A Sterile Hydroponic System for Characterizing Nitrogen Use Efficiency (NUE) and Plant Physiology of Bread Wheat

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IN THE NAME OF ALLAH THE BENEFICENT THE MERCIFUL

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This is to certify that the dissertation entitled "**A Sterile Hydroponic System for Characterizing Nitrogen Use Efficiency and Plant Physiology of Bread Wheat.**" submitted by (**Jawaria Fatima**) is accepted in its present form by the Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan, as to satisfy the partial fulfillment for the degree of M.Phil in Plant Science with specialization in Plant Physiology.

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DECLARATION

I hereby declare that no part of this thesis has been previously submitted to this or any other University as part of the requirement for a higher degree. The contents of this thesis are the results of my own work unless otherwise acknowledged in the text or by reference. The research work presented in this thesis was carried out by me in the Plant Physiology laboratory, Department of Plant Sciences, Quaid-i-Azam University Islamabad.

(Jawaria Fatima)

THIS WORK IS DEDICATED

TO MY LOVING PARENTS

(Mr. & Mrs. Muhammad Khurshid Khan)

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ABBREVIATIONS

Abbreviations	Descriptions
ANOVA	Analysis of Variance
C	Control
Chl	Chlorophyll
FW	Fresh Weight
GR	Germination rate
L.A	Leaf Area
N	Nitrogen
N.A	Network Area
NUE	Nitrogen Use Efficiency
NUpE	Nitrogen uptake Efficiency
NUtE	Nitrogen Utilization Efficiency
RW	Root Width
RWC	Relative Water Content
SL	Shoot Length
T1	Treatment 1
T2	Treatment 2
TW	Turgid Weight

Abstract

Nitrogen fertilizer is a major input for cereal crop production around the world. The control of this resource is a significant challenge to most agricultural system as it can have significant effects on yield and the climate. In Agro-ecological system with intensive cropping it is the delimiting factor that reduces both quantity and quality of the crops. Chemical fertilizers (such as Urea) have been the main source of nitrogen since last 7 decades. Due to high solubility most of the fertilizer (~50-70%) is leached to underground water reserves. To tackle such a serious issue, there is a need of time to increase the efficiency of N uptake and its use for better crop production. The current research was been carried out to identify varieties that perform well in both nitrogen deficient and efficient environments. Eleven cultivars PAK-13, PARWAZ-94, PISBK-91, BAKHAR-2000, FSD-83, KOHNOOR-83, LASANI-2008, NARC-11, SA-42, SOKOLL and WAFAQ were grown in sterile hydroponic environment specially designed for this experiment. Hoagland solution in the sterile system was modified to make three treatments i.e. Control (100%), Treatment 1 (66%), and Treatment 2 (33%). Three replicates of eleven cultivars were grown to estimate physiological and morphological traits. Highly significant variation was observed in both between the cultivars and treatments ($p<0.0001$). Nitrogen content of the plant was estimated using micro-Kjeldahl apparatus. Nitrogen Use Efficiency (NUE) was estimated to identify cultivars (NARC-11, Sokoll and SA-42) having capacity to grow efficiently under limited nitrogen condition. Some cultivars (KOHINOOR-83, FSD-83, Bhakhar-2001 and PIRSBK-91) were susceptible to limited nitrogen regime. Overall, the results indicated that wheat cultivars responded well to N application with medium rate of application within experiments.

1 Introduction

1.1 General introduction of wheat

Wheat is the world's third most valuable cereal crop, next to maize (*Zea mays* L.) and rice (*Oryza sativa* L.).Total production of the wheat among all major cereal crops account for 19 percent. Wheat provides 20% of the total food calories consumed by people around the globe (Bagge *et al.*,2007; P. K. Gupta *et al.*,1999). Wheat provides 78.10% carbohydrates, 14.70% protein, 2.10% fat and considerable proportions of minerals (zinc, iron) and vitamins i.e. thiamine and vitamin-B (Fraley,2003; Topping,2007).

Wheat is grown on around 237 million hectares annually, yielding 420 million tons (Olabanji *et al.*,2004), and accounting for at least one-fifth of man's calorie intake(Ohiagu *et al.*,1996). It has been cultivated for over 10,000 years probably and emerged in the "Fertile Crescent" along with other staple crops. However, ancestral wheat may have looked quite different with much small kernels than what we actually have today. The early domesticators of wheat obviously preferred to select for the plants with especially large kernels since more nutrients could be derived from each stalk (Oyewole,2010).

1.2 Origin and evolution of wheat

Wheat is a big cereal crop with annual harvests of more than 600 million tons. The total world harvest was approximately 607 million tons in 2007 as compared to 652 million tons of rice and 785 million tons of maize, respectively[1].Wheat was first cultivated about 10,000 years ago as a part of the Neolithic Revolution, which saw a transition from food collection and selection to settled agriculture. The earliest cultivated wheat varieties were diploid "genome AA" (einkorn) and tetraploid "genome AABB" (emmer), and their genetic associations indicate that they originated in Turkey's south-eastern region (Dubcovsky & Dvorak,2007; Heun *et al.*,1997; Nesbitt,1998).Agriculture extended the Near East about 9,000 years ago when hexaploid bread wheat made its first appearance (Bonjean *et al.*,2001).

[1] http://faostat.fao.org/

The earliest cultivated wheat was basically landraces picked by farmers from wild populations, probably an early and evidently non-scientific method of plant breeding due to their superior yield and other characteristics. However, the choice of genetic characteristics which differentiated them from their wild relatives was often correlated with domestication. Others have discussed this domestication phenomenon intimately, but two characteristics have enough interest to mention here. The first is the spike shattering failure at maturity, which results in seed loss during harvesting. This is obviously an essential feature in ensuring dispersal of seed in natural populations. The mutations at Br locus decide the non-shattering feature (Nalam *et al.*,2006).

Bread wheat is only existed in agriculture, having emerged from the hybridization of cultivated emmer with the unrelated wild grass *Triticum tauschii* (*Aegilopstauschii* and *Ae. squarosa*) einkorn and emmer. Obviously, it evolved to the domestication of natural populations. Formers selected the genome AABBDD for its superior properties. So this hybridization may have happened several times. Modern evolution of wheat product is shown in Figure 1.

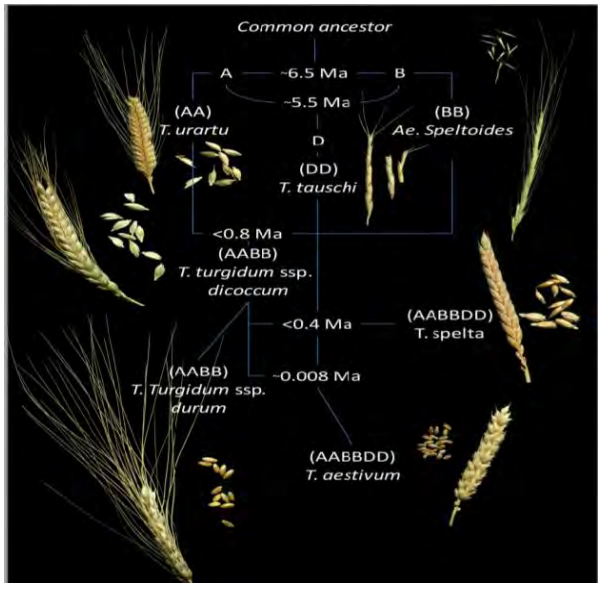

Figure 1: The evolutionary relationship between durum and cultivated bread as well as related wild diploid grasses, showing examples of spikes and grain(Wilson,2003).

Wheat's spread across the world from its origin was elegantly described by (Bonjean *et al.*,2001). The main route to Europe was through Anatolia to Greece (8000 BP) and then

north via the Balkans to the Danube (7000 BP) and then Italy, France, and Spain (7000 BP) to reach UK and Scandinavia around 500BP. Wheat spread to central Asia via Iran, eventually reaching China by about 3000 BP, and to Africa, initially through Egypt. It was brought to Australia in 1788Mexico in 1529 and by the Spaniards(Shewry,2009).

1.3 Economic importance of wheat

. Wheat is developed over a wide scope of ecological conditions. Subsequently, a hereditary agreement is of immense significance for hereditary qualities just as for plant rearing objectives. In most countries, wheat is the most important source of carbohydrates. It is also the leading source of vegetable protein in human food worldwide, with a 13% of protein content. It is comparatively high compared to other major cereals. Grain of wheat is also a source of micronutrients and dietary fiber. It contains minerals, vitamins and fats (lipids), and is highly nutritious with a limited amount of animal or legume protein added(Sarwar *et al.*,2013; Shewry & Hey,2015).Food primarily based on wheat is greater in fiber than a food based on meat (Waugh & Mrak,1988). It is noteworthy that many EFSA-approved health statements refer to fiber components in cereals, including wheat and barley. They have beneficial effects on intestinal function, glucose reactions, and cholesterol regulation[2].

