ALLEVIATION OF ADVERSE EFFECTS OF WATER DEFICIT STRESS ON WHEAT USING HUMIC ACID

A Thesis
By
Student ID: 1701145

MASTER OF SCIENCE
IN
CROP PHYSIOLOGY AND ECOLOGY

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Approved as the style and content by

Subrota Kumer Pramanik
Assistant Professor
(Supervisor)

Professor Dr. Md. Maniruzzaman Bahadur
(Co-Supervisor)

Professor Dr. Md. Hafizur Rahman Hafiz
Chairman
Examination Committee

DEPARTMENT OF CROP PHYSIOLOGY AND ECOLOGY
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DINAJPUR

DECEMBER 2023

DEDICATED

TO

MY BELOVED

PARENTS

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ABSTRACT

To investigate the ameliorative effect of exogenous application of humic acid on wheat (*Triticum aestivum* L.) under water deficit stress a pot experiment was conducted at the research shade house and laboratory of Crop Physiology and Ecology Department, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during the period of November, 2022 to March, 2023. The experiment was laid out in completely randomized design with three replications. Four growing conditions *viz.* well water (65% field capacity), water deficit stress (35% field capacity), soil application of humic acid (@ 0.044 g kg^{-1} soil) under water deficit stress and foliar application of humic acid (@ 200 ppm) under water deficit stress and two wheat varieties (BWMRI Gom 1 and BWMRI Gom 3) were assigned as experimental treatments. Water deficit stress significantly reduced different morphological traits (leaves $plant^{-1}$, flag leaf length and flag leaf breadth) physiological traits (relative leaf water content, SPAD value and leaf temperature), biochemical traits (flag leaf chlorophyll content and flag leaf proline content) and yield traits (plant height, spikes $plant^{-1}$, spike length, individual spike weight, spikelets $spike^{-1}$, grains $spike^{-1}$, 100 grains weight, grain yield $plant^{-1}$ and above ground biological yield $plant^{-1}$) final yield of wheat except proline content of flag leaf of wheat. Exogenous application of humic acid improved these traits at different degrees under water deficit stress. Foliar application of humic acid was found to be more effective to alleviate the adverse effects of water deficit stress on wheat as compared to soil application of humic acid. Soil application of humic acid improved grain yield by 9.13% and 13.86% and biological yield by 9.94% and 5.19% in BWMRI Gom 1 and BWMRI Gom 3, respectively, under water deficit stress condition. Foliar application of humic acid improved grain yield by 24.76% and 25.19% and biological yield by 19.23% and 6.50% in BWMRI Gom 1 and BWMRI Gom 3, respectively under water deficit stress condition. Therefore, it may be concluded that exogenous application of humic acid could alleviate the adverse effects of drought stress on wheat and foliar application of humic acid performed better than the soil application of humic acid.

ABBREVIATION AND ACRONYM

%	- Percentage
@	- At the rate
µg	- Microgram
µmole	- Micromole
0^0C	- Degree centigrade
AEZ	- Agro Ecological Zone
BBS	- Bangladesh Bureau of Statistics
BWMRI	- Bangladesh Wheat and Maize Research Institute
cm	- Centimeter
cv%	- Co-efficient of varience
DAS	- Days after sowing
et al.	- And others
FAO	- Food and Agriculture Organisation
FW	- Fresh weight
g	- gram
gkg^{-1}	- Gram per kilogram
HA	- Humic Acid
HSTU	- Hajee Mohammad Danesh Science and Technology University
Kg	- Kilogram
$Kgpot^{-1}$	- Kilogram per pot
M	- Mole
Mgg^{-1}	- Miligram per gram
ml	- Mililitre
Nos	- Number of spike
P	- Probability
SVglys	- Steviol glycosides
tha^{-1}	- Ton per hectare
USDA	- United States Department of Agriculture
WDS	- Water deficit stress
WW	- Well-water

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CHAPTER I

INTRODUCTION

The world population is expanding rapidly and food security has become a more vulnerable issue as compared to the past due to global climate change and increased uncertainties in weather patterns (FAO 2022). To feed the increasing world population and sustain wellbeing of human kind, world food production must be increased. To meet the food security, the global demand for wheat (a leading cereal) is expected to increase up to 40% by 2050 (Jahan *et al.* 2019).

Wheat (*Triticum aestivum* L.) is one of the most important cereals and consumed as a staple food by 1.20 billion people in 89 countries of the world (Iqbal *et al.* 2018). In Bangladesh, wheat is a leading cereal crop holding second position grown over an area of 0.34 million hectares with an annual production of 1.08 million metric tons (BBS 2022). The consumption of wheat in Bangladesh is increasing day by day due to rapid rising of population and their diversified food habit. Though, its consumption is increasing day by day, its average yield is low (3.45 tha^{-1}) (BBS 2022) compared to that of other wheat growing countries. The domestic production of the country can only encounter around 20% of total wheat demand and import is the only way for meeting her demand-supply gap (Timsina *et al.* 2018).

In view of the global climate change, drought, salinity and heat stresses are the major abiotic constraints for wheat productivity, that adversely affects the yield and quality of the crop through altering different physiological activities (Yassin *et al.* 2019). The production of wheat has been declined significantly over the last two decades due to various abiotic stresses (Yassin *et al.* 2019) and drought is the most prevalent one (Zhang *et al.* 2018). Drought inhibits nutrient uptake, shortens the phenological as well as the normal growth and development periods, changes physio-biochemical processes which finally reduce dry matter production, grain yield and grain quality in wheat (Fahad *et al.* 2017 and Ahmed and Fayyaz-ul-Hassan 2015). Water deficit at grain filling stage of wheat shortens grain-filling duration and ultimately reduces grain number and size resulted in final grain yield of wheat (Fahad *et al.* 2017).

Continuous rise in global temperature and uncertain rainfall pattern are increasing drought prone areas in the world; including the north-western wheat growing areas of Bangladesh (Mar *et al.* 2018). Drought has become a serious concern for wheat cultivation in Bangladesh as one third of the total area under wheat cultivation falls in the rainfed regions. In Bangladesh, most of the wheat is cultivated at Barind Tract region having warm and humid climate with comparatively little rainfall where most of the farmers use residual soil moisture for cultivating wheat after harvesting aman rice. The study area (Dinajpur) is comparatively hot and has already been designated as drought prone area which covers about 21% of the wheat-producing areas of the country (Sarker *et al.* 2015). It is a great concern that the drought associated problems are increasing day by day due to recurrent global warming and climate change. For sustaining wheat yield and to ensure food security for ever growing population of the country, it is essential to minimize the drought caused yield loss of wheat through different agronomic practices.

Now a days, consideration has been drawn to alleviate the detrimental effects of drought stress on wheat and to improve wheat production using effective agronomical strategy under water deficit stress condition. Among different agronomic practices, using anti-transpirant and osmo-protectants, organic fertigation, supplying adequate and balanced mineral nutrients, foliar feeding of nutrients, growth regulators and nano-particles have been proved as most effective approaches to mitigate the harmful effects of water deficit stress on crop production. But as most of them are environmentally harmful they may not be utilized on a large scale. Therefore, there arose a need to search for eco-friendly agents of natural origin having capacity to enhance drought tolerance in crop plants. In that case, humic acid is gaining its importance to improve abiotic stress tolerance in crop plants conserving ecological balance.

Humic acid is an important component of soil organic matter which improve physical, chemical and biological conditions of the soil as well as increase grain yield and quality of crop (Anwar *et al.* 2016 and Mutlu and Tas 2022). It appears that humic acid is the active constituent of organic fertilizers and its application may represent an alternative to conventional soil fertilization and a prompt source of N, especially in semi-arid conditions (Mackowiak *et al.* 2001 and Karakurt *et al.* 2009). The humic substances, the major component of soil organic matter, have both direct and indirect effects on plant growth (Sangeetha *et al.* 2006). Humic acid as organic fertilization plays an essential role in diminishing the utilization of chemical fertilizers and reducing its harmful impact on soil, the environment, and sustainable agriculture (Mekdad *et al.* 2021). Humic acid applied to leaves has significant effects on photosynthesis activity, gas exchange capacity, chlorophyll pigments, protein and starch ratios, improved physiological processes and most of the vital interactions in the plant which improve the tolerance of the plant to different abiotic stress (Khedr *et al.* 2022 and Vasenko and Haiyan 2018). Humic acid act in plants via a specific form of stress that is detected by anti-stress defense systems in plants and application of humic acid to plants can protect against water stress in degraded soils (Tohidi 2015). Researchers reported that application of humic acid under drought stress condition could reduce the drought caused adverse effect in wheat (El-Hashash *et al.* 2022, Pourmorad *et al.* 2018 and Tohidi 2015), in maize (Chen *et al.* 2022), in sunflower (Hatami 2017), in stevia (Zamani *et al.* 2021) and in rapeseed (Lotfia *et al.* 2015). Therefore, for sustaining wheat productivity under water deficit stress condition especially in the northern part of Bangladesh, the present investigation was conducted with the aim to mitigate the adverse effects of drought stress on wheat through application of humic acid. To meet up the aim of the investigation the experiments was carried out with the following objectives-

1. To observe the responses of morpho-physiological traits, yield components and yield of wheat under water deficit stress condition.
2. To examine the ameliorative effect of humic acid on morpho-physiological traits and productivity of wheat in relation to water deficit stress.

CHAPTER II
REVIEW OF LITERATURE

This chapter presents a comprehensive review of the works which have been done in home and abroad with regards to the effect of water deficit stress on wheat productivity as well as the ameliorative effect of humic acid to mitigate the adverse effect of drought stress on wheat. Emphasis has been given to the literature that has been published in the recent decades.

Drought is the most devastating abiotic stress factor, affecting growth and yield of various crops. Among the other environmental stresses, it is by far adversely reducing the crop productivity (Pennisi 2008 and Farooq *et al.* 2008), however, different mechanisms have been reported to cope with drought. It is now well evident that drought stressed plants exhibit various physiological, biochemical and molecular adaptations to thrive under drought stress (Arora *et al.* 2002).

A pot experiment was conducted by Pramanik *et al.* (2021) to analyze the changes in yield, yield attributes and physiology of four selected wheat genotypes due to water deficit stress. The plants of four selected genotypes, viz. BARI Gom 28, BARI Gom 29, BAW 1177 and ESWYT 29 were grown in pots, and two water regimes; control (65% of field capacity) and water deficit stress (35% of field capacity) were maintained. Relative water content (RWC) was decreased due to water deficit stress. However, all the genotypes showed slight increment in their water-use efficiency under water deficit stress condition compared to well water condition. Chlorophyll content also decreased in all the genotypes by the water stress though genotypic difference was obvious. At water deficit stress condition, spike length, spikes plant^{-1}, grains spike^{-1}, grains weight spike^{-1}, grain yield plant^{-1} and biological yield plant^{-1} were decreased at different magnitudes as compared to non-stressed condition. Genotype BAW 1177 showed better performance in most of the measured characters under water deficit stress condition as compared to other genotypes.

A pot experiment on screening of wheat genotypes against drought stress was conducted by Jahan and Ahmed (2017) at the research field of Sher-e-Bangla Agricultural University in earthen pots. Fifteen wheat genotypes were evaluated under drought and well irrigated condition. Exposure of plants to drought led to remarkable reduction in yield contributing characters which ultimate caused in reduced yield in all the genotypes. However, genotypes showed remarkable variability in yield reduction due to drought. The highest relative yield was observed in genotype SATYN-25 followed by SATYN-2, BARI Gom-26 and BARI Gom-28.

To assess the performance of sixteen durum wheat genotypes to drought stress an experiment was carried out by El Idrissi *et al.* (2023) at Morocco. They applied three levels of water treatment, corresponding to 100%, 66% and 33% of field capacity. Three agro-morphological, five physiological and three biochemical traits were investigated in those genotypes grown under controlled greenhouse conditions over two years (2020 and 2021). Their results showed that stomatal conductance, relative water content, leaf area, leaf temperature, SPAD values, proline, soluble sugars, glycine betaine and yield traits were significantly affected by the water treatment. Therefore, these lines could be selected as relevant and valuable germplasms to be used in durum wheat breeding programs aiming to develop drought-tolerant and high-yielding varieties. They also reported that high relative water content and stomatal conductance along with low leaf temperature can be taken as selection indices in this breeding program.

Jannat *et al.* (2023) assessed the physiological parameters and yield traits of wheat genotypes under water limiting drought condition following split plot design with three biological replications. The main plot treatment consisted of two water regimes (adequate water and drought condition) while four wheat genotypes were assigned as sub-plot treatments. They observed that physiological traits of wheat were adversely affected due to drought environment than normal condition. Even yield of genotypes greatly reduced (37.03-54.44%) because of water scarcity in drought plots.

To investigate the influence of water deficit stress on phenology, physiology and yield traits of wheat genotypes a pot trial was conducted by Pramanik *et al.* (2022). The experiment consisted with two factors i) water regimes, and ii) wheat genotypes and replicated thrice. The plants of four selected genotypes, *viz.* BARI Gom 28, BARI Gom 29, BAW 1177 and ESWYT 29 were grown in pots, and two water regimes; control (65% of field capacity) and water deficit stress (35% of field capacity) were maintained by weight basis throughout the growing period. They observed that water deficit stress significantly hampered the studied traits of wheat genotypes and caused meaningful reduction. At water deficit stress, plant height, effective tillers plant^{-1}, spikelets spike^{-1}, grains spikelet^{-1}, 100-grains weight and grain yield plant^{-1} were decreased at different magnitudes as compared to non-stressed condition. Genotype BAW 1177 showed better performance in most of the measured characters under water deficit stress as compared to other genotypes. Less disturbance in membrane permeability, greater stability of flag leaf SPAD value and greater ability to keep the canopy cooler under water deficit condition contributed to better tolerance of BAW 1177 than the other three genotypes.

