10.80bc	93.42 d
D_cS_1	33.50 b	5.50 b	11.10b	95.48 c
D_cS_2	31.90 c	5.40 c	10.90b	94.62 c
D_bS_0	17.30 g	4.90 f	5.60 g	48.61 i
D_bS_1	18.50 f	5.30 d	6.96 f	60.41 g
D_bS_2	18.30 f	5.20 e	6.6 f	57.29 h
D_aS_0	26.83 e	4.16 h	8.65 e	75.41 f
D_aS_1	28.00 d	4.26 g	9.79 d	81.68 e
D_aS_2	26.66 e	4.16 h	9.19 de	79.77 e
LSD $_{(0.05)}$	**0.90**	**0.07**	**0.71**	**2.05**
CV %	**5.45**	**5.26**	**5.61**	**5.99**

In a column, means with same letter (s) are not significantly different by LSD at 5% level of significance.
D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage; S_0: 0 mM SNP (control), S_1: 0.1 mM SNP, S_2: 0.2 mM SNP

4.11 Grain yield (g pot^{-1})

Effect of water deficiency

Water deficiency causes extensive loss to agricultural production worldwide, thus being a severe threat to sustainable agriculture. In this experiment, water deficiency was imposed at three different stages and the effect was observed in case of grain yield. Water deficiency at different stages showed significant effect on grain yield (g pot^{-1}) of wheat. Each stage under water deficiency showed reduction in yield compared to control (Figure 21 and Appendix VI). The lowest grain yield, 6.38 g pot^{-1} was recorded from D_b or water deficiency at booting stage whereas the highest grain yield, 12.30 g pot^{-1} was recorded from D_0 or control which significantly varied from others. In this study, grain yield was found to be severely reduced at booting stage by water deficiency which are consistent with the morphology and yield contributing characters of this experiment such as plant height (Figure 1), spike length (Figure 11), spikelets spike^{-1} (Figure 13) and grains spike^{-1} (Figure 17). The results are corroborated with several findings as Rashid *et al.* (2003) found that wheat plants subjected to terminal and pre-anthesis drought were severely damaged and the reduction in yield was 40% at pre-anthesis water stress. Atteya (2003) demonstrated that tassel emergence stage was more sensitive to drought than vegetative stage which reduced grain yield in maize. According to Blum and Pnuel (1990), water limitation during the terminal spikelets to booting stage affected yield and yield components in wheat. Chang and Suo (2007) studied the effects of water stress on grain yield of wheat and found that water deficiency before heading resulted in severe yield loss. All together, the experimental results suggest that booting stage is most affected by water deficiency causing significant reduction in grain yield.

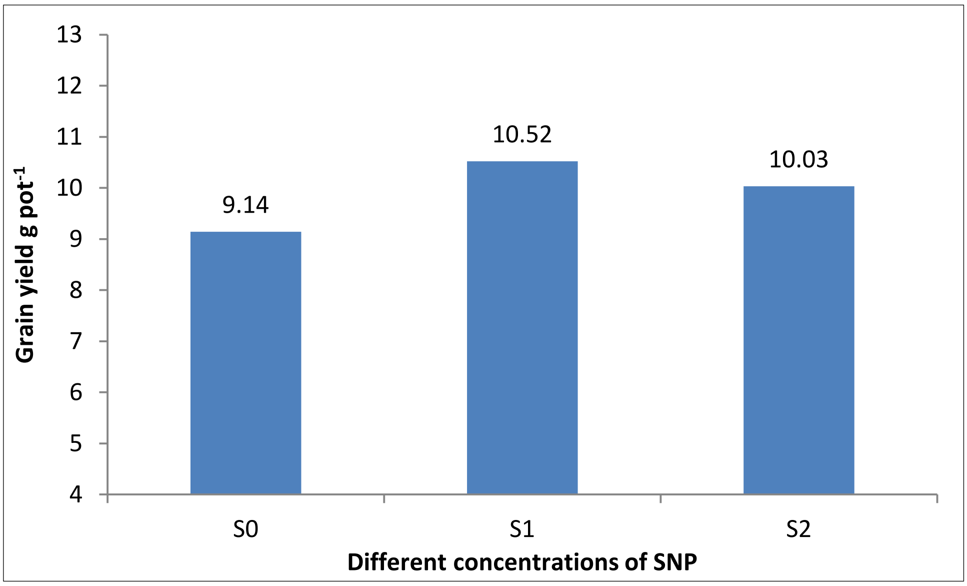


D_0: Control, D_c: Water deficiency at Crown root initiation stage, D_b: Water deficiency at booting stage, D_a: Water deficiency at after anthesis stage