1.4 Wheat genome

Wheat with a large genome size (16000 Mb) and high proportion about 80 percent of repetitive sequences, has been a complex crop for genomics research (P. Gupta *et al.*,2008). It is adapted to the world's temperate regions. About 1000 years ago, it was one of the first crops to be domesticated. Cytogenetically wheat has three subgenomes A, B and D. Each subgenome of wheat has 7 chromosomes making n=21.They are organized in 7 homoeologous groups. Each homoeologous group has 3 closely related chromosomes. One from each of the 3 related subgenomes is diploid. The diploid progenitors of the A, B, and D subgenomes have been identified. There has always been a debate regarding the progenitor of the genome B(Gill *et al.*,2004).

[2] http://ec.europa.eu/nuhclaims/

A gene triticum consists of 6 species: *T. urartu* (AA genome), *T. monococcum L.* (AA genome), *T. turgidum L.* (AABB genome), *T. timopheevii* (AAGG genome), *T. aestivum L.* (AABBDD genome), and *T. zhukovskyi*(AAAAGG genome) (Matsuoka,2011). Wheat is a largest cereal crop produced worldwilde. It has been studied that a wide range of agronomic traits is located in the wheat genome. Its large chromosome and the ability of the polyploidy genome to tolerate the addition and removal of chromosomes facilitate a rapid progress in early wheat genetics by using cytogenetic techniques (Lagudah *et al.*,2001).

1.5 Taxonomy of wheat

Wheat belongs to Poaceae Family. It is a monocot plant cultivated during the rabies season. The optimal planting season is between mid-October and mid-November, when it is harvested between late April and late May. Morphologically, it's about 2-5 feet long. It has a fibrous root system, i.e. the root, except radicle, which originates from some portion of the plant. The stem is made up of four or five branches called Tillers. Each tiller is composed of internodes and nodes. Nodes are the portion of the plant where leaves arise and the component between nodes is the internodes. The wheat stem is fistular, i.e., hollow.

The leaves are sessile, meaning that the leaves are without petioles and stalks. Leaves consist of two sheathing elements and a leaf comb. The sheathing section protects the stem and the leaf blade is the outer part. Leaf blades are found at the junction of the sheathing portion, called legules, and such leaves are called legulate leaves. Wheat has a venation in parallel. Spikes are the inflorescence of wheat. The spike's main axis is called the rachis. There are 15 to 25 spikelets or florets in each spike and each spikelet consists of its own rachilla-like axis. Two covers/layers, called glooms, cover each floret. Each floret consists of extensions or needles called awn-like structures.

1.6 Worldwide wheat consumption

Every month of the year, wheat crop is harvested somewhere in the world. However, the Global harvest occurs between April and September in the temperate zone of the Northern Hemisphere. Significantly less wheat is cultivated in the Southern Hemisphere where harvesting take place from October to January. The consumption of the wheat products is changing the world widely. The average annual rate of wheat consumption in the developed

countries of the world has remained stable at around 175 kg per capita since the early 1970s. However, in developing countries wheat is a major source of calories, and to a lesser extent, protein in millions of people's diets(Briggle & Curtis,1987).

In 2016, the wheat production was calculated as 749 million tons. This amount is 14 million tons more than the production of year 2015 and also 2.6 million tons greater than the previous expectations[3].World's top three wheat producing countries are China, India and USA, producing 126, 95 and 60 million metric tons per year respectively (www.worldatlas.com).

1.7 Wheat production in Pakistan

In Pakistan, wheat is produced in greater amount than all other cereal crops. From the overall GDP of agriculture sector in Pakistan which is 19.5%, wheat shares almost 1.9% GDP. Pakistan ranks 3rd in Asia and 8th in the world in the production of wheat. In years 2015-2016, Pakistan produced 25.45 million tons of wheat, which is almost equal to the estimated amount (25.5 million tons)[4].

In 2017, the estimated amount of wheat production was 25.8 million tons which preceded the production of previous year. In 2018, it was 26.8 million tons. Pakistan consumes almost 23 million tons of wheat per year if the wheat production comes below this amount then it will be difficult to satisfy the demand and will contribute to the import of wheat (https://www.world-grain.com).

1.8 Role of nutrients in plants

In nature, there are a huge number of elements. Sixteen are essential for the proper growth and development of the crop plants. The **macro or major nutrients** are called carbon (C), oxygen (O), hydrogen (H), nitrogen (N), phosphorus(P), potash(K), calcium(Ca), magnesium(Mg), and sulfur(S). They are required in comparatively large amounts. The **micro or minor nutrients** required in smaller quantities for the crop plant vegetative, and

[3]www.fao.org

[4]www.pabausa.com

reproductive growth are iron (Fe), copper (Cu), zinc (Zn), boron (B), molybdenum (Mo), manganese (Mn), and chloride (Cl). 85-9 percent of the total plant content is contributed by C, H, and O. **N** gives crop plants a dark-green color. It increases the vegetative growth of the crop plants. It is very important for leaf starch preparation and amino acid production.

P is the component of certain nucleic acids, chromosomes, coenzymes, and phosphatides. In about 60 enzymatic plant systems, P works as a catalyst. It reduces the negative effects of the plant salts. It also regulates the water in the plants.**Ca** is a major component of the wall of plant cells. It promotes early root growth, and growth of the plants. Micronutrients are always required in very small amount. They play a very important role in crop-plant physiological processes (Imran & Gurmani,2011).

1.9 Importance of nitrogen

Nitrogen (N) plays a prominent role in the metabolism system of the plants. All essential processes in the plants are associated with proteins. Nitrogen is a basic component in all of them. Nitrogen is found extensively in the cultivation of vegetables (Wang *et al.*,2008). It is an essential component of chlorophyll, protein, nucleic acids, and growth hormones (Barker *et al.*,1974). Nitrogen plays a vital role in agriculture by increasing the yield of the crop (Massignam *et al.*,2009). It also improves the food quality (Ullah *et al.*,2010).

Nitrogen being a major food for plants is an important constituent of protein (build from amino acids that involve in catalization of chemical responses and transportation of electrons) and chlorophyll (which enable the process of photosynthesis) present in many major parts of the plant. Nitrogen plays an important role in various physiological processes. It imparts dark green color in the plants. It promotes growth and development of leaves, stems and other vegetative parts. It also stimulates the growth of the roots. Nitrogen generates fast early growth and improves the production of fruit. It promotes the growth of the leafy vegetables. It also increases protein content of the fodder crops. It encourages the utilization and uptake of other nutrients including phosphorous, potassium. Nitrogen controls overall growth of the plants (Bloom,2015).

1.10 Deficiency of nitrogen

Nitrogen deficiency causes stunted growth of the plants. Red and purple spots are appeared on the leaves. It also causes chlorosis changing of the green color into the yellow color of leaves. It restricts lateral bud growth from which leaves, stems and branches develop. Symptoms of deficiency first appear on the older leaves of plant(Bianco *et al.*,2015), then leaf senescence begins. The excessive nitrogen application has an adverse effect on growth of the plant. It encourages extra dark-green color on the leaves. It makes the whole growth of plant succulent and prefers less quantity of fruit with less quality.

1.11 Nitrogen as a fertilizer

Nitrogen fertilizer is used in different farming systems to increase crop yields. The utilization of nitrogen fertilizer is increasing dramatically in recent decades to satisfy the rising food demands of the world's population (An *et al.*,2006). The increase in the global production of cereals is directly linked to the application of nitrogen fertilizers. Nitrogen fertilizers were extensively used in bread wheat to increase the protein content and yield of grain (Le Gouis *et al.*,2008). Nitrogen is used in most countries for plants in the form of urea, ammonium nitrate, anhydrous ammonia and ammonium sulfate (Andrews *et al.*,2013).

Animal manure is indeed an important component of nitrogen fertilization alongside commercial mineral types of nitrogen (Hooda *et al.*,2000; Körschens *et al.*,2013). Mineral nitrogen fertilizers are readily converted by crops, because of their solubility. Both ammonia and urea are converted to nitrate (NO_3) but their conversion depends on soil nature and climatic conditions as well (Jensen *et al.*,2011). If we provide organic or inorganic nitrogen to the plants, nitrate (NO_3) is the most common source of nitrogen for most crops (Gioseffi *et al.*,2011). The urea has higher concentration among all known solid nitrogen fertilizers (Schepers & Raun,2008).

1.12 Nitrogen use efficiency

Nitrogen is the most significant yield-limiting factor in the production of a crop. Increasing the efficiency with which plants use the nitrogen given to achieve yield is crucial for the growth of the sustainable agriculture (Tilman *et al.*,2002). However, crop growth in the past decades with excessive amounts of N application (Diouf *et al.*,2002) and the ability of pants

to recover only 50 % of the N applied have led to many environmental problems (Gastal *et al.*,2015). Thus, thinking about future difficulties of food security and fatigue of natural resources, further increases in wheat production will have to start from yield improvement in existent cropland with the most proficient utilization of available resources (N, water, and land) (Borlaug & Dowswell,2003; Lloyd T. Evans,1999; R. Fischer *et al.*,2014).