Mutlu and Tas (2022) investigated the effect of foliar application of humic acid on physiological and agronomic characteristics of durum wheat *(Triticum durum* L.). They used five different doses of humic acid, i.e., 0 (H_0), 1.5 (H_1), 2.5 (H_2), 3.5 (H_3) and 4.5 (H_4) L/ha at two different phenological stages, i.e., stem elongation and heading of wheat in organic field. The observed that physiological and agronomic traits were significantly affected by humic acid doses and growth stages. Grain yield was 3882.82 kg ha^{-1} in H_0 and increased to 4988.14 kg ha^{-1} (28 %) in H_4 dose of humic acid. In their experiment, the grain yield was decreased, whereas protein content and protein ratio increased in the second year of the experiment due to warmer and drier weather conditions. The highest protein content values were noted in H_0, and the protein content values decreased with the increase in humic acid doses. They recommended that application of 4.5 L/ha humic acid at heading stage could be used to improve durum wheat yield under organic farming.

Defline *et al.* (2005) conducted an experiment to observe the effect of foliar application of N and humic acids on growth and yield of durum wheat. They applied four fertilization treatments: a non-fertilized control, a crop fertilized with foliar application of humic acid, a crop fertilized with mineral N on soil at sowing, tillering and stem elongation, and a crop fertilized with foliar application of N. They reported that the foliar application of humic acid caused a transitional production of plant dry mass with respect to unfertilized control and split soil N application. According to their observation, humic acid never affected photosynthesis or stomatal conductance, while Rubisco activity and leaf protein content showed intermediate responses between unfertilized control and split soil N application. They conclude that humic acid had limited promoting effects on plant growth, grain yield and quality, and photosynthetic metabolism of durum wheat.

AL-zubaidy and Al-haidary (2019) conducted two field experiment at the research station of the college of Agriculture-Al-Qasim Green University during the two winter seasons 2017-2018 and 2018-2019. The different concentrations of the humic acid (0, 2, 4, and 6 g L^{-1}) were applied in the main plots and spraying dates in sub plots to study the effect of spraying dates of organic bio-fertilizer (humic acid) in some growth and dry matter properties of bread wheat. They found the highest response to growth traits was from the treatment of the reaction 6 g $L \times S_2$. The highest plant height (98.43) for the first season, the chlorophyll content (51.10 and 53.55 SPAD) during both seasons, respectively, and the dry matter of booting (6.63 and 6.92 t ha^{-1}) during both seasons, and dry matter at flowering (9.83 and 10.60 t ha^{-1}) during both seasons and dry matter at maturity (16.01 and 17.26 t ha^{-1}) during both seasons.

Lotfia *et al.* (2015) examined the effects of humic acid (HA, control, 3 and 6 mg/L) on some biochemical and physiological parameters of rapeseed (*Brassica napus* L.) plants under different water supply conditions (60, 100, and 140 mm evaporation from class a pan). Plants treated by HA had more Chl a and Chl T content under both well and limited water condition. HA treated plants had 42, 8.5, and 15% more soluble protein content under well watering, moderate and severe water stress conditions, respectively, compared with control plants. They reported that malondialdehyde increased with increasing the severity of water stress, in contrast, application of HA significantly reduced the amount of this trait under water stress conditions. They also observed that application of HA increased the activity of antioxidant enzymes, improved PSII activity and consequently decreased lipid peroxidation in rapeseed plants.

Slamani *et al.* (2022) examined the effect of humic acids on soybean (*Glycine max* (L.) Merr.) seedling growth under polyethylene-glycol-6000-induced drought stress. They assessed the influence of molecular fractions of humic acids (HA) (HA < 30 kDa and HA > 30 kDa) on reducing negative effects of drought stress in soybean seedlings. They observed significant influence of water deficit on most macro element content in dry matter leaves of studied cultivars, while its effect on micro element uptake by soybean plants was concluded and no effect of HA fraction on the macro and micro nutrients uptake system of the cultivars. They also observed that HA > 30 kDa fraction better upregulated the antioxidant defense system.

Poudineh *et al.* (2015) conducted an experiment at the Institute of Agriculture, University of Zabol to evaluate the effect of humic acid and folic acid on yield, yield components, and some qualitative characteristics of sunflower under drought stress. They included three levels of drought stress treatments: S_1 = optimum irrigation, 50% of farm field capacity (control), S_2 = 70% of farm field capacity, and S_3 = 50% of farm field capacity as the main factor. They found that drought could have a very significant impact and economical and biological yield and the effect of humic acid on 1000 seed weight and seed number per head was very significant.

Garcia *et al.* (2014) conducted an investigation on the potentialities of vermi compost humic acids to alleviate water stress in rice plants (*Oryza sativa* L.) They assessed the effects of applying humic acid (HA) to rice plants under water stress conditions. They used lipid peroxidation in the roots and leaves was lower in the treatments with HA compared to the plants that were under stress that were not treated with HA. They demonstrated that HA exerted a protective effect possibly through signaling mechanism independent of ABA, regulation of OsTIPs genes and potentially initiated by chemical and physical interactions between HA and the plant root system.

Hatami (2017) conducted an experiment to evaluate the effect of zinc and humic acid applications on yield and yield components of sunflower in drought stress condition during 2014 at the Islamic Azad University, Bojnourd. He applied three levels of irrigation factor (60, 100 and 140mm evaporation from evaporation pan class A) as the main factor; whereas zinc spray application (zero, 2.5 per thousand) and humic acid application (zero, 4 lit/h) as sub-factors. He found humic acid application significantly increased grain yield under water stress conditions. He also stated that the synergistic effect of zinc and humic acid applications improved sunflower yield under drought stress.

Abdulameer *et al.* (2019) carried out a field experiment to investigate the role of humic acid in improving some growth characters of corn (*Zea mays* L.) under water stress conditions during fall seasons of 2016 and 2017. Their experiment consisted of three levels of water stress, when depleting 50, 60 and 70% of available water, while the levels of humic acid 0, 40, 60 and 80 kg^{-1}. They observed, plants under water 70% depletion produced the lowest means for studied traits and the addition of humic acid with 80 kg h^{-1} had the highest crop growth rate. The concluded that in the case of limited irrigation water, irrigation with 60% of the available water with 80 Kg ha^{-1} humic acid could be an effective practice to sustain maize production under water deficit stress.

An experiment was conducted by Bijanzadeh *et al.* (2019) at Shiraz University, Iran, to alleviate drought stress in corn hybrid through application of humic acid and salicylic acid. They used 1 mM humic (HA) and salicylic (SC) acids on SC 260 and SC 705 corn seedlings to alleviate drought stress. They concluded that application of HA with SA could be an effective and low-cost approach to ensure seedling establishment and plant growth of corn hybrid in fields affected by soil drought in the early season, especially in semi-arid regions.

Zamani *et al.* (2021) conducted a pot trial to find the useful effect of the humic acid (HA) on stevia (*Stevia rebaudiana*) especially under drought stress condition. They used five levels of humic acid (0, 1, 1.5, 2, and 2.5 mg kg^{-1} soil) to the soil planted with stevia seedling under normal (well-watered) and drought stress condition (resulted from 10-day irrigation intervals). They reported drought stress significantly reduced the stevia leaf production and steviol glycosides (SVglys) content and compositions and, whereas HA (in concentrations more than 1.5 mg kg^{-1} soil) strongly increased leaf production in stevia and resulted in a significant increase in SVglys yield (44% in comparison with no HA). They clearly demonstrated that although HA showed an inhibitory effect for SVglys content in stevia leaf, it can increase the SVglys yield through a remarkable increase in leaf growth in stevia, especially under drought stress condition.

An experiment was carried out by Afrousheh and Javanshah (2020) to evaluate the effect of soil application of humic acid on growth and physiological indices of pistachio seedlings ("Badami Zarand" rootstock) under drought stress. Their experimental treatments consisted of humic acid (0, 30 and 60 g in 4 kg^{-1} soil in pot) and irrigation intervals (7, 20 and 30 days). They found that the application of humic acid increased the vegetative growth of pistachio seedling and they also showed that relative water content and relative water protection significantly increased with the application of humic acid ($p<0.05$). They concluded that the application of humic acid under drought stress could be an appropriate management strategy to improve the growth of seedlings in pistachio orchards in arid and semi-arid areas of Iran.

Shen *et al.* (2020) reported humic acid improves the physiological and photosynthetic characteristics of millet seedlings under drought stress. In their experiment, they soaked millet seeds in different concentrations of HA (0, 50, 10, 200, and 300 mg L^{-1}) for 12 h. They observed that, HA significantly promoted the growth of millet seedlings under drought stress. They also recorded that the accumulation of reactive oxygen species was reduced in HA treatments compared with that of the control. They observed that, 200 mg L^{-1} HA significantly increased the maximum P700, effective quantum yield of photosystem I and decreased non-photochemical energy dissipation. Finally, they concluded that, HA promoted the growth of millet seedlings under drought stress by promoting the osmotic adjustment ability and antioxidant capacity of seedlings and increased photosynthesis.

El-sayed *et al.* (2018) conducted a field experiment at Shandaweel Agricultural Research Station during the two successive growing seasons 2015/2016 and 2016/2017 using water treatments (water stress and well watered) and humic acid (soil application) and silicon (foliar application) on some wheat cultivars (Gemmeiza 11, Shandaweel1, and Sids 12). The results showed that wheat cultivars had a significant effect on the all studied traits. The interaction between irrigation treatments and wheat cultivars were significant or highly significant for most of the studied traits in both seasons. Using silicon didn’t affect or played any role on soil properties under normal or water stress condition, whereas using humic acid reduced water stress effect on soil properties.

Lotfi *et al.* (2018) conducted a research experiment to evaluate the effect of humic acid (HA) applications on photosynthesis efficiency of rapeseed plants under different watering conditions. They found that the application of HA improved plants net photosynthesis under water stress via increasing the rate of gas exchange and electron transport flux in plants.

Kiran *et al.* (2019) carried out an experiment under greenhouse condition. They used humic aid to mitigate the drought stress on two *Cucumis melo* L. genotypes. They observed that HA treatment increased the shoot fresh and dry weights and leaf area of both genotypes under drought stress. This effect was clearer in the Şemame genotype than in Ananas.

Zafar *et al.* (2023) conducted a pot trial in a completely randomized design with three replicates manner to investigate the role of humic acid, potassium dihydrogen phosphate and melatonin (osmo-regulators) synergistic application in ameliorating drought stress. The treatments applied were humic acid (400 ppm), potassium dihydrogen phosphate (20 ppm) and melatonin (0.5 mM) with two water levels i.e., Control (normal watering) and drought stress (stop watering). The maximum yield was noticed in Jau-17 plants given humic acid in control and drought stress in Jau-17.

Ehsan *et al.* (2016) conducted a field experiment at Institute of Soil Chemistry and Environmental Sciences, Ayub Agricultural Research Institute, Faisalabad, Pakistan during 2009 to 2012 to assess the improvement of the nutrients (Zn, Fe, Mn and B) availability for wheat crop with and without application of humic acid. There were six treatments of inorganic fertilizers with humic acid solid and liquid i.e. T_1 (No fertilizer), T_2 (NPK), T_3 (NPK+ solid HA), T_4 (NPK+ liquid HA), T_5 (NPK+B+Zn) and T6 (NPK+B+Zn+ solid HA). Maximum nutrients were recorded in case of soil application of humic acid along with T_4 showing 8 percent N, 82 percent Fe and 5 percent increase in Boron (B) over T_4. They concluded that humic acid increased N, Fe and B concentration and uptake in wheat grain.

CHAPTER III

MATERIALS AND METHODS

The present experiment was conducted to investigate the ameliorative effect of humic acid on wheat under water deficit stress. In this chapter the details of different materials used and methodologies followed during the experimental period are presented under the following heads.

3.1. Location, duration and experimental site

A pot experiment was conducted at the shade house of the Department of Crop Physiology and Ecology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during November, 2022 to March, 2023. The experimental area is a medium high land located at 25°39′ N latitude and 88°41′ E longitude with an elevation of 37.58 m above the sea level and under the Agro-ecological zone Old Himalayan Piedmont Plain (AEZ-1).

3.2. Weather and climate of experimental area

The experimental area belongs to the subtropical climate characterized by rainfall during the month of last April to October and scanty rainfall in the rest of the year. The monthly means of meteorological information like precipitation, relative humidity, maximum and minimum temperature and sunshine hours to the experimental site during the crop growing period are presented in Appendix-1.

3.3 Characteristics of the soil used

The type of soil used in the experiment was non-calcareous dark gray floodplain soil with sandy loam texture. The details about characteristics of the soil are presented in Appendix-2.