Figure 21. Effect of water deficiency at different stages on grain yield (g pot^{-1}) of wheat ($LSD_{0.05}$ = 0.41)

Effect of sodium nitroprusside (SNP)

It is well known that nitric oxide (NO) exists in plant cells and intercellular spaces widely and it is a ubiquitous signal molecule involved in multiple plant responses to environmental stress (He *et al.*, 2012). In this experiment, SNP was used as a NO donor that acted under water deficit condition of wheat. It was observed that grain yield (g pot^{-1}) of wheat was significantly influenced by different concentrations of SNP (Figure 22 and Appendix VI). In this study, SNP application was found to increase grain yield of wheat. The highest grain yield, 10.52 g pot^{-1} was recorded from S_1 or 0.1 mM SNP whereas the lowest grain yield, 9.14 g pot^{-1} was recorded from S_0 or control. These results are consistent with the morphology and yield contributing characters of this study such as plant height (Figure 2), number of leaves plant^{-1} (Figure 4), tillers plant^{-1} (Figure 6), effective tillers plant^{-1} (Figure 8), spike length (Figure 12), spikelets spike^{-1} (Figure 14), grains spike^{-1} (Figure 18) and 100 grains weight (Figure 20). The findings are corroborated with Kaur *et al.* (2006) who reported that exogenous application of SNP increased the total yield in mungbean since SNP has potential for conversion of flowers to pods. Chohan *et al*. (2012) observed maximum number of pods plant^{-1} with SNP treatment in chick pea plant under stress. Overall, the experimental results indicate that SNP application acts in increasing grain yield of wheat under water deficit condition.



S_0: 0 mM SNP (Control); S_1: 0.1 mM SNP; S_2: 0.2 mM SNP

Figure 22. Effect of different concentrations of SNP on grain yield (g pot^{-1}) of wheat (LSD $_{0.05}$= 0.35)

Interaction effect of water deficiency and sodium nitroprusside (SNP)

Interaction of water deficiency and SNP showed significant variation on the grain yield (g pot^{-1}) of wheat (Table 3 and Appendix VI). The highest grain yield, 12.77 g was recorded from D_oS_1 (no water deficiency and 0.1 mM SNP) which is statistically identical with D_0S_2 (12.61 g). On the other hand, the lowest grain yield, 5.60 g was recorded from D_bS_0 (water deficiency at booting stage and 0 mM SNP) which showed significant statistical variation from other treatment combinations. The result showed that booting stage was affected most by water deficiency in case of grain yield and SNP application was found to increase grain yield significantly. These results are consistent with the morphology and yield contributing characters of this study such as plant height (Table 1), spike length (Table 2), spikelets $spike^{-1}$ (Table 2) and grains $spike^{-1}$ (Table 3). It was reported by Zhang and Oweis (1999) that the first susceptible stage to drought stress was during the terminal spikelets and booting stages, whereas the second susceptible stage was between the booting stage and anthesis. Hochman (1982) determined the effect of water stress applied at different growth stages on grain yield in wheat and found booting stage to be most critical for grain yield. In this experiment, foliar application of SNP was found effective in increasing grain yield. The findings of this study corroborated with several researchers as Bavita *et al.* (2012) reported that foliar applications of sodium nitroprusside was effective for enhancing the productivity of wheat under adverse environmental conditions. As well, Suryavanshi *et al.* (2016) recorded significant higher grain yield in wheat than the control with foliar spraying of SNP and the increased grain yield was certainly related to higher number of spike/m^2, grains/spike and grain weight. Moreover, exogenous application of 0.1 mM NO enhanced grain yield per plant under saline conditions in wheat (Kausar *et al*., 2013). Habib *et al*. (2016) demonstrated that seed priming with SNP was effective in reducing the adverse effects of stress on grain yield per plant in all rice cultivars and of all SNP levels 0.1mM was more effective in reducing the adverse effects of stress on grain yield per plant. All together, these experimental results suggest that water deficiency reduces the grain yield severely in different stages while application of SNP shows efficiency in increasing grain yield of wheat under water deficit condition.