One approach to upgrade yield by N (reducing the yield gap between actual-yields and N-limited yields) without disturbing the climate is to improve crop N use efficiency (grain yield produced per unit of N applied) (Moll *et al.*,1982). NUE can be evaluated by the Nitrogen utilization efficiency (NUtE), (the amount of grain produced per unit of N uptake at maturity) (Ciampitti & Vyn,2014). However, because of the curvilinear connection between the yield and NupMAT in which increase of the yield is larger at lower NupMAT levels and arrive at an asymptote yield (Cassman *et al.*,2002; Singh,2002). The rate of increase in yield is not constantly connected with a similar rate of increase in NupMAT resulting in a decay in NUtE (Gastal *et al.*,2015).

A decrease in NUtE is clarified by the nonparallel increases in yield and NupMAT. However, factors underlying this relationship are not well understood. As a result, understanding the crop N uptake process, which is closely related with NUtE, is essential to direct agronomic and breeding strategies to increase yield and NUE at the same. Many research has investigated ways to increase yield by analyzing yield components (R. A. Fischer,2008; Slafer, Andrade, & Satorre,1990), and trends in NupMAT in wheat (Austin *et al.*,1980; Hamnér *et al.*,2017; Slafer, Andrade, & Feingold,1990). However, there is a need for further investigation of these two variables in a comprehensive manner (Barraclough *et al.*,2010).

1.13 Hydroponics

Hydroponics can be defined as the cultivation of plants without soil. Hydroponics, a Greek word meaning **hydro** (water) and **ponos** (labor) is the method of growing plants in various types of substrates (chemically inert), gravel, sand, and liquid, in which only nutrients are added, but no soil is used (Aires,2018; Savvas,2003). There are no differences between soil-grown and soilless plants. In both processes, the nutrients must be dissolved in water before plants can absorb them (Bridgewood,2002).

In hydroponics, the nutrients are dissolved in water and the solution goes into the plant roots, which uptake the water with minerals toward various plant parts (BENTON JONES,2005; Bridgewood,2002). Plants are allowed to contact directly with the nutrients in the soilless medium. It replaces the soil with growing media. The growing media can be Rockwool, perlite, sand, etc. Their important role is to make the roots oxygenated and to transfer nutrients to the water. The water pump is usually used to add nutrients in growing media which moves across the roots (Jones Jr,2016).

1.13.1 History of hydroponics

Hydroponics does not evolved over night but it has undergone many scientific researches by scientists. The detailed history of hydroponics is given as follows:

• The 600 BC: The Euphrates River and the hanging gardens of Babylon in Babylonia are ancient examples of hydroponics.

• The 1000 to 1100 AD: “Chinampas” floating gardens developed by Aztecs in the Island of Mexico.

• The late 1200s: Marco polo during his trip to China discovered floating gardens.

•1600s: The first experience was performed by Belgian Jan Van Helmont on the growth of plants and their constituents.

• 1699: John Woodward an English man grew a mixture of different soil particles in water. He came to know that plants absorb nutrients from minerals and certain substances in water, obtained from the soil. This is an incorrect statement.

• The first standard formula for mineral nutrients of plant dissolved in water was derived by German Scientists, Wilhelm Knop and Julius von Sachs in 1860s.

• The term “Hydroponics” coined by W.F Gericke (U.C. Berkley) in the years between 1920s and 1930s. He practiced growing plants in a water solution and performed many experiments regarding hydroponics.

• In 1940s, Hydroponics was used to supply troops stationed with fresh vegetables on the isolated and non-arable Wake Island.

• 1950s: Globally, Hydroponics was used for commercial farms and greenhouses. It gained much popularity in many countries such as Spain, England, France, Germany, Italy, etc.

• From 1960s to now, several Hydroponic systems are evolved and put into use, including the Drip System, Ebb & Flow, Aeroponics and Nutrient Film Technique. In the recent two decades, formers show interest in Hydroponics when applied to large-scale greenhouse farms to provide foods for millions of people worldwide.

1.13.2 Sterile hydroponics system

Sterilization is important for the removal and destruction of all microorganisms including fungi, viruses, protozoa, spore forming and non-spore forming bacteria etc, which are responsible for the contamination of any material of our interest (Goyal,2007). However, there are several types of hydroponic systems and can be sterilized by different methods. There are different methods to sterilize your material such as,

- Heating in an autoclave
- Dry heat sterilization
- Filtration
- Exposure to UV radiations

Different hydroponic systems required the different sterilization based on their design and infrastructure (Tzanakakis *et al.*,2014).

1.14 Aims and objectives

Overpopulation has increased the global food demand and thus the nutrient fertilization in cereal crops has increased enormously in the past 2 decades. The extensive use of nitrogen along with other nutritionally important macro-nutrients in cropping systems has not only caused environmental concerns but also increases the production cost to farmers. Considering these facts, the current study was designed to

- Phenotypic screening of the historical bread wheat cultivars of Pakistan for NUE.
- To evaluate correlation between SPAD, GY, FW, RWC, LA and NUE.
- To establish sterile hydroponic system to study the input use efficiency of major crops.
- Study the relationship between different morpho-physiological traits and NUE.

2. Materials and methods

The experiment was performed at Plant Physiology Laboratory in the Department of Plant Science, Quaid-e-Azam University Islamabad.

2.1. Plant material

Cultivars were selected based on their performance in nitrogen based experiment of plant physiology laboratory, Quaid-i-Azam University. Sokoll, Bakhar-2001, WAFAQ, NARC-11 and SA-42 were Nitrogen use efficient cultivars. LASANI-2008, PARWAZ-94, KOHINOOR-83, PIRSBK-91, and FSD-83 were Nitrogen use non-efficient cultivars and PAKISTAN-13 was high yielding cultivar used as control check. The seeds of the cultivars were attained from NARC wheat department in 2012. The seeds were increased and checked for phenotypic purity from 2014-2018.

Eleven wheat cultivars were used during the experiment (see supplementary table 9). Three treatments were given to these varieties under sterile conditions in hydroponic system named as Control (optimum amount of all nutrients were given), Treatment 1 and 2 (in which the concentration of nitrogen was low). The seeds of the cultivars were attained from NARC wheat department in 2012. The seeds were increased and checked for phenotypic purity from 2014-2018.Thehydroponic nutrient solution contained both micro and macro-nutrients.

The macro-nutrients include Potassium Nitrate, Magnesium Sulphate Heptahydrate, Calcium Nitrate Tetrahydrate, and Iron Chelate Fe EDTA. While the micro-nutrients includes Copper Sulphate Pentahydrate, Sodium Molybdate Dihydrate, Zinc Sulphate Heptahydrate, Manganese Chloride Tetrahydrate, Boric Acid and Potassium Dihydrate Phosphate. The nutrient solution pH was adjusted to 6.0.

2.2. Seed sterilization

The seed were washed twice with double distilled water. After this seeds were sterilized with 70% ethanol for 10 minutes on shaker. Later sterilized seeds were again washed with double distilled water and treated with 20% household bleach for about 10 minutes on shaker which was followed by multiple washings with autoclaved water. Afterwards we get sterilized seeds which

were further used in sterile hydroponic system. The above surface-sterilization of seeds was performed in laminar flow hood to prevent contamination.

2.3. Hydroponic system construct

To design a sterile hydroponic system the material listed in the table (Table 1) are required:

Table 1: List of materials for the construction of hydroponic system

Sr no.	Material	Quantity
1	Autoclave able transparent bottles (1L)	2
2	Plastic pipe	5 ft long
3	Pipe connector	2
4	Air pump	1
5	Air filters	2
6	Falcon tube	1
7	Silicon glue	2
8	Plastic cutter	1
9	Tape	2
10	T shaped pipe connector	4
11	Small tapes to control air	2
12	Sponge	1

The materials used for the construction of sterile hydroponic system were autoclaved separately according to their requirements. After this all the parts of this system were assembled very carefully using the laminar flow hood to avoid every kind of contamination. Two autoclave able bottles were used to construct two separate chambers, the upper chamber support the aerial part of the plant while the lower chamber mainly contain the plant root system and Hoagland nutrient solution.

- The lids of the bottles were separated and then scrapped well so that they become the rough and could be easily glued to gather by using silicon glue. When the glue got dried, a 30 mm whole was drilled at the middle of the lids where a plant holder is fitted.

- The plant holder was built from the 50ml falcon tube along with its lid. The pointed bottom of the falcon tube was removed by using the plastic cutter. Small holes were drilled in the lid of the falcon tube which allow the seeds to get nutrient and moisture from the lower chamber containing nutrient solution and from which the root move toward the nutrient solution. The plant holder was suspended upside down in the hole made in the glued lid and fixed tightly by using the silicon glue.

Figure 2: Falcon tubes containing seeds, fixed into the bottle

- Two holes were drilled in the bottom of the upper chamber to fix the air filters which facilitate ventilation of air from the upper chamber. Two autoclaved pipe connectors were fixed by using glue in those holes which are used to hold the air filters.
- Two holes were drilled vertically at little distance from each other in the sides of the lower chamber. The first hole was drilled about 3 cm down from the top of the bottle (used to aerate the solution) however the other hole was drilled near the bottom of the lower chamber (used for draining when solution needed to be change). A 150 mm plastic pipe was attached inside the lower chamber to the upper air inlet hole. The both holes of lower chamber were filled with the plastic tap adopters for the control.