3.4 Collection of wheat varieties

Seeds of two wheat varieties (BWMRI Gom 1 and BWMRI Gom 3) were collected from Bangladesh Wheat and Maize Research Institute, Nashipur, Dinajpur, Bangladesh and seeds were stored in dry condition with proper care till use for experimental purposes.

3.5. Experimental design and treatments

A factorial experiment was performed using completely randomised design comprising two factors with three replications.

The treatment factors were

i) Factor A: Four growing conditions

a. Well water condition (65% of field capacity of soil in the pots)

b. Water deficit stress condition (35% of field capacity of soil in the pots)

c. Soil application of humic acid @ 0.044 g kg^{-1} soil under water deficit stress condition

d. Foliar application of humic at @ 200 ppm under water deficit stress condition

ii) Factor B: Two wheat varieties

a. BWMRI Gom 1
b. BWMRI Gom 3

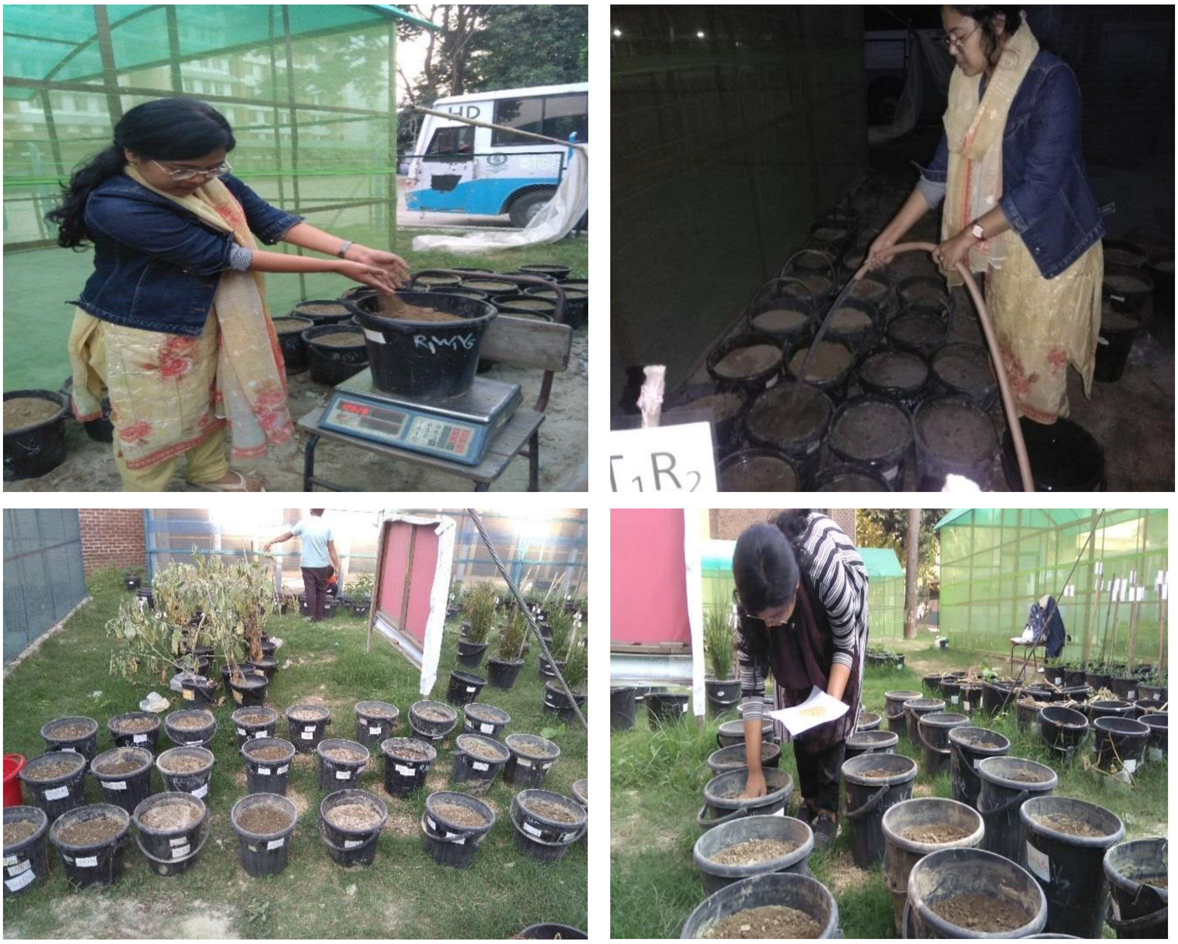

Plate 1: Photos showing preparation of experimental pots and sowing of seeds of wheat.

3.6 Preparation of experimental pots, application of fertilizer and sowing of seeds

Twenty-four plastic pots (26 cm diameter and 30 cm height) were collected and small pore was made at the bottom of each pot to drainage out the excess water from the pot. The soil was collected from 15 cm depth of the research field, well pulverized and dried in the sun. The pots were filled with equal amount (11 kg pot^{-1}) of a combined medium of soil and compost (weight ratio = 3:1) and labeled properly. Each pot was fertilized with Urea, TSP, MP and Gypsum on the basis of mass of soil per hectare area @ 100 kg N, 60 kg P_2O_5, 40 kg K_2O and 20 kg S ha^{-1}. After pot preparation, the whole quantity of TSP, MP and Gypsum and one third of

Urea were incorporated into the soil as basal dose. The remaining amount of Urea was top dressed in two equal splits, at 20 days and 45 days after sowing. Seeds of two wheat varieties were sown in the pots on November 20, 2022 at the rate of 12 seeds per pot. After sowing, slight irrigation was given to facilitate uniform germination.

3.7 Regulation of irrigation water

Field capacity of soil in the pots was measured by weight basis after 72 hours of flooding of soil in the pots. Soil moisture content of all experimental pots were kept at field capacity until 30 days after sowing to allow a good plant development (Six plants per pot). After 30 days of sowing two water regimes: well water (65% of field capacity) and water stress (35% of field capacity) were applied at 1 day interval by regulating irrigation water by weight basis. Water loss was estimated based on daily measurements of pot weight. No precipitation was allowed during experimental period by taking plastic covering before raining.

Plate 2: Photos showing regulation of irrigation water based on daily measurements of pot weight.

3.8 Preparation and application of humic acid

As soil application, humic acid was applied at pot soil @ 0.044 g kg^{-1} soil at 30 days after sowing when imposing of water deficit stress will be started. For making 200 ppm humic acid solution for foliar application, 200 mg of humic acid powder was taken in 2 ml methanol and 1 ml of Tween-20 which was properly mixed in it prior to dilution with distilled water. Then distilled water was added to make the volume 1 liter. The foliar spraying was done at tillering, booting and anthesis stage with the help of a hand sprayer until all leaves were completely wetted. Tween-20 was used as a sticky substance which helps solution's droplet in maintaining a close contact with plant leaves.

Plate 3: Photos showing foliar application of humic acid on wheat plant.

3.9 Intercultural operations and plant protection

Necessary measures were taken to protect the crop from various insect-pest and diseases during the entire growing period of the crop when and as necessary.

3.10 Data collection: Data were collected on following observations-

3.10.1 Morphological traits

3.10.1.1 Number of leaves plant^{-1}

The number of leaves plant^{-1} were recorded at 65 days after sowing from three plants of each treatment replication and means were calculated.

3.10.1.2 Length of leaf blade of flag leaf

Length of leaf blade of flag leaf was measured manually by centimeter scale at 65 days after sowing from three plants of each treatment replication and means were calculated.

3.10.1.3 Breadth of leaf blade of flag leaf

Breadth of leaf blade of flag leaf was measured manually by centimeter scale at 65 days after sowing from three plants of each treatment replication and means were calculated.

Plate 4: Photos showing data collection on morphological traits.

3.10.2 Physiological traits

3.10.2.1 Determination of relative leaf water content (RLWC)

Relative leaf water content of flag leaf was determined at 10 days after anthesis according to Kocheva *et al.* (2014). For determination of the trait flag leaves for each treatment combination collecting from the pot was sealed immediately in plastic bags and quickly transferred to the laboratory. Fresh weight was taken within 2 hours after excision. Turgid weight was obtained after soaking leaves in distilled water in test tubes for about 24 hours at room temperature. Dry weight was obtained after oven drying the leaf samples for 72 h at $70^{o}C$. Relative water content of flag leaf was calculated using the following formula-

$$\text{RLWC}\left(\%\right) = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

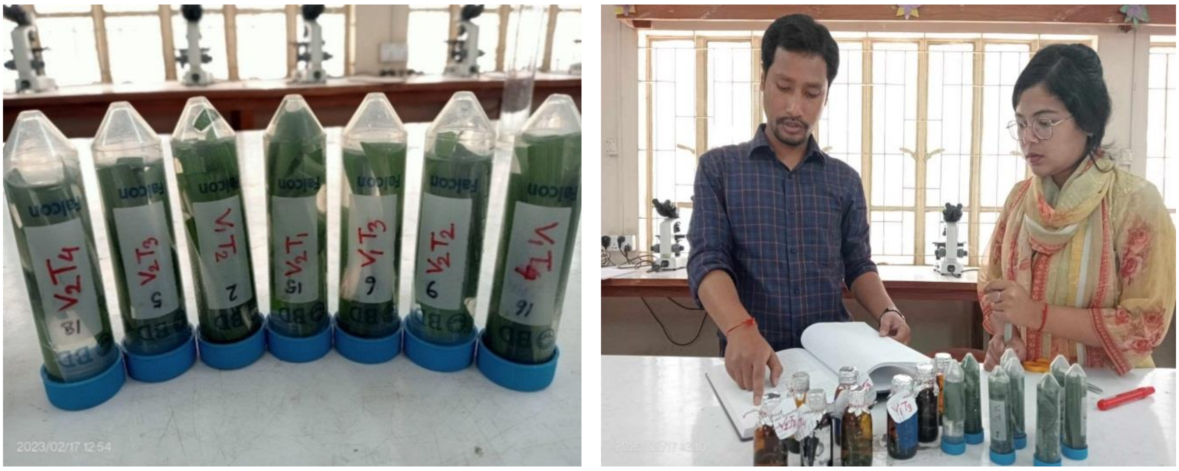

Plate 5: Photos showing determination of relative water content of flag leaf.

3.10.2.2 Measurement of SPAD value

SPAD value of flag leaf was recorded at 15 days after anthesis with a hand held SPAD meter (Model: SPAD-502 Plus, Konica Minolta, Inc.).

Plate 6: Photos showing measurement of SPAD value of flag leaf using SPAD meter.

3.10.2.3 Measurement of leaf temperature

A hand held infra-red thermometer (Model: Crop TRAC item no. 2955L-Spectrum Technologies, Inc.) was used to measure this trait at 16 days after anthesis during noon period under bright sunlight and less wind.

Plate 7: Photos showing measurement of temperature of flag leaf using infra-red thermometer.

3.10.3 Biochemical traits

3.10.3.1 Estimation of chlorophyll

Photosynthetic pigment (Chlorophyll) was estimated at 10 days after anthesis on fresh weight basis according to Witham *et al.* (1986). For the estimation of total chlorophyll of the flag leaf, 0.25g of leaf was taken from middle position of the flag leaf in a brown bottle containing 25ml of 80% aqueous acetone. The bottles were kept in dark for 48 hours. The optical density of this green colored solution was determined against 80% acetone as blank using spectrophotometer (SPECTRO UV-VIS RS Spectrophotometer, Labo Med, Inc.) at 645 and 663 nm. Total chlorophyll of fresh leaf was determined using following formula-

Total chlorophyll (mg g^{-1} FW) = $[20.2(D_{645}) + 8.02(D_{663})] \times [V/(1000 \times W)]$

Where,

V = Volume of 80% aqueous acetone (ml)

W = Weight of fresh leaf (g)

D_{645} = Absorbance at 645nm wavelength

D_{663} = Absorbance at 663nm wavelength

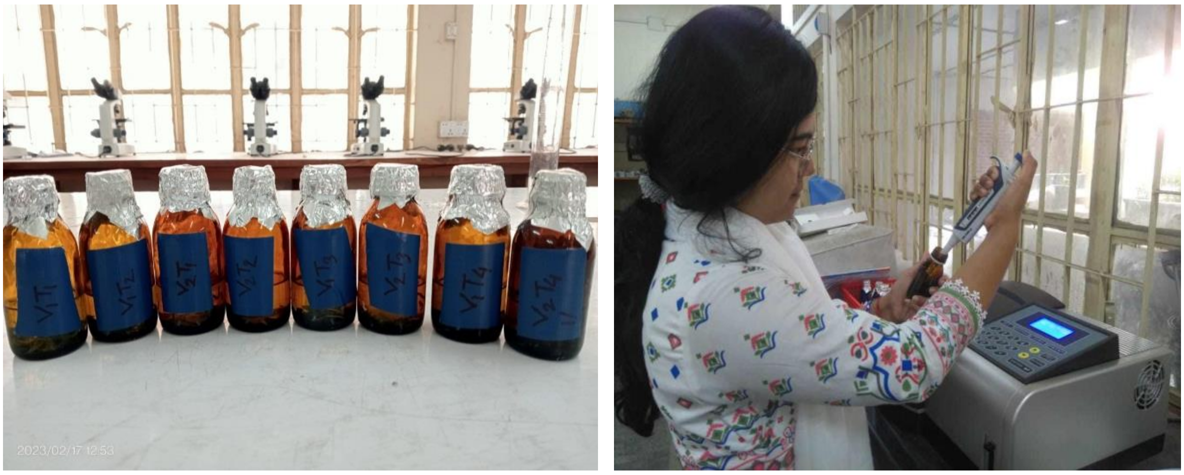

Plate 8: Photos showing estimation of chlorophyll of flag leaf.