4.12 Mitigation (%)

Interaction effect of water deficiency and sodium nitroprusside (SNP)

Combined effect of water deficiency and SNP showed significant variation on mitigation percentage (Table 3 and Appendix VI). The maximum mitigation percentage, 110.85 was found from D_0S_1 (no water deficiency and 0.1 mM SNP) which is statistically similar with D_0S_2 (109.46) whereas the minimum mitigation percentage, 48.61 was found from D_bS_0 (water deficiency at booting stage and 0 mM SNP) which significantly differed from other treatment combinations. In this study, percentage of mitigation was found to be improved by application of SNP. The results are corroborated with the findings of Boyarshinov and Asafova (2011) and Farooq *et al.* (2009) who observed that exogenous NO improved drought tolerance and enhanced net photosynthetic rate in wheat and rice by improving stability of membrane, enhancing activities of antioxidant enzymes and reducing H_2O_2 and MDA contents. Moreover, it was exhibited that treatment of SNP plays a crucial role in plant growth and development starting from germination to flowering, ripening of fruit and senescence of organs (Arasimowicz and Wieczorek, 2007). All together, from these experimental findings it can be specified that water deficiency affects different stages of wheat severely whilst exogenous application of SNP has the efficacy to mitigate adverse effects of water deficiency in wheat plant.

CHAPTER V

SUMMARY AND CONCLUSIONS

The experiment was conducted in the net house at the field laboratory of Agricultural Botany department, Sher-e-Bangla Agricultural University, Dhaka during the period from November 2015 to March 2016 to examine the mitigation of water deficiency in wheat with sodium nitroprusside (SNP). In this experiment, the treatment consisted of four different stages of water deficiency *viz.* D_0 = control, D_c= water deficiency at crown root initiation stage, D_b= water deficiency at booting stage, D_a= water deficiency at after anthesis stage and three different concentrations of sodium nitroprusside (SNP) viz. S_0= 0 mM, S_1= 0.1 mM and S_2= 0.2 mM of SNP. The experiment was laid out in two factors Completely Randomized Design (CRD) with four replications. Data on different morpho-physiological parameters and yield contributing characters of wheat were recorded. The collected data were statistically analyzed for assessment of the treatments effect. A significant variation among the treatments was found while different periods of water deficiency and different concentrations of SNP were applied in different combinations.

Significant differences were recorded among the effect of different periods of water deficiency in case of almost all the parameters. In this experiment, water stress was imposed at three different growth periods of wheat. Plant grown on normal condition or control treatment showed the highest height more or less over the growth period. However, at harvest, the highest plant height, 77.13 cm was found from D_0 or control whereas the lowest value, 59.43cm was observed from D_b or water deficiency at booting period. The maximum number of leaves plant^{-1} (13.56), tillers plant^{-1} (2.76), effective tillers plant^{-1} (2.40) were recorded from D_0 or control condition without water deficiency. On the other hand, the minimum number of leaves plant^{-1} (11.10), tillers plant^{-1} (1.93), effective tillers plant^{-1} (1.76) were observed from D_c or water deficiency at crown root initiation period. The SPAD value was not statistically affected by different periods of water deficiency. But numerically the highest value, 55.20 was recorded from D_0 or control whereas the lowest value, 51.66 was recorded from D_b or water deficiency at booting period of wheat. The maximum non-effective spikelets spike^{1} (3.50) was recorded from D_b or water deficiency at booting period whereas the lowest number of non-effective spikelets spike^{-1} (1.53) was found from

D_0 or control. The highest spike length (12.33 cm) , spikelets spike^{-1} (17.86), grains spike^{-1} (34.00), 100 grain weight (5.53 g), grain yield (12.30 g pot^{-1}), mitigation percentage (106.77%) were recorded from D_0 or control whereas the lowest spike length (10.10 cm), spikelets spike^{-1} (12.83), grains spike^{-1} (18.03), grain yield (6.38 g pot^{-1}), percentage of mitigation (55.44%) was recorded from D_b or water deficiency at booting stage but the lowest 100 grain weight (4.20 g) was recorded from D_a or water deficiency at after anthesis period.