Figure 3: Fully organized sterile hydroponics system

- The each and every part of the system was wrapped with foil and autoclaved. After autoclave all the parts of the system were assembled in laminar flow hood to maintain the sterility.

2.4. Nutrient solution preparation

The Hoagland solution used as a hydroponic nutrient solution was developed by Hoagland and Snyder in 1933(D. HOAGLAND,1933). It's revised by Arnon in 1950 and refined by Hoagland and Arnon in 1938(D. R. Hoagland & Arnon,1950).It is one of the most known solution compositions for the growing plants (in the scientific world) with more than 16,000 citations listed by Google Scholar(Zhao *et al.*,2012). The Hoagland solution contains all of the necessary nutrients that green plants need. It is ideal for promoting the growth of a large range of species of plants (Metali *et al.*,2012).

The solution defined by Hoagland in 1933 was personalized several times, primarily to include Fe-EDTAs and modify the concentrations and numbers of micro-nutrients. In the 1950 revision, only one concentration (Mo 0.01 ppm) was changed relative to 1938, while the composition and concentrations of macronutrients remained the same since 1933. The concentration of the nutrients used in Hoagland are N 210ppm, P 31 ppm, K 235 ppm, Ca 200 ppm, S 64 ppm, Mg 48.6 ppm, Fe 2.9 ppm, Na 1.2 ppm, Cl 0.65 ppm, B 0.5 ppm, Mn 0.5 ppm, Zn 0.05 ppm, Mo 0.05 ppm and Cu 0.02 ppm.

To estimate the impact of Nitrogen, Hoagland solution was modified to have three different concentrations of Nitrogen (70 ppm, 140ppm and 210 ppm) as given:

Table 2: Hoagland solution concentration per litre

Concentration(ppm)	**Nutrients**	N	K	Ca	P	S	Cl	Na	Mg	B	Fe	Mn	Zn	Cu	Mo
	Control	210	235	200	31	64	0.65	1.2	48.6	0.5	2.9	0.5	0.05	0.02	0.05
	Treatment 1	140	235	200	31	64	0.65	1.2	48.6	0.5	2.9	0.5	0.05	0.02	0.05
	Treatment 2	70	235	200	31	64	0.65	1.2	48.6	0.5	2.9	0.5	0.05	0.02	0.05

Hoagland solution required following salts and acids:

1. Potassium nitrate (KNO_3)
2. Calcium nitrate tetrahydrate($Ca(NO_3)_2.4H_2O$)
3. Magnesium sulfate heptahydrate($MgSO_4.7H_2O$)
4. Potassium dihydrogen phosphate(KH_2PO_4) or
5. Ammonium dihydrogen phosphate $(NH_4)H_2PO_4$
6. Iron chelate (Fe-EDTA)
7. Boric acid (H_3BO_3)
8. Copper sulfate pentahydrate($CuSO_4.5H_2O$)
9. Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$)
10. Manganese chloride tetrahydrate($MnCl_2.4H_2O$)
11. Molybdic acid monohydrate ($H_2MoO_4.H_2O$) or
12. Sodium molybdate dihydrate($Na_2MoO_4.2H_2O$)

Initially, the stock solution for each nutrient was prepared in 40 ml or 50 ml. later it is used for the nutrient solution. This solution was prepared by the following weighed chemicals in required concentration and then dissolved in distilled water.

Table 3: To prepare the stock solutions and a full Hoagland solution

Components	Stock solution per liter	Stock solution per 100ml
Macronutrients		
$2MKNO_3$	202g/L	20.2g/100ml
$2MCa(NO_3)_2.4H_2O$	236g/L	47.2g/100ml
2M $MgSO_4.7H_2O$	493g/L	49.3g/100ml
Fe-EDTA	15g/L	1.5g/100ml
Micronutrients		
H_3BO_3	2.86g/L	0.286g/100ml
$MnCl_2.4H_2O$	1.81g/L	0.181g/100ml
$ZnSO_4.7H_2O$	0.22g/L	0.22g/100ml
$CuSO_4.5H_2O$	0.08g/L	0.008g/100ml
$Na_2MoO_4.2H_2O$	0.12g/L	0.012g/100ml
1M KH_2PO_4	136g/L	13.6g/100ml

The Hoagland solution formulation based on (NH_4) H2PO4 rather than KH_2PO_4 must be prepared in compliance with a separate protocol referred to as the solution in the 1938 and 1950 circulars. As sodium Fe-EDDHA, Sprint 138 iron chelate is produced, while the original solution formulation of Hoagland (1933) optionally contains ferric or ferrous tartrate but no sodium ions. Other micro-nutrients (e.g: Ni. Co) and fairly non-essential elements (e.g. Hg, Pb) indicated in Hoagland's 1933 pioneer publication (termed as A-Z solutions a and b(Schropp & Arenz,1942) are omitted from his later circulars. These organic compounds and elements are not essential for normal plant nutrition(Murashige & Skoog,1962).

As an exception, there is evidence that, for example, some algae require cobalt for the synthesis of vitamin B12. On the other hand, it is evident that the modified Hoagland solutions of 1938 and beyond are balanced nutrient solutions that address the question how to compose and concentrate the solutions ideally suited to the plant growth (D. Hoagland,1920).

2.5. Experimental Design

The setup was assembled in laminar air flow. 5 surface sterilized healthy seeds were planted in each system. Five replicates to each treatment and cultivars were planted on same date.

Two replicates were used for estimation of morpho-physiological data, while other three were used to estimate relative water content and nitrogen content.

2.6. Phenotypic traits

The following phenotypic traits were evaluated during the hydroponic experiment.

2.6.1. Germination rate

The phenomenon in which seeds sprout is known as germination. Days to germination were recorded when 50% of the plants were germinated during the experiment.

2.6.2. Chlorophyll content

The second leaf of 3 plants from each replicates was taken and the chlorophyll content of those leaves was measured by the hand held chlorophyll measuring device SPAD-502 plus. Chlorophyll was measured at the day 21th after germination.

2.6.3. Fresh weight (FW)

We measured the 3 replicates seedlings with the help of electric balance and took the mean of all 3 replicates.

2.6.4. Shoot length (SL)

Shoot length was measured with the help of ruler in centimetre.

2.6.5. Turgid weight (TW)

To gain turgid weight we submerged the 3 replicates in distilled water for 72 hours and took the mean of these 3 replicates after 72 hours in grams.

2.6.6. Dry weight (DW)

To obtain dry weight we put the 5 replicates in the oven at 70C for 72 hours after that we took the average weight of 5 replicate in grams.

2.6.7. Relative water content (RWC)

RWC was measured by the given formula:

$RWC\% = Freshweight - Dryweight / Turgidweight - Dryweight \times 100$

2.6.8. Leaf area (LA)

Leaf area was calculated by the following formula (Yoshida,1976).

$Leaf area = length of leaf \times width\ of leaf \times 0.725$

2.6.9. Shoot length (SL)

Shoot length was measured with the help of ruler in centimetre.

2.6.10. Shoot weight (SW)

Shoot weight was measured by the electric balance in grams.

2.7. Root analysis

GIA root software was used to study different root parameters(Kumar *et al.*,2014)such as root area, root average width and root length etc.

2.8. Determination of nitrogen

Nitrogen concentration was determined by Micro-Kjeldahl digestion method. The Kjeldahl method has to be carried out in proper sequence. The steps include digestion, distillation, and titration. For **digestion** a 0.5g sample was heated in the presence of sulphuric acid. Potassium sulphate as a catalyst was also added to increase the boiling point of the medium. The samples were decomposed and we obtain a clear and colorless solution.

Then the **distillation** of the colorless solution took place and a small quantity of sodium hydroxide (NaOH) was added to convert the ammonium salt to ammonia. The distilled vapors were then trapped in a special trapping solution of HCl (hydrochloric acid) and water.

The concentration of N present in the sample was then estimated by back **titration**. HCl was neutralized when ammonia dissolves in the acid trapping solution. A standard solution of a base, such as NaOH, used to titrate acid that was left behind.

2.9. Nitrogen uptake efficiency (NUpE)

NUP is defined as the amount of nitrogen (N) absorbed by the plants from the given dosage and it is calculated by the formula,

NUp/N (Hoagland solution) = Acquired N/N available

Acquired nitrogen (amount of nitrogen that was measured from the plant)

2.10. Nitrogen utilization efficiency (NUtE)

Nitrogen utilization efficiency is defined as the impact of the nitrogen uptake efficiency on the yield of plants and it calculated by this formula,

NUtE = yeild / NUpE

2.11. Nitrogen use efficiency (NUE)

It is calculated by a given formula;

NUE= Yield / N supplied

2.12. Statistical analysis

Statistical analysis was conducted for all phenotypic traits using XLSTAT 2014. It includes correlation and analysis of variance (ANOVA). Descriptive statistics was calculated from Microsoft Excel 2007.