3.10.3.2 Quantification of proline

Proline content of flag leaf was estimated at 15 days after anthesis according to Bates (1973). For quantification of the trait, flag leaves from each treatment combination were collected and immediately kept in the ice-bag and brought to

Crop Physiology and Ecology Laboratory of HSTU. 0.5g of fresh weight of flag leaf was taken for proline estimation. At first Ninhydrin reagent was prepared in such a way so that it was utilized for proline estimation within two hours of preparation. To prepare Ninhydrin reagent, addition of 30 ml glacial acetic acid and 20 ml 6M orthophosphoric acid was mixed with 1.25 g of Ninhydrin. It was subsequently heated and stirred gently to dissolve but the temperature was not allowed to exceed 70°C. Proline standards were prepared for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/ ml with distilled water. 0.5g of fresh sample was crushed in mortar and pestle and homogenized the material in 10 ml 3% sulphosalicylic acid until no large segments of plant material remained. Homogenate was filtered through Whatman No. 2 filter paper. Two ml of the filtrate and each standard proline solutions were then reacted with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid in a pyrex test tube, boiled for one hour at 100°C in water bath covering the tube with aluminium foil to prevent excess evaporation. Subsequently, it was cooled in ice bath and 4 ml of toluene was added to each tube using a dispenser. Each tube was then shaken vigorously for 15 to 20 seconds in an electrical shaker and allowed the layer to separate for 30 minutes. The absorbance of layer was measured through spectrophotometer (SPECTRO UV-VIS RS Spectrophotometer, Labo Med, Inc.) at 520 nm with pure toluene as a blank. The proline content was determined from a standard curve and calculated on a fresh weight basis as follows-

µmoles proline / g of fresh plant material = {(µg proline / ml × ml toluene) / 115.5 µg / µmoles} / (g sample/5)

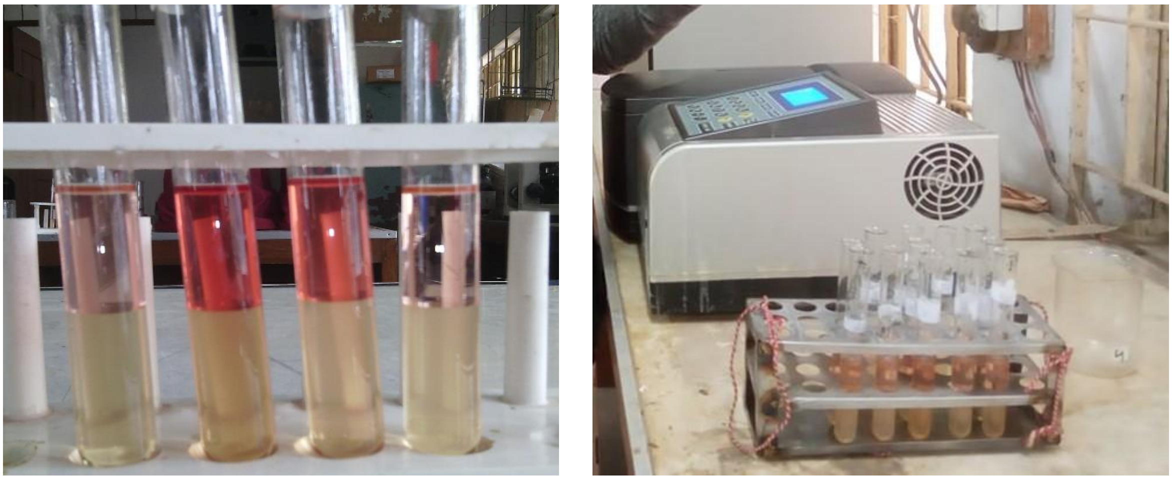

Plate 9: Photos showing determination of proline content of flag leaf.

3.10.4 Post-harvest observations

3.10.4.1 Plant height

Plant height was measured in centimeters from the base of the plant to the tip of the spike excluding the awn. Height of three plant samples from each replication of each treatment was measured and means were calculated.

3.10.4.2 Number of spikes plant^{-1}

The number of spikes plant^{-1} were recorded from three plants of each treatment replication and means were calculated.

3.10.4.3 Spike length

Spike length was measured in centimeters from the base of the spike to the tip of the spike excluding the awn of six spikes of each treatment replication and means were calculated.

Plate 10: Photos showing harvesting of crop and data collection on yield components.

3.10.4.4 Individual spike weight

Spike weight of three individual spike from each treatment replication were taken using an electric balance and means were calculated.

3.10.4.5 Number of spikelets spike^{-1}

Number of spikelets spike^{-1} were counted manually after harvest from six spikes of each treatment replication and means were calculated.

3.10.4.6 Number of grains spike^{-1}

Grains were separated manually from spike and counted for six spikes from each of their sample plants from each treatment replication and means were calculated.

3.10.4.7 100 grains weight

100 grains were taken randomly from dried sample of each replication and weighed using an electric balance and means were calculated.

3.10.4.8 Grain yield plant^{-1}

The grains were separated by threshing plant wise for each replication and then sun dried for adjusting to 12% moisture. Grain yield plant^{-1} of three plants for each replication was weighed using an electric balance and the means were calculated.

3.10.4.9 Above ground biological yield plant^{-1}

Above ground biological yield plant^{-1} of three plants for each replication was weighed using an electric balance and the means were calculated.

3.11 Statistical analysis

The recorded data were analyzed by partitioning the total variance with the help of software package “Statistix 10” program and the treatment means were compared using Tukey’s test.

CHAPTER IV
RESULTS AND DISCUSSION

The results of the present study in several tables with adequate discussion and possible interpretation whenever suitable have been provided in this chapter.

4.1 Number of leaves plant^{-1}

The interaction effect of varieties and growing conditions was not significant on number of leaves plant^{-1} of wheat (Table 1). The maximum number of leaves plant^{-1} (5.76) was found in BWMRI Gom 3 under well water condition followed by that of same variety when treated with foliar application of humic acid under water deficit stress. The minimum leaves plant^{-1} of wheat (4.63) was found in BWMRI Gom 1 under water deficit stress condition. Water deficit stress reduced the leaves number plant^{-1} of wheat in both varieties at different magnitude (0.86% in BWMRI Gom 1 and 7.12% in BWMRI Gom 3). Foliar and soil application of humic acid increased the number of leaves plant^{-1} of wheat in both varieties. Soil application of humic acid increased the number of leaves plant^{-1} of wheat by 16.85% in BWMRI Gom 1 and 5.23% in BWMRI Gom 3 and foliar application of humic acid caused 6.91% and 6.54% increased in number of leaves plant^{-1} in BWMRI Gom 1 and BWMRI Gom 3, respectively.

Photosynthesis of carbohydrate is the primary source of grain yield crop plant and plant leaves are considered as the important determinant and characterized for higher photosynthetic capacities (Asana 1968). Though the interaction effect was not significant on number of leaves of wheat but comparatively more leaves were

Table 1. Interaction effect of growing conditions and varieties on number of leaves plant^{-1} of wheat at grain filling stage

Wheat varieties	Growing conditions	Number of leaves plant^{-1}	
		Nos.	% Change over WW
BWMRI Gom 1	Well water (WW)	4.67a	-
	Water deficit stress (WDS)	4.63a	-0.86
	WDS + Soil application of humic acid	5.41a (+16.85)	+15.85
	WDS + Foliar application of humic acid	4.95a (+6.91)	+5.99
BWMRI Gom 3	Well water (WW)	5.76a	-
	Water deficit stress (WDS)	5.35a	-7.12
	WDS + Soil application of humic acid	5.63a (+5.23)	-74.88
	WDS + Foliar application of humic acid	5.70a (+6.54)	-1.04
Level of significance		NS	-
Co-efficient of variation (%)		8.19	-
Critical value for comparison		1.2419	-
Standard error for comparison		0.3521	-

NS indicates not significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

found due to application of humic than that of stressed plant. The increase in number of leaf in wheat plant under humic acid application possibly due to the stimulatory effect of humic acid on vegetative growth of wheat plant. The application of humic acid caused down-regulation of GRF gene, a drought susceptible gene in wheat, indicating that humic acid can serve as a signaling molecule and trigger wheat growth under drought conditions (Arslan *et al.* 2020). Therefore, growth might be enhanced in wheat due to application of humic acid.

4.2 Length of flag leaf

The interaction effect of growing conditions and varieties on leaf length of wheat was significant (Table 2). Among the treatment combinations, the highest leaf length (27.78 cm) was obtained in BWMRI Gom 1 under well water condition which was statistically similar to that of BWMRI Gom 3 under well water condition. The shortest leaf length (19.83 cm) was observed in BWMRI Gom 3 under water deficit stress condition. Water deficit stress reduced the flag leaf length by 21.59% in BWMRI Gom 1 and 28.43% in BWMRI Gom 3. Under water deficit stress condition foliar and soil application of humic acid improved the flag leaf length of BWMRI Gom 3. Foliar application of humic acid improved the flag leaf length of wheat by 17.36% in BWMRI Gom 1 and 10.49% in BWMRI Gom 3. Soil application of humic acid improved the flag leaf length of wheat by 5.95% in BWMRI Gom 3 and decreased by 3.17% in BWMRI Gom 1.

Grain filling is sustained by current photosynthesis of the flag leaf, penultimate leaf and the ear (Tambussi *et al.* 2007). Flag leaf provides the most important source of

Table 2. Interaction effect of growing conditions and varieties on length of flag leaf of wheat at grain filling stage

Wheat varieties	Growing conditions	Length of flag leaf	
		cm	% Change over WW
BWMRI Gom 1	Well water (WW)	27.78a	-
	Water deficit stress (WDS)	21.78c	-21.59
	WDS + Soil application of humic acid	21.09cd (-3.17)	-24.08
	WDS + Foliar application of humic acid	25.56b (+17.36)	-7.99
BWMRI Gom 3	Well water (WW)	27.71a	-
	Water deficit stress (WDS)	19.83d	-28.43
	WDS + Soil application of humic acid	21.01cd (+5.95)	-24.19
	WDS + Foliar application of humic acid	21.91c (+10.49)	-20.93
Level of significance		**	-
Co-efficient of variation (%)		2.77	-
Critical value for comparison		1.8635	-
Standard error for comparison		0.5283	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. ** indicates significant at 1% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

photosynthetic energy during reproduction which is metabolically active and has proved as the closest source of food to the grain (Ramadas and Rajendrudu 1977). As leaf has important role on grain yield it is prerequisite to analyze the morphological and the physiological characteristics of functional leaves to improve grain yield in crop (Yue *et al.* 2006). Water deficit stress substantially hamper the physiological as well as morphological processes results in hampered growth of crop plant leads to reduced morphological traits of wheat plant. On the other hand, humic acid accelerates the nutrients uptake, increases rate of photosynthesis, accelerates translocation and efficiency of utilization of photosynthase thus resulting in the cell elongation and rapid cell division which might be the reason behind the increase in leaf length.

4.3 Breadth of flag leaf

Breadth of flag leaf of wheat was significantly influenced by the combined effect of varieties and growing conditions (Table 3). Water deficit stress reduced the breadth of flag leaf of wheat in two wheat varieties at different magnitudes, while application of humic acid was found to alleviate the adverse effect of water stress in different extends. Water deficit stress reduced the breadth of flag leaf by 13.34% in BWMRI Gom 1 and 14.29% in BWMRI Gom 3. The increment in leaf breadth (3.85% and 9.02%) were recorded in BWMRI Gom 1 and BWMRI Gom 3, respectively when humic acid was applied in soil under stress condition. Foliar feeding of humic acid was found to increase the breadth of flag leaf by 10.26% and 16.67% in BWMRI Gom 1 and BWMRI Gom 3, respectively under water deficit

Table 3. Interaction effect of growing conditions and varieties on breadth of flag leaf of wheat at grain filling stage

Wheat varieties	Growing conditions	Breadth of flag leaf	
		cm	% Change over WW
BWMRI Gom 1	Well water (WW)	1.80a	-
	Water deficit stress (WDS)	1.56cd	-13.34
	WDS + Soil application of humic acid	1.62bc (+3.85)	-10.00
	WDS + Foliar application of humic acid	1.72ab (+10.26)	-4.45
BWMRI Gom 3	Well water (WW)	1.68bc	-
	Water deficit stress (WDS)	1.44d	-14.29
	WDS + Soil application of humic acid	1.57c (+9.02)	-6.55
	WDS + Foliar application of humic acid	1.68bc (+16.67)	0.0
Level of Significance		*	-
Co-efficient of variation (%)		2.52	-
Critical value for comparison		0.1188	-
Standard error for comparison		0.0337	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

stress condition. However, the longest breadth of flag leaf of wheat (1.72 cm) was observed in BWMRI Gom 1 under foliar application of humic acid at water deficit stress condition, whereas the shortest breadth (1.44 cm) was observed in BWMRI Gom 3 under water deficit stress condition. Though the water deficit stress reduced the breadth of flag leaf of wheat at different extends but application of humic acid meaningfully increased the trait. The increased breadth of flag leaf under humic acid application might be due to positive regulatory effect of humic acid on vegetative growth of wheat plant. Humic acid accelerates cell division and cell elongation consequently improved vegetative growth (plant height, leaf length, leaf breadth, leaf area, stem diameter) of plant.