Foliar application of sodium nitroprusside of different concentrations showed significant variations in case of almost all the parameters. Plant height of wheat differed significantly in response of the application of SNP. At harvest, the highest plant height was 72.29 cm, which was recorded from S_1 or 0.1 mM sodium nitroprusside (SNP) whereas the shortest 69.03cm was found from S_0 or control. The maximum number of leaves plant^{-1} (12.90), tillers plant^{-1} (2.65), effective tillers plant^{-1} (2.32) were found from S_1 or 0.1 mM SNP. On the other hand, the minimum number of leaves plant^{-1} (12.03), tillers plant^{-1} (2.29), effective tillers plant^{-1} (1.95) were observed from S_0 or control. The SPAD value was not statistically significant in response to application of SNP. But numerically the highest SPAD value, 55.50 was found from S_1 or 0.1 mM SNP whereas the lowest value, 53.08 was found from S_0 or control. The highest number of non-effective spikelets spike^{-1}, 2.17 was recorded from S_0 whereas the lowest number of non-effective spikelets spike^{-1}, 2.01 was found from S_1. The highest spike length (11.63 cm) , spikelets spike^{-1} (16.45), grains spike^{-1} (28.65), 100 grain weight (5.19 g), grain yield (10.52 g pot^{-1}), mitigation percentage (87.10%) were recorded from S_1 or 0.1mM SNP whereas the lowest spike length (11.07 cm), spikelets spike^{-1} (15.40), grains spike^{-1} (27.18), 100 grain weight (4.94 g), grain yield (9.14 g pot^{-1}), percentage of mitigation (80.40 %) was recorded from S_0 or control.

The combinations of water deficiency and different concentrations of SNP had significant effect on almost all the parameters. The highest plant height, 78.40 cm was recorded from D_0S_1 treatment combination whereas the lowest height, 56.90 cm was found from D_bS_0 treatment combination. The maximum number of leaves plant^{-1} (14.60), tillers plant^{-1} (3.00), effective tillers plant^{-1} (2.70) were recorded from D_0S_1. On the other hand, the minimum number of leaves plant^{-1} (10.80), tillers plant^{-1} (1.80), effective tillers plant^{-1} (1.60) were recorded from D_cS_0 . The SPAD value was not statistically differed by combinations of water deficiency and different concentrations of SNP. But numerically the highest value (56.30) was recorded from D_0S_2 and the lowest value (50.93) was recorded from D_bS_0 treatment combination. The highest number of non-effective spikelets spike^{-1} (3.60) was recorded from D_bS_0 and lowest number of non-effective spikelets spike^{-1} (1.50) was found from three treatment combinations D_0S_1, D_0S_2 and D_cS_1.The highest spike length (12.80 cm), spikelets spike^{-1} (18.60), grains spike^{-1} (34.60), 100 grain weight (5.70 g), grain yield (12.77 g pot^{-1}), mitigation percentage (110.85 %) were recorded from D_0S_1 whereas the lowest spikelets spike^{1} (12.60), grains spike^{-1} (17.30), grain yield (5.60 g pot^{-1}), percentage of mitigation (48.61 %) was recorded from D_bS_0. However, the lowest spike length (10.10 cm) recorded from three treatment combinations D_bS_0, D_bS_1 and $D_bS_2$and lowest 100 grain weight (4.16 g) was found from two treatment combinations D_aS_0 and D_aS_2.

Considering the above mentioned results, it may be concluded that the yield of wheat was significantly reduced by the induced water deficiency and the reduction in yield varied under water deficit condition with different stages of wheat. Among the different concentrations of SNP, 0.1 mM SNP showed the highest result in growth, physiology and yield parameters. Morphological parameters, yield contributing parameters and grain yield of wheat are found consistent with water deficiency and SNP application. Therefore, the present experimental results suggest that the 0.1 mM SNP increased grain yield of wheat variety BARI Gom-24 at both water deficit and non-deficit conditions.