3. Results

3.1. Summary statistics (Quantitative data)

I have taken total 99 observation of each of the parameters i.e. growth rate, chlorophyll, fresh weight, shoot length, leaf area, relative water content, root width, Nitrogen content, Nitrogen uptake efficiency and nitrogen utilization.

Table 4 Quantitative data of phenotypic traits of bread wheat.

Variables	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Std. deviation
GR	99	0	99	80.00	990.00	105.354	90.05
Chl-14DAG	99	0	99	17.03	35.300	25.847	4.381
FW	99	0	99	46.50	174.00	108.061	38.818
SL	99	0	99	9.85	25.10	17.660	3.531
RWC	99	0	99	54.912	94.580	73.714	8.082
N.A.	99	0	99	25939.500	443938.500	97441.591	102625.092
LAIGAR	99	0	99	0.325	6.660	3.957	1.099
RW	99	0	99	2.910	7.600	5.277	0.987
N	99	0	99	0.085	0.385	0.227	0.077
NUpE	99	0	99	0.078	0.386	0.181	0.068
NUtE	99	0	99	0.230	1.351	0.661	0.296
NUE	99	0	99	0.290	2.064	0.888	0.455

3.2. Correlations Matrix

This test was used in this particular research to evaluate the positive and negative correlation and the linear and non linear relationship between different parameters of plants in control, treatment 1 and treatment 2.

3.2.1. Correlation Matrix of Control

To analyze the correlation among the different traits “Correlation matrix” is used. A trait show positive relationship when its value ranges from 0-1 however, it is interpreted as negative when it value falls between 0-(-1). The value of correlation may also be zero.

Chl showed positive correlations with N and NUpE at value of r= 0.653 that also showed positive linear correlation. Fresh Weight (FW) also showed significant correlation with RWC at a value of r=0.427 that showed that it has positive correlation but it is non-linear. It also showed positive correlation with N and NUpE at a value of r=0.687 that showed linear correlation and that also predicted linear correlation with NUtE at a value of r= 0.612 that indicated their linear correlation and with NUE at a value of r= 1.000 that showed a linear correlation.SL presented significant positive correlation with LA at a significant value of r=0.537 that showed linearity.

Relative water content (RWC) showed significant correlation with the fresh weight (FW) and LAIGAR at a value of r= 0.427, that shows non linear correlation between them .It also showed non-linear correlation with NUtE and NUE at a value of r= 0.394 and 0.427 respectively. Leaf Area (LA) parameter showed non linear significant positive correlation with SL and RWC at a values of r=0.537 and 0.427 respectively. Root width (RW) showed a significant negative correlation with N.A at a value of r=-0.512 .Nitrogen (N) showed significant positive, non linear correlation with Chl, FW, NUpE and NUE at the values of r = 0.653, 0.687, 1.000 and 0.687 respectively .Among them NUpE is linear. Nitrogen uptake efficiency (NUpE-C) showed significant positive correlation with Chl, FW, N and NUE at the values of r= 0.653, 0.687, 1.000 and 0.687 respectively.

Nitrogen utilization efficiency (NUtE-C) showed positive significant correlation with FW, RWC and NUE at the values of r = 0.612, 0.394 and 0.612 respectively. Nitrogen use efficiency (NUE-C) showed perfectly linear, positive and significant correlation with FW and NUE at the values of r= 1.000 and 1 respectively and non linear significant positive correlation with RWC, N, NUpE and NUtE at the values of r =0.427, 0.687, 0.687 and 0.612 respectively. The above data is given in (Table 4).

Correlation matrix (Pearson)/ group control

Table 5: Correlation matrix (person) of phenotypic traits in control group of bread wheat

Variables	GR	Chl-14DAG	FW	SL	RWC	N.A.	LAIGAR	RW	N	NUpE	NUtE	NUE
GR	**1**	0.018	0.294	0.216	0.244	0.164	0.306	-0.042	0.331	0.331	0.105	0.294
Chl-14DAG	0.018	**1**	0.273	0.175	0.054	0.242	0.058	0.052	**0.653**	**0.653**	-0.313	0.273
FW	0.294	0.273	**1**	0.334	**0.427**	0.177	0.105	-0.016	**0.687**	**0.687**	**0.612**	**1.000**
SL	0.216	0.175	0.334	**1**	0.217	0.162	**0.537**	0.251	0.299	0.299	0.160	0.334
RWC	0.244	0.054	**0.427**	0.217	**1**	0.127	**0.427**	-0.335	0.216	0.216	**0.394**	**0.427**
N.A.	0.164	0.242	0.177	0.162	0.127	**1**	0.196	**-0.512**	**0.507**	**0.507**	-0.236	0.177
LAIGAR	0.306	0.058	0.105	**0.537**	**0.427**	0.196	**1**	0.078	0.061	0.061	0.113	0.105
RW	-0.042	0.052	-0.016	0.251	-0.335	**-0.512**	0.078	**1**	-0.113	-0.113	0.028	-0.016
N	0.331	**0.653**	**0.687**	0.299	0.216	**0.507**	0.061	-0.113	**1**	**1.000**	-0.130	**0.687**
NUpE	0.331	**0.653**	**0.687**	0.299	0.216	**0.507**	0.061	-0.113	**1.000**	**1**	-0.130	**0.687**
NUtE	0.105	-0.313	**0.612**	0.160	**0.394**	-0.236	0.113	0.028	-0.130	-0.130	**1**	**0.612**
NUE	0.294	0.273	**1.000**	0.334	**0.427**	0.177	0.105	-0.016	**0.687**	**0.687**	**0.612**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

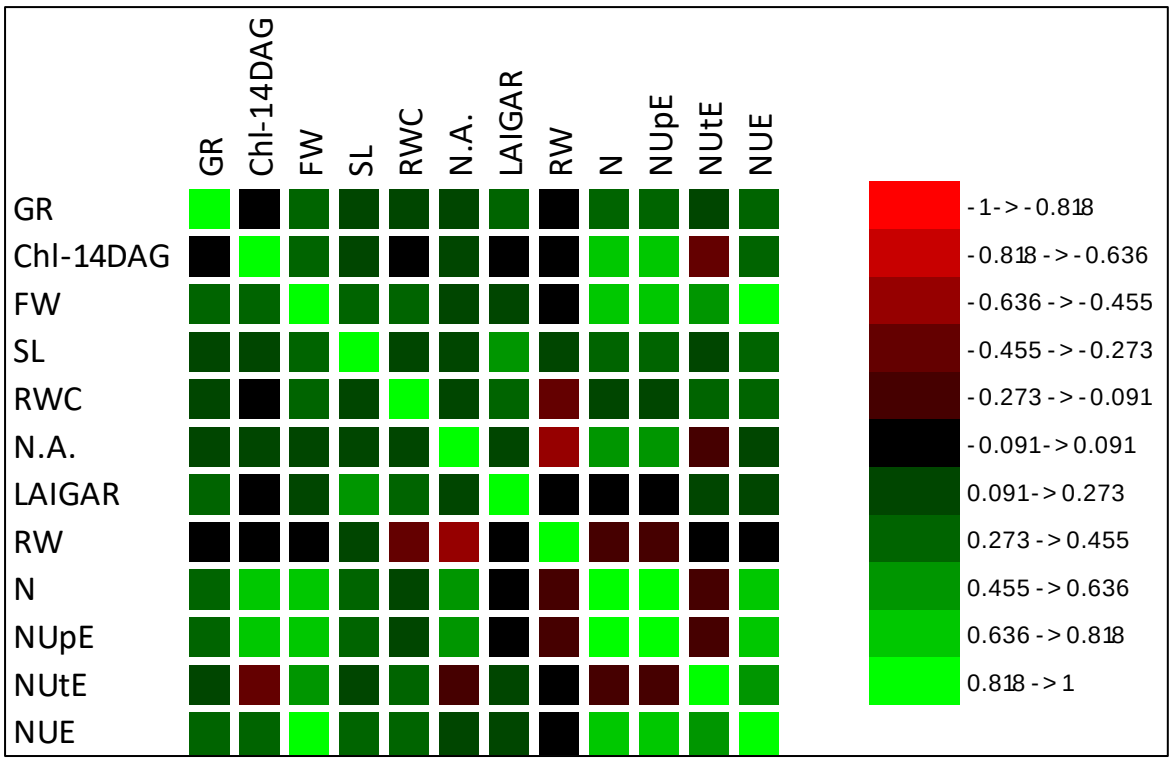

Figure 4: Correlation (person) of phenotypic traits in bread wheat.