4.4 Relative leaf water content (RLWC) of flag leaf

The relative leaf water content of flag leaf of wheat at 10 days after anthesis was significantly affected by the interaction effect of wheat varieties and growing conditions (Table 4). Relative water content of flag leaf of wheat was significantly decreased under water stress as compared with unstressed plants. Among different treatment combinations, the maximum RLWC (78.62%) was found in BWMRI Gom 1 under well water condition and the minimum RLWC (60.52%) was found in BWMRI Gom 3 under water stress condition. Water stress reduced the water content of flag leaf of wheat by 14.78% in BWMRI Gom 1 and 17.58% in BWMRI Gom 3. Application of humic acid alleviated the adverse effect of water deficit stress on RLWC in wheat. Soil application of humic acid increased the RLWC by 6.48% in BWMRI Gom 1 and 1.75% in BWMRI Gom 3, whereas foliar application

Table 4. Interaction effect of growing conditions and varieties on relative leaf water content of flag leaf of wheat at 10 days after anthesis

Wheat varieties	Growing conditions	Relative leaf water content of flag leaf	
		%	% Change over WW
BWMRI Gom 1	Well water (WW)	78.62a	-
	Water deficit stress (WDS)	67.00c	-14.78
	WDS + Soil application of humic acid	71.34b (+6.48)	-9.25
	WDS + Foliar application of humic acid	75.89a (+13.27)	-3.47
BWMRI Gom 3	Well water (WW)	73.43b	-
	Water deficit stress (WDS)	60.52d	-17.58
	WDS + Soil application of humic acid	61.58d (+1.75)	-16.14
	WDS + Foliar application of humic acid	65.16c (+7.67)	-11.26
Level of significance		*	-
Co-efficient of variation (%)		5.21	-
Critical value for comparison		2.7335	-
Standard error for comparison		0.8258	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

of humic acid increased the trait by 13.27% in BWMRI Gom 1 and 7.67% in BWMRI Gom 3 as compared to water deficit stress condition. The most pronounced improvement in RLWC (13.27%) was detected in BWMRI Gom 1 under foliar application of humic acid as compared to water deficit stress condition. The RLWC is directly related to soil water content imitating the metabolic events in cells and utilized as an utmost significant index for selecting drought tolerant wheat genotypes. Other authors (Farshadfar *et al.* 2014, Pramanik *et al.* 2021, Haque *et al.* 2022 and Sindabad *et al.* 2023) also found that water deficit stress significantly reduced the relative leaf water content in wheat. These findings support our results on RLWC of flag leaf of wheat plant to water deficit stress. In our current enquiry, we observed that application of humic acid significantly increased RLWC of flag leaf of wheat than that of water deficit stress. Furthermore, previous authors (Abou-Tahoun *et al.* 2022 and Shen *et al.* 2020) recommended that humic acid molecules promote the osmotic adjustment ability, increase leaf water retention, as well the photosynthetic and antioxidant metabolism of plants under drought stress which are congruent to our present findings of the study.

4.5 Chlorophyll content of flag leaf

The interaction effect of growing conditions and varieties was significant on total chlorophyll content of flag leaf of wheat (Table 5). Among different interactions, the highest chlorophyll (2.29 mg g^{-1} FW) in leaf of wheat was found in BWMRI Gom 1 under well water condition. The lowest chlorophyll content of leaf (1.23 mg g^{-1} FW) was found in BWMRI Gom 3 under water deficit stress condition. Water stress significantly reduced the chlorophyll content of flag leaf of wheat in both of the varieties. The degree of reduction was 14.84% in BWMRI Gom 1 and 29.31% in BWMRI Gom 3. Moreover, soil and foliar application of humic acid under water stress showed significant increment in chlorophyll content of wheat leaf as compared to stressed plants. The degree of increment was 3.07% in BWMRI Gom 1 and 30.08% in BWMRI Gom 3 under soil application of humic acid, whereas 16.92% and 31.70% increment were observed in chlorophyll content in BWMRI Gom 1 and BWMRI Gom 3, respectively under foliar application of humic acid. The decline in photosynthetic pigment (chlorophyll) under water limiting drought situation has been deliberated as a distinctive indicator of oxidative strain that might be the consequence of photo-oxidation and destruction of leaves chlorophyll (Keyvan 2010). Our outputs are in parallel with the findings of Farshadfar *et al.* (2014) and Pramanik *et al.* (2021) who conveyed that drought stress decreased chlorophyll content of wheat leaf and the deterioration was further obvious in drought prone genotypes. Limited water supply significantly decreased different physiological processes as well total chlorophyll content but by contrast humic acid application improve the traits in a meaningful rate. Due to their effective components, humic compounds can alter biochemical processes in plants, resulting in higher photosynthesis and respiration rates, as well as increased hormone and protein production (Olk *et al.* 2018). They concluded that increase in chlorophyll of plants exposed to drought stress with application of humic acid might be due to its key role in stimulating its biosynthesis and elevating ascorbate peroxidase activity for scavenging ROS in thylakoids and delaying chlorophyll degradation (Arjumend

Table 5. Interaction effect of growing conditions and varieties on total chlorophyll content of flag leaf of wheat at 10 days after anthesis

Wheat varieties	Growing conditions	Total chlorophyll content of flag leaf	
		mg g^{-1} FW	% Change over WW
BWMRI Gom 1	Well water (WW)	2.29a	-
	Water deficit stress (WDS)	1.95bc	-14.84
	WDS + Soil application of humic acid	2.01b (+3.07)	-12.22
	WDS + Foliar application of humic acid	2.28a (+16.92)	-0.43
BWMRI Gom 3	Well water (WW)	1.74bcd	
	Water deficit stress (WDS)	1.23e	-29.31
	WDS + Soil application of humic acid	1.60d (+30.08)	-8.04
	WDS + Foliar application of humic acid	1.62d (+31.70)	-6.89
Level of significance		**	-
Co-efficient of variation (%)		4.22	-
Critical value for comparison		0.2526	-
Standard error for comparison		0.0437	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. ** indicates significant at 1% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

et al. 2015). Improvement of chlorophyll content of wheat under water deficit stress condition due to application of humic acid was also reported by Tohidi (2015) which is supportive to our present findings.

4.6 Proline content of flag leaf

Significant variation was found in flag leaf proline content of wheat at 15 days after anthesis due to interaction effect of varieties and growing conditions (Table 6). However, the maximum proline content of flag leaf (2.41 µmole g^{-1} FW) was observed in BWMRI gom 1 under water deficit stress condition, whereas the minimum proline content of flag leaf (1.43 µmole g^{-1} FW) was observed in BWMRI Gom 3 under well water condition. Water deficit stress increased the proline content of flag leaf in two wheat varieties at different magnitude (21.71% in BWMRI Gom 1 and 65.73% in BWMRI Gom 3). Under water deficit stress condition, the proline content of flag leaf was reduced by soil and foliar application of humic acid. Soil application of humic acid reduced the proline content of flag leaf by 7.47% in BWMRI Gom 1 and 18.99% in BWMRI Gom 3, whereas foliar application of humic acid reduced the flag leaf proline content by 17.01% in BWMRI Gom 1 and 29.53% in BWMRI Gom 3. Proline plays a role as an enzyme stabilizing agent which regulates and reduces water loss from the cell under water deficit circumstances and it has the capability to conciliate osmotic regulation and attributes to substantial sub-cellular configuration (Kaur and Asthir 2015, Liang *et al.* 2013, Hayat *et al.* 2012 and Hassanein 2004). Proline is well known to rise dramatically in higher crop plants in response to different abiotic stresses like drought (Nowsherwan *et al.* 2018) which is in agreement with the present findings.

Table 6. Interaction effect of growing conditions and varieties on proline content of flag leaf of wheat at 15 days after anthesis

Wheat varieties	Growing conditions	Proline content of flag leaf	
		µmole g^{-1} FW	% Change over WW
BWMRI Gom 1	Well water (WW)	1.98bc	
	Water deficit stress (WDS)	2.41a	+21.71
	WDS + Soil application of humic acid	2.23ab (-7.47)	+12.63
	WDS + Foliar application of humic acid	2.00bc (-17.01)	+1.01
BWMRI Gom 3	Well water (WW)	1.43d	
	Water deficit stress (WDS)	2.37a	+65.73
	WDS + Soil application of humic acid	1.92c (-18.99)	+34.27
	WDS + Foliar application of humic acid	1.67cd (-29.53)	+16.78
Level of Significance		*	-
Co-efficient of variation (%)		3.19	-
Critical value for comparison		0.3253	-
Standard error for comparison		0.0532	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % change over water deficit stress in respective variety.

Dramatic rose of proline in wheat leaf due to water deficit stress was also observed by Pramanik *et al.* (2021) and Haque *et al.* (2022) that supports our present findings.

4.7 SPAD value of flag leaf

Interaction effect of wheat varieties and growing conditions significantly influenced the SPAD value of flag leaf in wheat (Table 7). The SPAD value of flag leaf of wheat was decreased considerably due to adverse effect of water deficit stress but this could be increased by soil and foliar application of humic acid under water deficit stress. The extend of reduction of SPAD value due to water deficit stress was more in BWMRI Gom 3 (7.41%) than that of BWMRI Gom 1 (1.13%%) as compared to well water. The degree of increase in SPAD value was 0.17% in BWMRI Gom 1 and 3.05% in BWMRI Gom 3 due to soil application of humic acid; 2.89% in BWMRI Gom 1 and 4.50% in BWMRI Gom 3 due to foliar application of humic acid as compared to water deficit stress. The decline in greenness (SPAD value) under water deficit stress might be the consequence of photo-oxidation and destruction of leaves chlorophyll (Keyvan 2010). Drought mediated reduction in SPAD value was notable in camelina and canola (Ahmad *et al.* 2021) and in wheat (Pramanik *et al.* 2022 and Sindabad *et al.* 2023) that are parallel to our present findings and suggest that water deficit stress impairs the greenness as well as the photosynthetic machinery of green plant. In our study, application of humic acid under water deficit stress significantly increased the SPAD value of wheat leaf. This might be due to humic acid increase nitrogen and potassium content in plant shoot which helps in increasing the number of

Table 7. Interaction effect of growing conditions and varieties on SPAD value of flag leaf of wheat at 15 days after anthesis

Wheat varieties	Growing conditions	SPAD value of flag leaf	
		Value	% Change over WW
BWMRI Gom 1	Well water (WW)	54.40abc	-
	Water deficit stress (WDS)	53.78bc	-1.13
	WDS + Soil application of humic acid	53.87abc (+0.17)	-0.97
	WDS + Foliar application of humic acid	55.33ab (+2.89)	1.70
BWMRI Gom 3	Well water (WW)	56.67a	-
	Water deficit stress (WDS)	52.47c	-7.41
	WDS + Soil application of humic acid	54.07abc (+3.05)	-4.59
	WDS + Foliar application of humic acid	54.83abc (+4.50)	-3.24
Level of significance		*	-
Co-efficient of variation (%)		1.80	-
Critical value for comparison		2.8246	-
Standard error for comparison		0.8008	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

chloroplasts per cell, thus contributing to increased synthesis of chlorophyll as well as greenness of green plant like wheat.

4.8 Leaf temperature of flag leaf

The interaction effect of growing conditions and varieties was significant on leaf temperature of flag leaf of wheat (Table 8). Among different interactions, the highest temperature (22.49 °C) in leaf of wheat was found in BWMRI gom 1 under water deficit condition. The lowest temperature content of leaf (16.89 °C) was found in BWMRI Gom 3 under well water condition. Water stress significantly increased the temperature of flag leaf of wheat in both of the varieties. The degree of increment in temperature due to stress was 20.59% in BWMRI Gom 1 and 24.99% in BWMRI Gom 3. Moreover, soil and foliar application of humic acid under water deficit stress showed significant reduction of temperature of wheat leaf as compared to stressed plants. The degree of reduction was 1.69% in BWMRI Gom 1 and 0.28% in BWMRI Gom 3 under soil application of humic acid, whereas 6.67% and 5.87% reduction were observed temperature in BWMRI Gom 1 and BWMRI Gom 3, respectively under foliar application of humic acid. Leaf temperature is an interesting indicator of drought tolerance associated with the central role played by stomata in controlling water loss. One of the responses to drought in plants is a relative decrease in water content, which leads to a reduction in leaf water potential, causing stomatal closure to reduce transpiration. As a result, there is an increase in leaf temperature, a decrease in leaf area, and a loss of yield and its stability (Sankarapandian *et al.* 2013 and Buckley 2019). The temperature

Table 8. Interaction effect of growing conditions and varieties on leaf temperature of flag leaf of wheat at 15 days after anthesis

Wheat varieties	Growing conditions	Leaf temperature of flag leaf	
		°C	% Change over WW
BWMRI Gom 1	Well water (WW)	18.65cd	-
	Water deficit stress (WDS)	22.49a	+20.59
	WDS + Soil application of humic acid	22.11a (-1.69)	+18.55
	WDS + Foliar application of humic acid	20.99abc (-6.67)	+12.55
BWMRI Gom 3	Well water (WW)	16.89d	-
	Water deficit stress (WDS)	21.11ab	+24.99
	WDS + Soil application of humic acid	21.05ab (-0.28)	+24.63
	WDS + Foliar application of humic acid	19.87bc (-5.87)	+17.64
Level of significance		*	-
Co-efficient of variation (%)		4.22	-
Critical value for comparison		2.3610	-
Standard error for comparison		0.6694	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % change over water deficit stress in respective variety.

change of the leaves is mainly caused by disturbances related to the stomatic function under water stress and following the reduction of the relative water content of leaf. In fact, the increase in temperature is related to the reduction of transpiration and the closure of stomata by reducing the outflow of water vapor to the atmosphere in water-limiting condition (Degu *et al.* 2019 and Ru *et al.* 2020). Application of humic acid especially the foliar application improves the stomatal conductance which leads to stomatal transpiration results in cooling of plant leaf and reduced leaf temperature.