Considering the situation of the present experiment, further studies in the following areas may be recommended:

1. Such study is needed in different agro-ecological zones (AEZ) of Bangladesh for analogy the accurateness of the experiment.

2. It is needed to conduct more experiments with water deficiency and different concentrations of SNP whether they can regulate the morpho-physiology, yield and seed quality of different varieties of wheat.

3. There is a scope to conduct advance research with other varieties of wheat for the development of new drought tolerant varieties with climate change.

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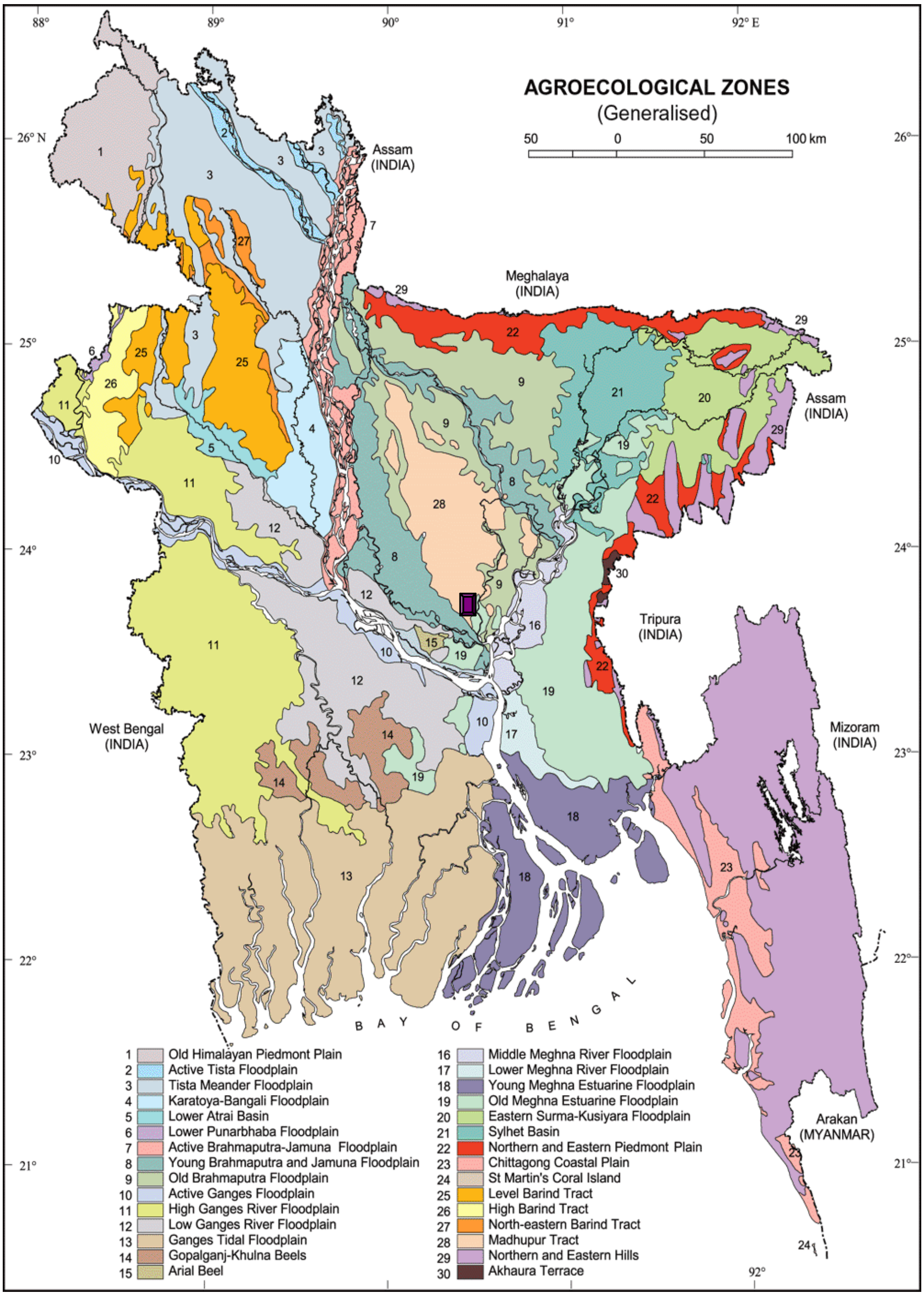
APPENDICES