3.3. Correlation Matrix of Treatment 1

Chl showed positive and significant correlation with FW, SL, LA, RW, N, NUpE and NUE at the values of r = 0.470, 0.614, 0.514, 0.559, 0.654, 0.654 and 0.470 respectively. Fresh weight (FW-T1) showed positive, significant and perfectly linear correlation with itself. While with others it shows positive significant relationship with Chl, FW, SL, RWC, LA, RW, N, NUpE, NUtE and NUE at the values of r = 0.470, 0.532, 0.607, 0.355, 0.436, 0.614, 0.614, 0.489, and 1.000 respectively .Among these SL, RWC, LA, N, NUpE, and NUE showed linear correlation while others showed non linear correlation. Shoot Length (SL) also showed a positive, linear and significant correlation with itself. While with others it shows a significant positive correlation with Chl, FW, RWC, LA, N, NUpE and NUE at the values of r = 0.614, 0.532, 0.812, 0.726, 0.523, 0.523 and 0.532 respectively . All of them are linear in their correlation.

Relative water content (RWC) represented to have a positive significant and linear correlation with itself. While it has linear correlation with FW, SL, LA and NUE at the values

of r= 0.607, 0.812, 0.609 and 0.607 respectively. LA showed a positive significant and linear correlation with Chl, FW, SL, RWC, N, NUpE and NUE at the values of r = 0.154, 0.826, 0.726, 0.609, 0.506, 0.506, and 0.826 respectively but it has a non linear positive and significant correlation with RW and NUtE at the values of r= 0.429 and 0.364 respectively. Root Width (RW) showed only one parameter positive, significant and in linear correlation i.e. Chl at a value of r = 0.559 and a non linear relationship with FW, LA and NUE with values of r = 0.436, 0.429 and 0.436 respectively.

Nitrogen(N-T1) showed a positive significant and linear relationships with Chl , FW, SL, LA, NUpE and NUE at the values of r= 0.654, 0.614, 0.523, 0.506 , 1.000 and 0.614 respectively Nitrogen uptake efficiency (NUpE) showed linear positive significant relationship with Chl, FW, SL, LA, N and NUE at the values of r = 0.654, 0.614, 0.523, 0.506, 1.000, 0.614 respectively. Nitrogen Utilization Efficiency (NUtE) showed positive, non-linear and significant correlation i.e. FW, LA and NUE at the values of r = 0.489, 0.364 and 0.489 respectively .Nitrogen Use Efficiency (NUE) formed a significant , linear and positive correlation with parameters that are FW, SL, RWC, LA,N and NUpE at the values of r =1.000 ,0.532, 0.607, 0.826, 0.614 and 0.614 respectively. It also formed non linear, positive and significant correlation with Chl-14day, RW and NUtE at the values of r = 0.470, 0.436 and 0.489 respectively. The above data is given in (Table 6).

Table 6 : Correlation matrix (person) value of phenotypic traits in treatment 1 in bread wheat

Variables	GR	Chl-14DAG	FW	SL	RWC	N.A.	LAIGAR	RW	N	NUpE	NUtE	NUE
GR	**1**	0.064	0.157	0.041	0.048	0.217	0.145	0.219	0.001	0.001	0.221	0.157
Chl-14DAG	0.064	**1**	**0.470**	**0.614**	0.242	**0.444**	**0.514**	**0.559**	**0.654**	**0.654**	-0.136	**0.470**
FW	0.157	**0.470**	**1**	**0.532**	**0.607**	**0.355**	**0.826**	**0.436**	**0.614**	**0.614**	**0.489**	**1.000**
SL	0.041	**0.614**	**0.532**	**1**	**0.812**	0.344	**0.726**	0.276	**0.523**	**0.523**	-0.011	**0.532**
RWC	0.048	0.242	**0.607**	**0.812**	**1**	**0.416**	**0.609**	0.117	0.324	0.324	0.238	**0.607**
N.A.	0.217	**0.444**	**0.355**	0.344	**0.416**	**1**	0.170	0.241	**0.490**	**0.490**	-0.114	**0.355**
LAIGAR	0.145	**0.514**	**0.826**	**0.726**	**0.609**	0.170	**1**	**0.492**	**0.506**	**0.506**	**0.364**	**0.826**
RW	0.219	**0.559**	**0.436**	0.276	0.117	0.241	**0.492**	**1**	0.239	0.239	0.157	**0.436**
N	0.001	**0.654**	**0.614**	**0.523**	0.324	**0.490**	**0.506**	0.239	**1**	**1.000**	-0.340	**0.614**
NUpE	0.001	**0.654**	**0.614**	**0.523**	0.324	**0.490**	**0.506**	0.239	**1.000**	**1**	-0.340	**0.614**
NUtE	0.221	-0.136	**0.489**	-0.011	0.238	-0.114	**0.364**	0.157	-0.340	-0.340	**1**	**0.489**
NUE	0.157	**0.470**	**1.000**	**0.532**	**0.607**	**0.355**	**0.826**	**0.436**	**0.614**	**0.614**	**0.489**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

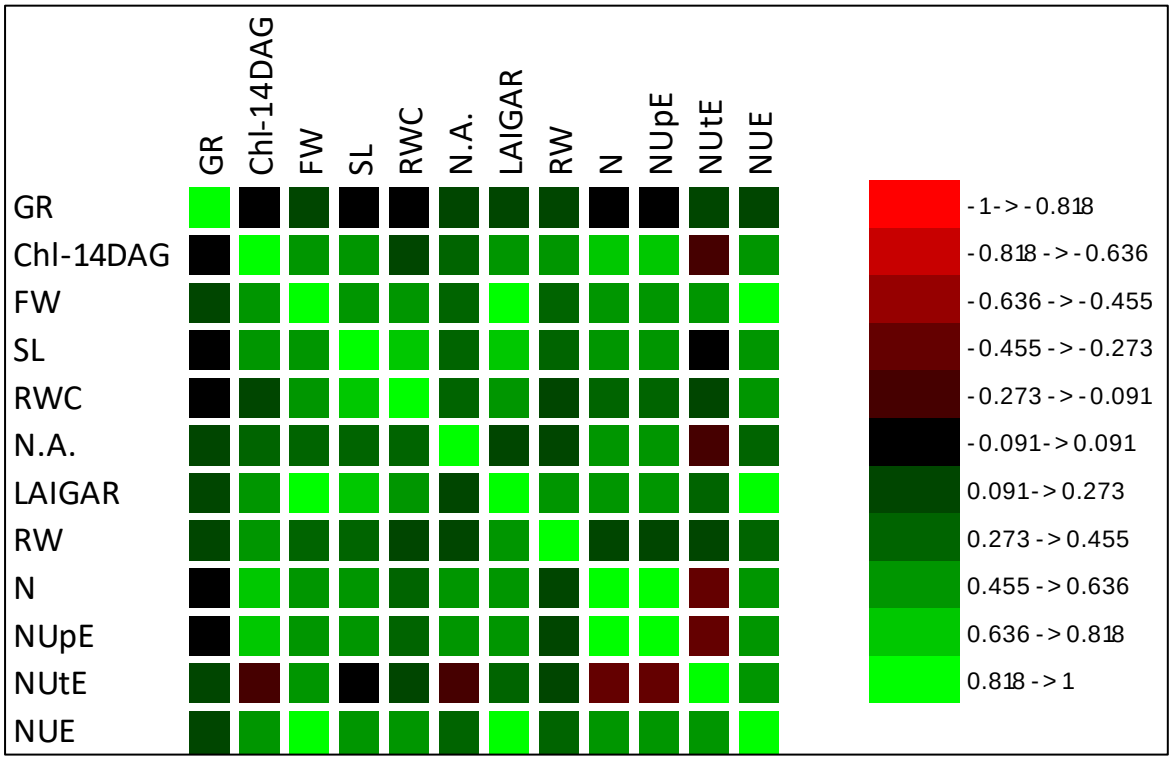

Figure 5 Correlation (person) of phenotypic traits of bread wheat.

3.4. Correlation Matrix of Treatment 2

Chl-T2 showed significant positive correlation with SL, N and NUpE at the values of r= 0.518, 0.618 and 0.618 respectively and non-linear positive and significant correlation with FW, and NUE at the values of r = 0.402 and 0.402 respectively. FW-T2 showed significant positive and non-linear correlation with Chl at a value of r= 0.402 and linear correlation with SL, LA, N, NUpE, NUtE and NUE at the values of r = 0.674, 0.615, 0.772, 0.772, 0.623 and 1.000 respectively. SH-T2 had linear, positive and significant correlation with Chl, FW, LAIGAR, N, NUpE, NUtE and NUE at the values of r = 0.518, 0.674, 0.680, 0.812, 0.812 and 0.674 respectively.

RWC-T2 has also a significant positive and perfectly linear relationship with itself. LA showed positive linear correlation with FW, SL, N, NUpE and NUE at a value of r=0.615, 0.680, 0.536, 0.536 and 0.615 respectively. RW-T2 showed negative correlation with N.A at the value of r= -0.588. N-T2 showed positive and linear correlation Chl, FW, SL and LA and

NUpE at the value of r=0.618, 0.772, 0.812 and 0.536 respectively. NUpE-T2 showed positive significant and linear correlation with Chl, FW, SL, LA, N and NUE at the value of r=0.618, 0.772, 0.812, 0.536, 1.000 and 0.772 respectively.