4.9 Number of tillers plant^{-1}

The interaction effect of varieties and growing conditions was significant on number of tillers plant^{-1} of wheat (Table 9). The maximum number of tillers plant^{-1} (4.88) was found in BWMRI Gom 1 under well water condition followed by that of BWMRI Gom 1 when treated with foliar application of humic acid under water deficit stress. The minimum tillers plant^{-1} of wheat (3.30) was found in BWMRI Gom 3 under water deficit stress condition. Water deficit stress reduced the tillers number plant^{-1} of wheat in both varieties at different magnitude (18.23% in BWMRI Gom 1 and 14.29% in BWMRI Gom 3). Foliar application of humic acid increased the number of tillers plant^{-1} of wheat in both varieties but soil application of humic acid increased the trait of wheat only in BWMRI Gom 3. Foliar application of humic acid increased the number of tiller plant^{-1} of wheat by 8.52% in BWMRI Gom 1. In case of BWMRI Gom 3, same increment (14.24%) was recorded due to both soil and foliar application of humic acid. The reduction in

Table 9. Interaction effect of growing conditions and varieties on number of tillers plant^{-1} of wheat

Wheat varieties	Growing conditions	Number of tillers plant^{-1}	
		Nos.	% Change over WW
BWMRI Gom 1	Well water (WW)	4.88a	-
	Water deficit stress (WDS)	3.99bc	-18.23
	WDS + Soil application of humic acid	3.99bc	-18.23
	WDS + Foliar application of humic acid	4.33ab (+8.52)	-11.27
BWMRI Gom 3	Well water (WW)	3.85bc	-
	Water deficit stress (WDS)	3.30c	-14.29
	WDS + Soil application of humic acid	3.77bc (+14.24)	-2.07
	WDS + Foliar application of humic acid	3.77bc (+14.24)	-2.07
Level of significance		*	-
Co-efficient of variation (%)		5.99	-
Critical value for comparison		0.6879	-
Standard error for comparison		0.1950	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

number of tillers plant^{-1} might be due to the reduction of the availability of water and nutrients to the roots under water deficit stress which disturbs the plant tissue leading to decreased in meristematic activity and cell expansion. Ali *et al.* (2018) and Pramanik *et al.* (2022) also found reduced number of tillers plant^{-1} in wheat due to drought stress that clarify the results of our study. Humic acid significantly increased the number of tillers plant^{-1} which might be due to the role of humic acid that increases the number of cells, cell size, chloroplasts development, and synthesis of chlorophyll that assist vegetative growth of plant. The application of organic products such as humic acid reduce irrigation intervals, improve the water use efficiency and decrease the effect of drought stress on plants which ultimately improves vegetative growth of crop plants (Haghighi *et al.* 2011). El-sayed *et al.* (2018) observed the positive effect of humic acid application under water stress condition on yield component like number of tillers plant^{-1} on some bread wheat cultivars. These results are in a line with our investigated results.

4.10 Plant height

The interaction effect of growing conditions and varieties on plant height of wheat at harvest was significant (Table 10). Among the treatment combinations the tallest plant (81.86 cm) was obtained in BWMRI Gom 1 under well water condition which was statistically similar to that of BWMRI Gom 1 under water deficit stress treated with foliar application of humic acid. The shortest plant (68.87 cm) was observed in BWMRI Gom 3 under water deficit stress condition. Water deficit stress reduced the plant height by 4.83% in BWMRI Gom 1 and 5.82% in BWMRI Gom 3. Under

Table 10. Interaction effect of growing conditions and varieties on plant height of wheat at harvest

Wheat varieties	Growing conditions	Plant height	
		cm	% Change over WW
BWMRI Gom 1	Well water (WW)	81.86a	-
	Water deficit stress (WDS)	77.90b	- 4.83
	WDS + Soil application of humic acid	78.34b (+0.56)	- 4.30
	WDS + Foliar application of humic acid	81.19a (+4.22)	- 0.82
BWMRI Gom 3	Well water (WW)	73.13c	-
	Water deficit stress (WDS)	68.87d	- 5.82
	WDS + Soil application of humic acid	70.49d (+2.35)	-3.61
	WDS + Foliar application of humic acid	69.17d (+0.43)	-5.62
Level of significance		**	-
Co-efficient of variation (%)		2.16	-
Critical value for comparison		2.5081	-
Standard error for comparison		0.7111	-

In the column, means having similar letter(s) did not differ significantly at $P \leq 5\%$ level by Tukey. ** indicates significant at 1% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

water deficit stress condition foliar and soil application of humic acid improved the plant height of wheat. Soil application of humic acid improved the plant height of wheat by 0.56% in BWMRI Gom 1 and 2.35% in BWMRI Gom 3. Foliar application of humic acid improved the plant height of wheat by 4.22% in BWMRI Gom 1 and 0.43% in BWMRI Gom 3. Plant height of wheat was decreased under water deficit condition and it might be due to dehydration of protoplasm, decrease in relative turgidity associated with turgor loss and decreased cell expansion and cell division. Under water deficit stress condition, plant suffers from physical drought consequently growth and development processes are hampered which might be the responsible for reduction in plant height. Our findings match with findings of Sikder *et al.* (2011), Pramanik *et al.* (2022) and Jannat *et al.* (2023) who reported that water deficit stress significantly reduced the height of wheat plant. Application of humic acid positively improved the plant height of wheat, which might be due to the fact that humic acid enhances the photosynthetic area of the plants which maximize the photosynthesis and finally it increases the plant height. Humic acid has been shown to improve nutrient transport and availability consequently growth and development processes which assist vegetative as well as reproductive growth and development of crop plants (Olk *et al.* 2018). Our results on plant height of wheat are in line with the findings of Khan *et al.* (2010) who observed that application of humic acid increased the plant height of rainfed wheat.

4.11 Number of spikes plant^{-1}

The combined effect of growing conditions and varieties was significant on number of spikes plant^{-1} of wheat (Table 11). In present study water deficit stress significantly reduced the number of spikes plant^{-1} in wheat, whereas soil and foliar application of humic acid caused meaningful increment of the spike number under water deficit stress. The maximum number of spikes plant^{-1} (4.67) was found in BWMRI Gom 1 under water deficit stress treated with soil application of humic acid which was statistically at par with that of BWMRI Gom 1 under foliar feeding of humic acid. The minimum number of spikes of wheat (2.89) was found in BWMRI Gom 3 under water deficit stress condition which was statistically similar to that of BWMRI Gom 3 under soil application of humic acid. Water deficit stress reduced the spikes number plant^{-1} of wheat in two wheat varieties at different magnitude (16.59% in BWMRI Gom 1 and 21.25% in BWMRI Gom 3), whereas soil application of humic acid increased the yield component by 27.24% and 3.80%; foliar application of humic acid increased the trait by 25.61% and 26.98% in BWMRI Gom 1 and BWMRI Gom 3, respectively. The yield component *viz*. number of spikes plant^{-1} in wheat may have considered as one of the most important yields contributing characters that is positively correlated with final grain yield of wheat (Forgone 2009). In the present study, water deficit stress significantly reduced the component. These results are in line with the results of Sikder *et al.* (2011), Pramanik *et al.* (2021) and Haque *et al.* (2022) who found water deficit stress reduced spike number plant^{-1} compared to non-stress condition. In our investigation, humic acid enhanced the spike number in wheat than that of stressed condition. Enhancement of number of spikes plant^{-1} in wheat was also recorded by Nasiroleslami *et al.* (2021) that support our present findings related to spike number plant^{-1} of wheat.

Table 11. Interaction effect of growing conditions and varieties on number of spikes plant^{-1} of wheat

Wheat varieties	Growing conditions	Number of spikes plant^{-1}	
		Nos.	% Change over WW
BWMRI Gom 1	Well water (WW)	4.40ab	-
	Water deficit stress (WDS)	3.67bc	-16.59
	WDS + Soil application of humic acid	4.67a (+27.24)	+6.14
	WDS + Foliar application of humic acid	4.61a (+25.61)	+4.78
BWMRI Gom 3	Well water (WW)	3.67bc	-
	Water deficit stress (WDS)	2.89c	-21.25
	WDS + Soil application of humic acid	3.00c (+3.80)	-18.25
	WDS + Foliar application of humic acid	3.67bc (+26.98)	0.0
Level of significance		*	-
Co-efficient of variation (%)		7.56	-
Critical value for comparison		0.8324	-
Standard error for comparison		0.2360	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

4.12 Spike length

Spike length of wheat was significantly influenced by the combined effect of varieties and growing conditions (Table 12). Water deficit stress reduced the spike length of wheat in two wheat varieties at different magnitudes, while application of humic acid was found to alleviate the adverse effect of water stress in different extends. Water deficit stress reduced the spike length by 24.83% in BWMRI Gom 1 and 12.82% in BWMRI Gom 3. The increment (2.24% and 8.52%) were recorded in BWMRI Gom 1 and BWMRI Gom 3, respectively when humic acid was applied in soil under stress condition. Foliar feeding of humic acid was found to increase the spike length by 9.25% and 12.37% in BWMRI Gom 1 and BWMRI Gom 3, respectively under water deficit stress condition. However, the longest spike of wheat (13.65 cm) was observed in BWMRI Gom 1 under well water condition, whereas the shortest spike (9.86 cm) was observed in BWMRI Gom 3 under water deficit stress condition that was statistically similar to spike length recorded from other treatment combinations. It is clear that water shortage apparently declined spike length of wheat and the results are in accordance with the results of Jannat *et al.* (2023) who observed water shortage significantly reduced spike length of wheat. The shorter spike under water deficit stress might be due to decreased in cell expansion as well as reduced cell division resulted from lack of water for growth and development of wheat plants. Application of humic acid positively improved the spike length of wheat which might be due to humic acid improves the supply of water and the absorption of important nutrients by adjustment of osmotic pressure

Table 12. Interaction effect of growing conditions and varieties on spike length of wheat at harvest

Wheat varieties	Growing conditions	Spike length	
		cm	% Change over WW
BWMRI Gom 1	Well water (WW)	13.65a	-
	Water deficit stress (WDS)	10.26b	-24.83
	WDS + Soil application of humic acid	10.49b (+2.24)	-23.15
	WDS + Foliar application of humic acid	11.21b (+9.25)	-17.88
BWMRI Gom 3	Well water (WW)	11.31b	-
	Water deficit stress (WDS)	9.86b	-12.82
	WDS + Soil application of humic acid	10.70b (+8.52)	-5.39
	WDS + Foliar application of humic acid	11.08b (+12.37)	-2.03
Level of significance		**	-
Co-efficient of variation (%)		5.21	-
Critical value for comparison		1.6590	-
Standard error for comparison		0.4703	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. ** indicates significant at 1% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

at the cellular level and enhances the enzymatic and antioxidant activities which results in enhanced reproductive part of plant. Other researcher (Khan *et al.* 2010) reported improved spike length of rainfed wheat resulted from application of humic acid that match with our findings regarding interaction of spike length of wheat and humic acid under limited water stress condition.

4.13 Individual spike weight

Spike weight of wheat was significantly influenced by the interaction effect of wheat varieties and growing conditions (Table 13). Individual weight of spike of wheat was substantially decreased due to water deficit stress as compared to unstressed condition. Water stress reduced 29.78% and 27.03% weight in spike of BWMRI Gom 1 and BWMRI Gom 3, respectively than that of well water. Soil and foliar and application of humic acid under water stress can alleviate the adverse effect of water shortage in respect of spike weight in wheat. The most pronounced increment in spike weight (30.15%) was recorded in BWMRI Gom 1 when treated with foliar humic acid under water deficit stress. However, the maximum spike weight (18.80 g) was noted in BWMRI Gom 1, whereas the minimum spike weight (12.20 g) was noted in BWMRI Gom 3 under water deficit stress condition. Spike weight is an important yield component which is closely related to spike length, number of spikelets spike^{-1}, number of grains spike^{-1} and grain size which ultimately contributes on final grain yield. In our study water deficit stress knowingly hampered morpho-physiological traits of wheat resulted in reduced vegetative and reproductive development as in spike weight. This

Table 13. Interaction effect of growing conditions and varieties on individual spike weight of wheat at harvest

Wheat varieties	Growing conditions	Individual spike weight	
		g	% Change over WW
BWMRI Gom 1	Well water (WW)	18.80a	-
	Water deficit stress (WDS)	13.20cd	-29.78
	WDS + Soil application of humic acid	15.07bc (+14.17)	-19.84
	WDS + Foliar application of humic acid	17.18ab (+30.15)	-8.62
BWMRI Gom 3	Well water (WW)	16.72ab	-
	Water deficit stress (WDS)	12.20d	-27.03
	WDS + Soil application of humic acid	13.55cd (+11.06)	-18.96
	WDS + Foliar application of humic acid	13.97cd (+14.50)	-16.45
Level of significance		*	-
Co-efficient of variation (%)		4.89	-
Critical value for comparison		2.1229	-
Standard error for comparison		0.6019	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

reduction may be resulted due to the hamper in photosynthetic activity and lower supply of assimilates that support reproductive development (Farooq *et al.* 2014). Tahir *et al.* (2011) found that humic acid application increased wheat agronomic parameters in non-calcareous relative to calcareous soils. Khan *et al.* (2018) found higher wheat spike weight and grain yield on clayey loam compared to sandy loam soil. These findings prove the positive effect of humic acid on yield and yield components of wheat that is supportive to our findings of the present study.