Appendix I: Map showing the experimental sites under study



The experimental site under study

Appendix II. Monthly average temperature, relative humidity and total rainfall of the experimental site during the period from October 2015 to March 2016

Month	Air temperature (^0C)		R. H. (%)	Total rainfall (mm)
	Maximum	Minimum		
October,15	29.18	18.26	81	39
November,15	25.82	16.04	78	0
December,15	22.4	13.5	74	0
January,16	24.5	12.4	68	0
February,16	27.1	16.7	67	3
March,16	31.4	19.6	54	11

Source: Bangladesh Meteorological Department (Climate and weather division) Agargaon, Dhaka

Appendix III. Results of mechanical and chemical analysis of soil

A. Mechanical analysis

Constituents	Percentage (%)
Sand	28.78
Silt	42.12
Clay	29.1

B. Chemical analysis

Soil properties	Amount
Soil pH	5.8
Organic carbon (%)	0.95
Organic matter (%)	0.77
Total nitrogen (%)	0.075
Available P (ppm)	15.07
Exchangeable K (%)	0.32
Available S (ppm)	16.17

Appendix-IV. Analysis of variance of data on plant height, number of leaves plant^{-1}, number of tillers plant^{-1} and number of effective tillers plant^{-1} of wheat

Source of variation	Degrees of freedom (df)	Plant height (cm)	Number of leaves plant^{-1}	Number of tillers plant^{-1}	Number of effective tillers plant^{-1}
Replication	3	3.70E^{-28}	1.03E^{-29}	4.51E^{-31}	3.16E^{-31}
Factor A (Drought stress)	3	552.02*	13.482*	1.340*	0.669*
Factor B (SNP)	2	31.990*	2.555*	0.385*	0.403*
Interaction(A X B)	6	2.454*	0.355**	0.0091*	0.020*
Error	33	0.284	0.055	0.015	9.70E^{-03}
** : Significant at 1% level of probability; * : Significant at 5% level of probability					

Appendix-V. Analysis of variance of data on SPAD value, spike length, spikelets spike^{-1} and number of non-effective spikelets spike^{-1} of wheat

Source of variation	Degrees of freedom (df)	Mean square of			
		SPAD value	Spike length (cm)	Spikelets Spike^{-1}	No. of non-effective spikelets spike^{-1}
Replication	3	7.61E^{-28}	1.26E^{-29}	2.27E^{-29}	3.34E^{-31}
Factor A (Drought stress)	3	81.115	10.129*	48.936*	7.926*
Factor B (SNP)	2	16.585	0.935*	3.557**	0.075*
Interaction(A X B)	6	7.526	0.165**	0.324**	0.0058**
Error	33	0.450	0.066	0.045	0.0042
** : Significant at 1% level of probability; * : Significant at 5% level of probability					

Appendix-VI. Analysis of variance of data on number of grains spike^{-1}, 100 grains weight, grain yield and mitigation (%) of wheat

Source of variation	Degrees of freedom (df)	Mean square of			
		Number of grains spike^{-1}	100 grains weight (g)	Grain yield (g pot^{-1})	Mitigation (%)
Replication	3	2.78E^{-28}	1.01E^{-29}	2.56E^{-29}	3.92E^{-28}
Factor A (Drought stress)	3	460.737*	3.253*	65.494*	4408.32**
Factor B (SNP)	2	6.613*	0.187*	5.862*	144.168**
Interaction(A X B)	6	0.486**	0.017**	0.106**	27.177*
Error	33	0.287	1.82E^{-03}	0.177	6.337
** : Significant at 1% level of probability; * : Significant at 5% level of probability					