NUE showed positive significant and linear correlation with FW, NUE at the value of r =0.623, 0.623 respectively. Nitrogen Utilization Efficiency showed positive significant and linear relationship with FW, SL, LA, N, NUpE and NUtE at the value of r = 1.000, 0.674, 0.615, 0.772, 0.772 and 0.623 respectively and it showed a positive significant and non-linear correlation with Chl at the value of r =0.402. The above data is given in (Table 7).

Table 7 : Correlation matrix (person) value of phenotypic traits in treatment 1 of bread wheat

Variables	GR	Chl-14DAG	FW	SL	RWC	N.A.	LAIGAR	RW	N	NUpE	NUtE	NUE
GR	**1**	0.087	0.255	0.176	0.130	**0.377**	0.093	-0.292	0.116	0.116	0.207	0.255
Chl-14DAG	0.087	**1**	**0.402**	**0.518**	0.212	0.044	0.279	-0.082	**0.618**	**0.618**	-0.090	**0.402**
FW	0.255	**0.402**	**1**	**0.674**	0.244	0.070	**0.615**	-0.214	**0.772**	**0.772**	**0.623**	**1.000**
SL	0.176	**0.518**	**0.674**	**1**	0.250	0.233	**0.680**	-0.155	**0.812**	**0.812**	0.068	**0.674**
RWC	0.130	0.212	0.244	0.250	**1**	-0.143	0.157	-0.147	0.321	0.321	-0.020	0.244
N.A.	**0.377**	0.044	0.070	0.233	-0.143	**1**	0.187	**-0.588**	0.000	0.000	0.054	0.070
LAIGAR	0.093	0.279	**0.615**	**0.680**	0.157	0.187	**1**	-0.226	**0.536**	**0.536**	0.339	**0.615**
RW	-0.292	-0.082	-0.214	-0.155	-0.147	**-0.588**	-0.226	**1**	-0.070	-0.070	-0.202	-0.214
N	0.116	**0.618**	**0.772**	**0.812**	0.321	0.000	**0.536**	-0.070	**1**	**1.000**	0.000	**0.772**
NUpE	0.116	**0.618**	**0.772**	**0.812**	0.321	0.000	**0.536**	-0.070	**1.000**	**1**	0.000	**0.772**
NUtE	0.207	-0.090	**0.623**	0.068	-0.020	0.054	0.339	-0.202	0.000	0.000	**1**	**0.623**
NUE	0.255	**0.402**	**1.000**	**0.674**	0.244	0.070	**0.615**	-0.214	**0.772**	**0.772**	**0.623**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

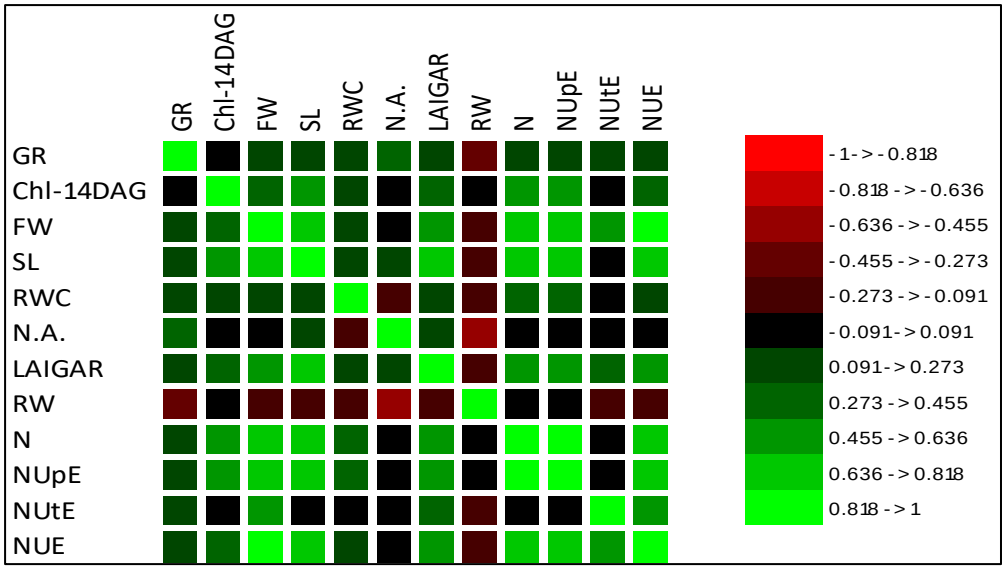

Figure 6 : Correlation (person) of phenotypic traits of bread wheat

3.5. Analysis of Variance (ANOVA)

ANOVA (analysis of variance) is the statistical analysis tool used for the comparison between more than two groups and tells the difference between them.

Table 8: Complete Data (ANOVA) Analysis Overview

Variables	Min	Max	Mean	Std.deviations	overall	Cultivar	Replicate	treatment	cultivar* treatment
GR	80	100	95.354	90.049	0.457	0.409	0.363	0.34	0.475
Chl	17.03	35.3	25.847	4.381	5.086	<0.0001	0.865	<0.0001	0.735
FW	46.5	174	108.061	38.818	546.913	<0.0001	0.755	<0.0001	<0.0001
SL	9.85	25.1	17.66	3.531	2.658	<0.0001	<0.0001	0.144	0.037
LA	0.325	6.66	3.957	1.099	42.105	<0.0001	<0.0001	<0.0001	<0.0001
RW	2.91	7.60	5.277	0.987	33.617	<0.0001	<0.0001	0.029	<0.0001
N	0.085	0.385	0.227	0.077	19.544	<0.0001	<0.0001	<0.0001	<0.0001
NUpE	0.078	0.386	0.181	0.086	16.071	<0.0001	0.864	<0.0001	<0.0001
NUtE	0.23	1.351	0.661	0.296	49.813	<0.0001	0.239	<0.0001	<0.0001
NUE	0.29	2.064	0.888	0.455	807.231	<0.0001	0.865	<0.0001	<0.0001

3.5.1. ANOVA for GR

The overall mean value for germination rate was 105.353%. The mean of germination rate in control group was 96.061% while in treatment 1 germination rate reduced to 95.758% and in treatment 2 was 124.242%.

So overall in treatment 2, the variety BAKHAR-2000 showed maximum germination rate 394.667 and SA-42 showed minimum germination rate 93.333. The variety PARWAZ-94 had minimum growth rate 90.000 in control group and in treatment 1. Differences between these varieties were significant as shown in the graph below.