4.14 Number of spikelets spike^{-1}

Interaction effect of varieties and growing conditions was significant on number of spikelets spike^{-1} of wheat (Table 14). The maximum number of spikelets spike^{-1} of wheat (19.33) was observed in BWMRI Gom 3 under well water condition which was statistically similar to that of BWMRI Gom 3 treated with foliar application of humic acid under water deficit stress condition, whereas the minimum number of spikelets spike^{-1} of wheat (16.67) was observed in BWMRI Gom 1 under water deficit stress condition. Water deficit stress reduced the number of spikelets spike^{-1} of wheat at different magnitudes (1.94% in BWMRI Gom 1 and 3.51% in BWMRI Gom 3). Under water deficit stress conditions, soil application of humic acid improved the number of spikelets spike^{-1} of wheat by 1.91% and 0.69% in BWMRI Gom 1 and BWMRI Gom 3, respectively. Foliar application of humic acid improved the number of spikelets spike^{-1} of wheat by 8.58% in BWMRI Gom 1 and 3.64% in BWMRI Gom 3 under water deficit stress condition. Number of spikelets spike^{-1} in wheat may have considered being most important yield contributing components

Table 14. Interaction effect of growing conditions and varieties on number of spikelets spike^{-1} of wheat

Wheat varieties	Growing conditions	Number of spikelets spike^{-1}	
		Nos.	% Change over WW
BWMRI Gom 1	Well water (WW)	17.00cd	-
	Water deficit stress (WDS)	16.67d	-1.94
	WDS + Soil application of humic acid	16.99cd (+1.91)	-0.05
	WDS + Foliar application of humic acid	18.10bc (+8.58)	6.47
BWMRI Gom 3	Well water (WW)	19.33a	-
	Water deficit stress (WDS)	18.65ab	-3.51
	WDS + Soil application of humic acid	18.78ab (+0.69)	-2.84
	WDS + Foliar application of humic acid	19.33a (+3.64)	0.0
Level of significance		*	-
Co-efficient of variation (%)		2.27	-
Critical value for comparison		1.1824	-
Standard error for comparison		0.3352	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

that is positively correlated with final grain yield. Anjum *et al.* (2011) reported water deficit stress significantly reduced spike length results in smaller number of spikelets spike^{-1} and grains per spike^{-1} which ultimately reduced grain yield. Reduction in number of spikelets spike^{-1} of wheat was also noted by Pramanik *et al.* (2022) and Sindabad *et al.* (2023). These findings support our present findings regarding reduction of spikelets spike^{-1} in wheat resulted from inadequate irrigation supply.The development of spikelet in wheat was significantly enhanced by humic acid application which might be due to an increase in the availability and consumption of water and essential nutrients, resulting in an increase in key nitrogen metabolism enzyme activities and improved nitrogen transport, which has increased the rate of photosynthesis, growth and plant development of reproductive part of wheat plant.

4.15 Number of grains spike^{-1}

Number of grains spike^{-1} of wheat was significantly varied due to interaction effect of varieties and growing conditions (Table 15). Among the different treatment combinations, the maximum number of grains spike^{-1} of wheat (50.50) was observed in BWMRI Gom 3 under well water condition followed by that of BWMRI Gom 3 (49.61) grown under foliar humic acid treated condition. The minimum number of grains spike^{-1} of wheat (41.94) was observed in BWMRI Gom 1 under water deficit stress condition. Water deficit stress reduced the number of grains spike^{-1} of wheat in two wheat varieties at different magnitude (8.38% in BWMRI Gom 1 and 4.85% in BWMRI Gom 3). Soil application of humic acid improved the number of grains spike^{-1} under water deficit stress condition by

0.71% in BWMRI Gom 1 and 0.52% in BWMRI Gom 3. Foliar application of humic acid improved the trait of wheat by 5.31% in BWMRI Gom 1 and 3.24% in BWMRI Gom 3. The yield component *viz.* grains spike^{-1} in wheat may have considered to be the most important yield contributing character that is positively correlated with final grain yield of wheat and the most important parameter for screening wheat genotypes (Forgone 2009). Water deficit stress limits grains number either by influencing the amount of dry matter produced by the time of flowering or by directly influencing pollen or ovule function, which leads to decreased seed set (Prasad *et al.* 2008). Similar response of wheat genotypes to different moisture levels was found by Ali *et al.* (2017) and Ali *et al.* (2018) which agree to the findings of the present study. Humic acid significantly stimulated number of grains spike^{-1} in wheat which might be due to the capacity of the humic acid to enhance the photosynthetic pigments and biochemical activities in plants that results in increasing photosynthates that are directed towards the grain development and resulted in a greater number of grains spike^{-1} consequently number of grains plant^{-1}. On the other hand, exogenous application of humic acid under water deficit stress alleviated the adverse effects of water stress and positively improved the spike length and number of spikelets spike^{-1} which in turn improved the number of grains spike^{-1} in wheat under limited water condition. Our results are congruent with the results of Mehrinfar *et al.* (2023) who reported the favorable effect of foliar application of humic acid on grain yield and grain quality of bread wheat cultivars under conditions of water stress.

Table 15. Interaction effect of growing conditions and varieties on number of grains spike^{-1} of wheat

Wheat varieties	Growing conditions	Number of grains spike^{-1}	
		Nos.	% Change over WW
BWMRI Gom 1	Well water (WW)	45.78c	-
	Water deficit stress (WDS)	41.94d	-8.38
	WDS + Soil application of humic acid	42.24d (+0.71)	-7.73
	WDS + Foliar application of humic acid	44.17c (+5.31)	-3.52
BWMRI Gom 3	Well water (WW)	50.50a	-
	Water deficit stress (WDS)	48.05b	-4.85
	WDS + Soil application of humic acid	48.30b (+0.52)	-4.36
	WDS + Foliar application of humic acid	49.61ab (+3.24)	-1.76
Level of significance		*	-
Co-efficient of variation (%)		2.27	-
Critical value for comparison		1.6802	-
Standard error for comparison		0.4763	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

4.16 100 grains weight

The interaction effect of wheat varieties and growing conditions significantly affected the 100 grains weight of wheat (Table 16). Among different treatment combinations, the maximum 100 grains weight (5.09 g) was recorded in BWMRI Gom 1 at well water condition, whereas the lowest 100 grains weight (4.21 g) was recorded in BWMRI Gom 3 under water deficit stress condition. Water stress significantly declined 100 grains weight of wheat by 8.05% and 14.60% in BWMRI Gom 1 and BWMRI Gom 3, respectively compared to well water condition. Exogenous application of humic acid mitigated the adverse effect of water deficit stress and significantly accelerated the 100 grains weight of wheat as compared to stress condition. Moreover, 6.62% and 1.90% increase in 100 grains weight were observed in BWMRI Gom 1 and BWMRI Gom 3, respectively due to soil application of humic acid under water deficit stress condition, while 5.12% and 0.48% increase in 100 grains weight were observed in BWMRI Gom 1 and BWMRI Gom 3, respectively due to foliar application of humic acid under water deficit stress condition. 100 grains weight is an important yield component which represent the characters of the grain. The reduction in 100 grains weight might be due to the reduction in grain size spike^{-1} which favors the reduced grain weight. The reduction or increase in weight of 100 grains depends on the size and weight of the single grain. Water deficit stress affects the rate of photosynthesis that led to decrease in photosynthates and ultimately decrease the grain development which results in reduced grain size and less grain weight caused reduction in 100 grains

Table 16. Interaction effect of growing conditions and varieties on 100 grains weight of wheat

Wheat varieties	Growing conditions	100 grains weight	
		g	% Change over WW
BWMRI Gom 1	Well water (WW)	5.09a	-
	Water deficit stress (WDS)	4.68ab	-8.05
	WDS + Soil application of humic acid	4.99a (+6.62)	-1.97
	WDS + Foliar application of humic acid	4.92a (+5.12)	-3.33
BWMRI Gom 3	Well water (WW)	4.93a	-
	Water deficit stress (WDS)	4.21b	-14.60
	WDS + Soil application of humic acid	4.29b (+1.90)	-12.98
	WDS + Foliar application of humic acid	4.23b (+0.48)	-14.19
Level of significance		*	-
Co-efficient of variation (%)		4.41	-
Critical value for comparison		0.5933	-
Standard error for comparison		0.1682	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

weight. Reduction in grain weight due to water deficit stress was also reported by Zaman *et al.* (2016) and Rana *et al.* (2017) that support our present findings. Humic acid enhances the photosynthetic pigments and biochemical activities in plants that results in increasing photosynthates that are directed towards the seed resulted in improved seed characters. Arjumend *et al.* (2015) and Khan *et al.* (2010) reported significant effect of different humic acid rates and recommended NPK mineral fertilizer on thousand grain weight and grain yield in wheat that partially support our investigated results on 100 grains weight of wheat.

4.17 Grain yield plant^{-1}

Grain yield plant^{-1} was significantly influenced by the interaction effect of varieties and growing conditions (Table 17). The maximum grain yield plant^{-1} of wheat (5.76 g) was observed in BWMRI Gom 1 under well water condition, whereas the minimum grain yield plant^{-1} of wheat (3.97 g) was observed in BWMRI Gom 3 under water deficit stress condition. Water deficit stress reduced the grain yield plant^{-1} of wheat in two wheat varieties at different magnitude (27.78% in BWMRI Gom 1 and 24.38% in BWMRI Gom 3). Foliar application of humic acid improved the grain yield plant^{-1} of wheat by 24.76% in BWMRI Gom 1 and 25.19% in BWMRI Gom 3, whereas soil application of humic acid improved the grain yield plant^{-1} of wheat by 9.13% in BWMRI Gom 1 and 13.86% in BWMRI Gom 3. Yield is basically the complex integration of the different yield components. In the present study, water deficit stress reduced different yield components which ultimately reflected on grain yield plant^{-1} of wheat varieties (Pramanik *et al.* 2022).

Table 17. Interaction effect of growing conditions and varieties on grain yield plant^{-1} of wheat

Wheat varieties	Growing conditions	Grain yield plant^{-1}	
		g	% Change over WW
BWMRI Gom 1	Well water (WW)	5.76a	-
	Water deficit stress (WDS)	4.16c	-27.78
	WDS + Soil application of humic acid	4.54bc (+9.13)	-21.18
	WDS + Foliar application of humic acid	5.19ab (+24.76)	-9.89
BWMRI Gom 3	Well water (WW)	5.25ab	-
	Water deficit stress (WDS)	3.97c	-24.38
	WDS + Soil application of humic acid	4.52bc (+13.86)	-13.90
	WDS + Foliar application of humic acid	4.97ab (+25.19)	-5.34
Level of significance		*	-
Co-efficient of variation (%)		5.84	-
Critical value for comparison		0.8066	-
Standard error for comparison		0.2287	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

Both the wheat varieties reduced their grain yield plant^{-1} at water deficit stress condition. This reduction might be due to the reduction in photosynthetic activity and lower supply of assimilates that cause reduction in number and size of grain at stress condition (Farooq *et al.* 2014). Yield reduction in wheat due to water deficit stress was also found by Daryanto *et al.* (2017), Matiu *et al.* (2017), Pramanik *et al.* (2022) and Sindabad *et al.* (2023). These findings are very much consistent to our present findings. Seed yield improvement of wheat due to application of humic acid under water deficit stress condition was also reported by Tohidi (2015). Mutlu and Tas (2022) recommended that application of 4.5 L/ha humic acid at heading stage could be used to improve durum wheat yield as humic acid application created favorable growing environments for plant growth. El-Hashash *et al.* (2022) also reported that application of 60 kg humic acid per hectare improved wheat productivity under drought conditions. In line with this study, Hegab *et al.* (2020) have already stated that wheat grain yield and its components increased with an increasing the application of humic acid rates. These findings are supportive to our present findings.

4.18 Above ground biological yield plant^{-1}

Data regarding above ground biological yield in Table 18 depict that the above ground biological yield plant^{-1} of wheat was significantly interacted by the combined effect of wheat varieties and growing conditions. The results present that, BWMRI Gom 1 produced the highest yield (15.85 g) under well water condition, while the lowest yield of the trait (12.14 g) was recorded in BWMRI Gom 3. The biological yield plant^{-1} was substantially reduced in both wheat varieties under water deficit stress condition compared to well water condition. The more reduction (22.59%) due to water stress was recorded in BWMRI Gom 1 which indicates more susceptibility of the variety to water stress regarding the trait. On the contrary, comparatively less reduction (16.04%) due to water stress was found in BWMRI Gom 3 which indicates more tolerance of the variety to water stress in relation to above ground biological yield. Soil and foliar application of humic acid alleviated the adverse effect of water deficit stress resulted in increase of biological yield as compared to water deficit stress. The maximum alleviation (19.23%) in biological yield was observed in BWMRI Gom 1 due to foliar application of humic acid which expresses more synergistic effect of foliar humic acid on the variety under water deficit stress regarding the trait. The least increment (5.19%) in the trait was found in BWMRI Gom 3 due to soil application of humic acid which indicates less stress mitigating effect of soil application of humic acid on the respective variety under water deficit stress condition. In our present investigation, the above ground biological yield decreased and it was probably due to unfavorable condition (water stress) at vegetative stage; as a result, crop became thinned and produced less tillers which in turn decreased the biological yield (Pramanik *et al.* 2021). Water deficit stress experienced by wheat crop during growth stages is known to have cumulative effects expressed as a reduction in total biomass as compared to well water conditions (Zhang *et al.* 2018) is an agreement of the present study. Zhang *et al.* (2018) also observed drought decreased wheat biomass and yield by 25.00% and 27.50%, respectively that is supportive to our findings regarding above ground

Table 18. Interaction effect of growing conditions and varieties on above ground biological yield plant^{-1} of wheat

Wheat varieties	Growing conditions	Above ground biological yield plant^{-1}	
		g	% Change over WW
BWMRI Gom 1	Well water (WW)	15.85a	-
	Water deficit stress (WDS)	12.27e	-22.59
	WDS + Soil application of humic acid	13.49cd (+9.94)	-14.89
	WDS + Foliar application of humic acid	14.63b (+19.23)	-7.69
BWMRI Gom 3	Well water (WW)	14.46bc	-
	Water deficit stress (WDS)	12.14e	-16.04
	WDS + Soil application of humic acid	12.77de (+5.19)	-11.68
	WDS + Foliar application of humic acid	12.93de (+6.50)	-10.58
Level of significance		*	-
Co-efficient of variation (%)		2.75	-
Critical value for comparison		1.0755	-
Standard error for comparison		0.3049	-

In the column, means having similar letter(s) did not differ significantly at P≤5% level by Tukey. * indicates significant at 5% level of probability. Values in parenthesis indicate % improvement over water deficit stress in respective variety.

biological yield of wheat under water stress. It is worth noting that humic acid application in wheat promoted plant growth, yields (grain, straw, and biological), nutrient uptake in the soil and resistance to biotic and abiotic stress (El-Hashash *et al.* 2022). Thalooth *et al.* (2016) and Khan *et al.* (2010) reported that humic acid applied alone at 3 kg/ha the most economical rate to obtain the maximum yield of wheat under rainfed conditions and improve soil fertility.

CHAPTER V

SUMMARY AND CONCLUSION

A pot experiment was conducted at the research shade house of Crop Physiology and Ecology Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of November 2022 to March 2023 to investigate the ameliorative effect of exogenous application of humic acid on wheat under water deficit stress condition. The experiment was laid out in completely randomized design with three replications. Four growing conditions (well water, 65% field capacity; water deficit stress, 35% field capacity; soil application of humic acid @ 0.044 g kg^{-1} soil under water deficit stress and foliar application of humic acid @ 200 ppm under water deficit stress) and two wheat varieties (BWMRI Gom 1 and BWMRI Gom 3) were set up as experimental treatments. Observation were made on number of leaves plant^{-1}, length of flag leaf, breadth of flag leaf, relative leaf water content of flag leaf, chlorophyll content of flag leaf, proline content of flag leaf, SPAD value of flag leaf, leaf temperature, number of tillers plant^{-1}, plant height, number of spikes plant^{-1}, spike length, individual spike weight, number of spikelets spike^{-1}, number of grains spike^{-1}, 100 grains weight, grain yield plant^{-1} and above ground biological yield plant^{-1}. Interaction effect of growing conditions and wheat varieties significantly influenced the different morphological traits, physiological traits, yield and yield attributes of wheat except number of leaves plant^{-1}. The maximum length of flag leaf (27.78 cm), breadth of flag leaf (1.80 cm), relative leaf water content of flag leaf (78.62%), chlorophyll content of flag leaf (2.29 mg g^{-1} FW), number of tillers plant^{-1} (4.88), plant height (81.86 cm) number of spikes plant^{-1} (4.67) spike length (13.65 cm), individual spike weight (18.80 g), 100 grains weight (5.09 g), grain yield plant^{-1} (5.76 g) and above ground biological yield plant^{-1} (15.85 g) were observed under well water condition but proline content of flag leaf (2.41 µmole g^{-1} FW) and leaf temperature (22.49^{0}C) was observed under water deficit stress in BWMRI Gom 1, whereas the number of leaves plant^{-1} (5.76), SPAD value of flag leaf (56.67), number of grains spike^{-1} (50.50) and number of spikelets spike^{-1} (19.33) were observed in BWMRI Gom 3 under water deficit condition applied with foliar humic acid .

The minimum length of flag leaf (19.83 cm), breadth of flag leaf (1.44 cm), relative leaf water content of flag leaf (60.52%), chlorophyll content of flag leaf (1.23 mg g^{-1} FW), SPAD value of flag leaf (52.47), leaf temperature (16.89 ^{0}C), number of tillers plant^{-1} (3.30), plant height (68.87 cm), number of spikes plant^{-1} (2.89), spike Length (9.86 cm), individual spike weight (12.20 g), 100 grains weight (4.21 g), grain yield plant^{-1} (3.97 g) and above ground biological yield plant^{-1} (12.14 g) at water deficit stress condition in BWMRI Gom 3 but minimum proline content of flag leaf (1.43 µmole g^{-1} FW) was observed in BWMRI Gom 3 at well water condition. The minimum number of leaves plant^{-1} (4.63), number of spikelets spike^{-1} (16.67) and number of grains spike^{-1} (41.94) were observed in BWMRI Gom 1 under water deficit stress condition.

Water deficit stress reduced the number of leaves plant^{-1}, length of flag leaf, breadth of flag leaf, relative leaf water content of flag leaf, chlorophyll content of flag leaf, SPAD value of flag leaf, number of tillers plant^{-1}, plant height, number of spikes plant^{-1}, spike length, individual spike weight, number of spikelet spike^{-1}, number of grains spike^{-1}, 100 grains weight, grain yield plant^{-1} and above ground biological yield plant^{-1} by 0.86%, 21.59%, 13.34%, 14.78%, 14.84%, 1.13%, 4.83%, 16.59%, 24.83%, 29.78%, 1.94%, 8.38%, 8.05%, 27.78% and 22.59% in BWMRI Gom 1 and by 7.12%, 28.43%, 14.29%, 17.58%, 29.31%, 7.41%, 5.82%, 21.25%, 12.82%, 27.03%, 3.51%, 4.85%, 14.60%, 24.38% and 16.04 % in BWMRI Gom 3, respectively.

Soil application of humic acid improved number of leaves plant^{-1}, breadth of flag leaf, relative leaf water content of flag leaf, chlorophyll content of flag leaf, SPAD value of flag leaf, plant height, number of spikes plant^{-1}, spike length, individual spike weight, number of spikelets spike^{-1}, number of grains spike^{-1}, 100 grains weight, grain yield plant^{-1}, and above ground biological yield plant^{-1} by 16.85%, 3.85%, 6.48%, 3.07%, 0.17% ,0.56%, 27.24%, 2.24%, 14.17%, 1.91% 0.71%, 6.62%, 9.13% and 9.94%, in BWMRI Gom 1 and 5.23% , 5.95%, 9.02%, 1.75%, 30.08%, 18.99%, 3.05%, 0.28%, 14.24%, 2.35%, 3.80%, 8.52%, 11.06%, 0.69%, 0.52%, 1.90%, 13.86% and 5.19% in BWMRI Gom 3.

Foliar application of humic acid improved number of leaves plant^{-1}, length of flag leaf, breadth of flag leaf, relative leaf water content of flag leaf, chlorophyll content of flag leaf, SPAD value of flag leaf, number of tillers plant^{-1} , plant height, number of spikes plant^{-1} , spike length, individual spike weight, number of spikelets spike^{-1}, number of grains spike^{-1} , 100 grains weight , grain yield plant^{-1} , and above ground biological yield plant^{-1} by 6.91%, 17.36%, 10.26%, 13.27%, 16.92%, 2.89%, 8.52%, 4.22%, 25.61%, 9.25%, 30.15%, 8.58%, 5.31%, 5.12%, 24.76% and

19.23% respectively in BWMRI Gom 1 and by 6.54%, 10.49%, 16.67%, 7.67%, 31.70%, 29.53%, 4.50%, 5.87%, 14.24%, 0.43%, 26.98%, 12.37%, 14.50%, 3.64%, 3.24%, 0.48%, 25.19% and 6.50% in BWMRI Gom 3.

From the overall results of the present study, it was concluded that-

- Morpho-physiological traits, yield components and yield of tested wheat varieties were significantly affected under water deficit stress condition and BWMRI Gom 1 was comparatively more sensitive than the BWMRI Gom 3.

- Exogenous application of humic acid had ameliorative effect on morpho-physiological traits and productivity of wheat in relation to water deficit stress.
- Both soil and foliar application of humic acid improved morpho-physiological traits, grain yield as well as biological yield of wheat under water deficit stress condition but foliar application was comparatively more effective in alleviating the adverse effect of water deficit stress.
- Therefore, foliar application of humic acid could improve wheat productivity under water deficit stress condition.

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APPENDICES

Appendix I: Weather data during the growing season (2022-2023) wheat genotypes

Month	Relative Humidity (%)	Temperature		Rainfall (mm)
		Maximum ^{0}C	Minimum ^{0}C	0
November	95.763	30.63	16.6444	0
December	95.62	26.50	13.15	0
January	96.98	25.56	10.14	0
February	94.81	28.16	13.98	0
March	92.70	31.29	18.36	0

Source: Bangladesh Wheat and Maize Research Institute, Dinajpur

Appendix II: Morphological, physical and chemical characteristics of initial soil samples used in the pot experiment

Morphology	**Description**
Location	Crop Physiology and Ecology, HSTU, Dinajpur
AEZ	Old Himalayan Piedmont Plain (AEZ-1)
General Soil type	Non-Calcareous Brown Flood Plain Soil
Parent material	Piedmont alluvium
Soil series	Ranishankail
Drainage	Moderately well drained
Flood level	Above flood level
Topography	High land

Physical characteristics	**Value**
Bulk density (g cm^{-3})	0.86-1.07
Particle size (%)	
Sand (2-0.02mm)	60.0
Silt (0.02-0.002mm)	27.0
Clay (< 0.002mm)	13.0
Textural class	Sandy loam

Chemical characteristics	**Content**	**Interpretation**
pH	5.25	Moderately acidic
Organic carbon (%)	0.97	Low
Organic matter (%)	1.67	Low
Total N (%)	0.084	Very low
Available P (µg/g)	42.68	Medium
Exchangeable K (meq/100g soil)	0.33	Medium low

Source: Analysis of initial soil samples was done in SRDI, Dinajpur, Bangladesh

Appendix III: Analysis of variance (ANOVA)

Sources of variation	Degrees of freedom	Mean square								
		Number of leaves $plant^{-1}$	Length of flag leaf	Breadth of flag leaf	Relative leaf water content	Total chlorophyll content of flag leaf	Proline content of flag leaf	SPAD value	Leaf temperature of flag leaf	Number of tillers $plant^{-1}$
Growing conditions	3	0.29387^{NS}	62.4837**	0.06629**	7.4750**	1.2429**	0.7887*	7.16722**	15.9695**	0.53044**
Wheat varieties	1	2.90510*	12.4560**	0.03920**	3.1550**	0.5823**	0.5861*	0.16667*	7.3261**	2.28784**
Growing conditions × Wheat varieties	3	0.19266^{NS}	4.4769**	0.00258*	1.0360*	0.1329**	0.0146*	3.50333*	3.5185*	0.16664*

** Significant at the 1% probability level
* Significant at the 5% probability level
NS Not significant at the 5% probability level

Appendix III: Contd.

Sources of variation	Degrees of freedom	Mean Square								
		Plant height	Number of spikes plant^{-1}	Spike length	Individual spike weight	Number of spikelets spike^{-1}	Number of grains spike^{-1}	100 grains weight	Grain yield plant^{-1}	Above ground biological yield plant^{-1}
Growing conditons	3	18.315**	0.88589**	6.47057**	27.3458**	1.2491**	12.991**	0.35662**	2.37690**	9.22625**
Wheat varieties	1	530.912**	6.36540**	2.74727*	22.8345**	20.1667**	186.986**	1.53520**	0.33135*	5.82135**
Water conditions× Wheat varieties	3	4.919**	0.28494*	1.91881**	1.3340*	0.3157*	0.635*	0.09515	0.06205*	0.73825*

** Significant at the 1% probability level
* Significant at the 5% probability level
NS Not significant at the 5% probability level