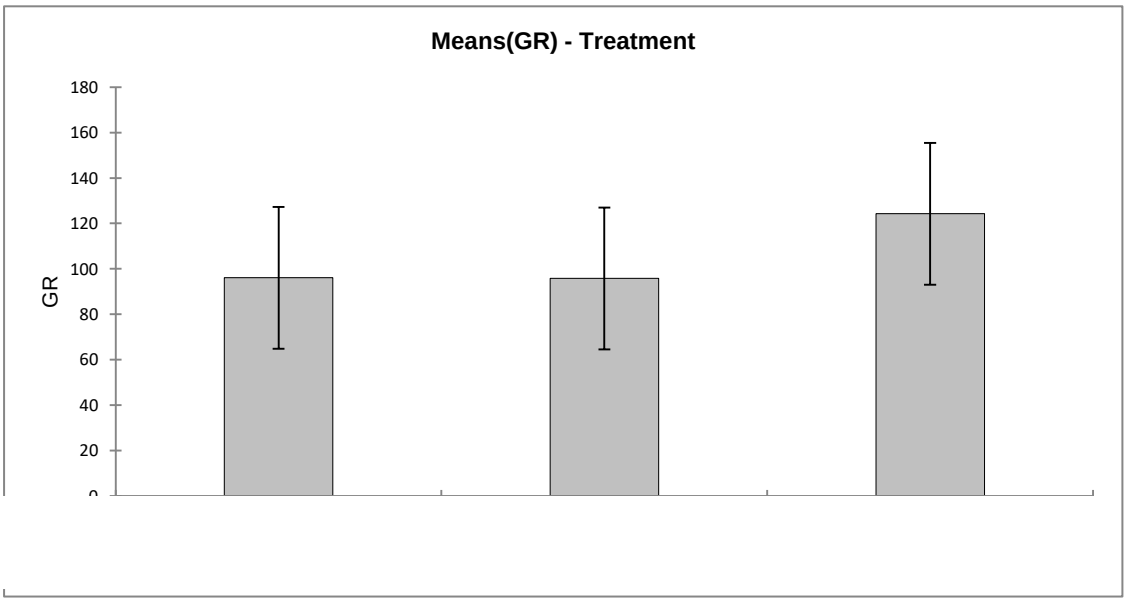

Figure 7: Graphical representation of Means (GR) of all treatment groups

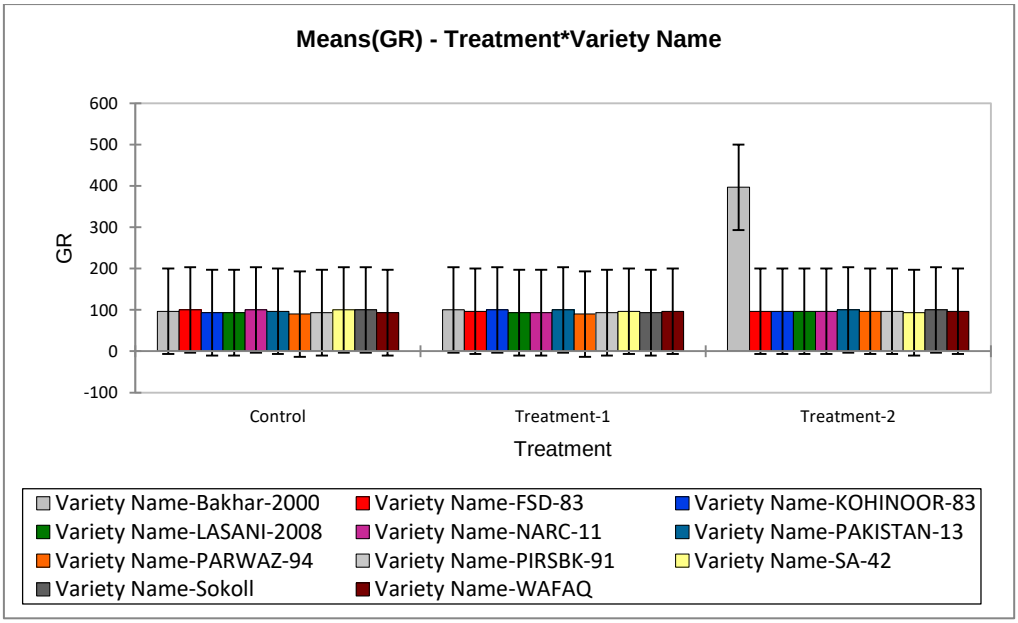

Figure 8 : Graphical representation of Means (GR) of all Varieties.

3.5.2. ANOVA for Chlorophyll

The overall mean value of chlorophyll content at the 21 day of germination was 25.847, with minimum chlorophyll value was 17.030 and maximum chlorophyll value was 35.300.The mean value for 21 days chlorophyll content in controlled group was 29.292, significantly higher than treatment 1 (26.26) which was significantly higher than treatment 2 (21.892).

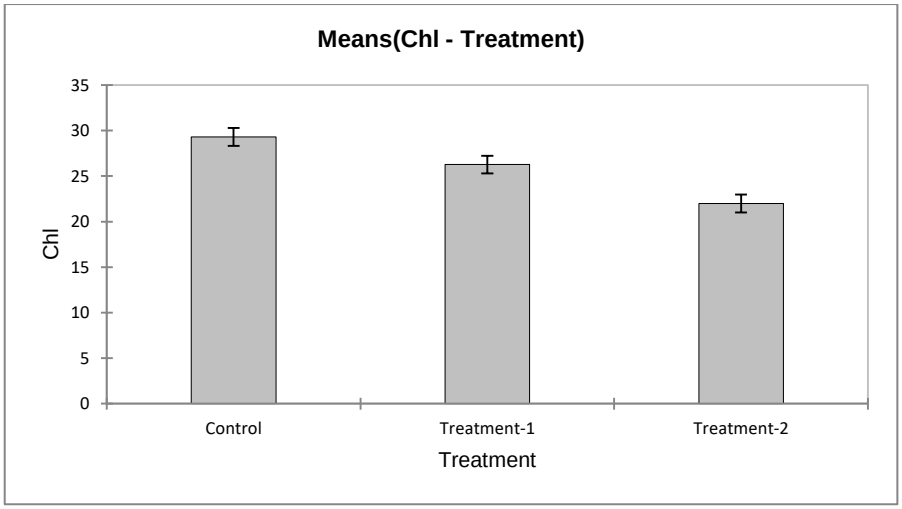

Figure 9 : Graphical representation of Means (Chl) of all treatment groups

Overall in control group NARC-11 has maximum chlorophyll value 33.333whereasPAKISTAN-13 has minimum chlorophyll value 26.700. In control treatment 1 and treatment 2 and varieties the difference in Chl was found to be highly significant ($p<0.0001$).

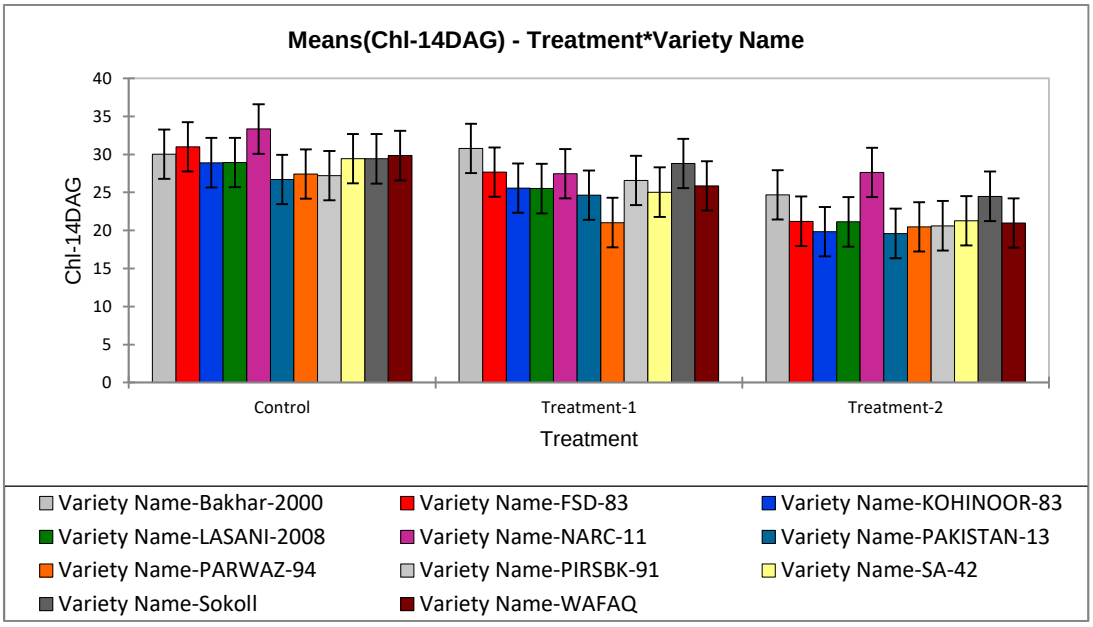

Figure 10 Graphical representations of Means (Chl) in all Varieties.

3.5.3. ANOVA for Fresh Weight

The overall mean value of fresh weight of hydroponics-grown plant was 108.061 mg. The maximum mean value of fresh weight was 174.000 and the minimum mean value was 46.500 that showed a significant difference. In Control group the mean of the fresh weight was 125.909, which showed a significant reduction to 107.727 in Treatment 1 and to the 90.545 in Treatment 2 as shown in graph below.

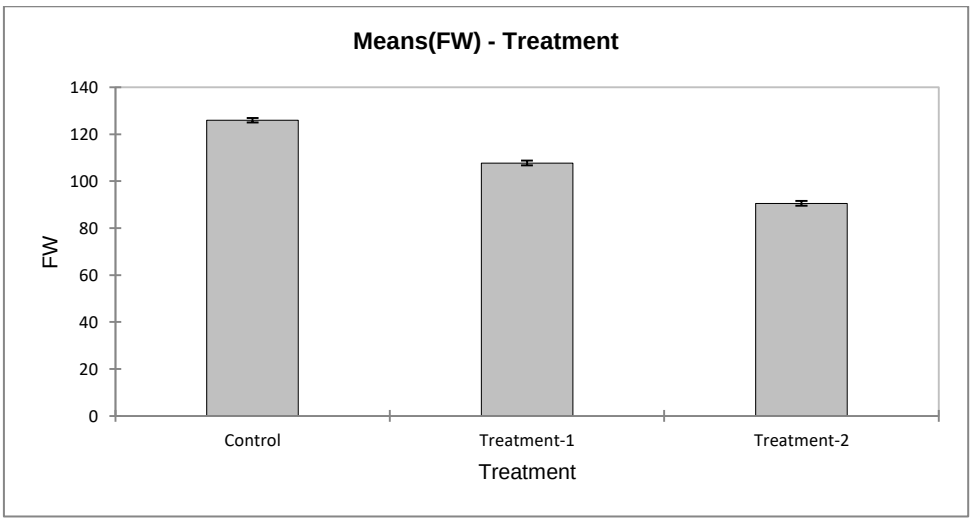

Figure 11 : Graphical representation of Means (FW) of all treatment groups.

In treatment 2 the VarietiesBakhar-2000, SOKOLL and WAFAQ had maximum fresh weight as compared to the other Varieties like PIRSBK-99, FSD-83 and KOHINOOR-83 that showed minimum fresh weight. In treatments and varieties the difference in FW was found to be highly significant ($p<0.0001$). These varieties showed huge difference as represented in the graph below.

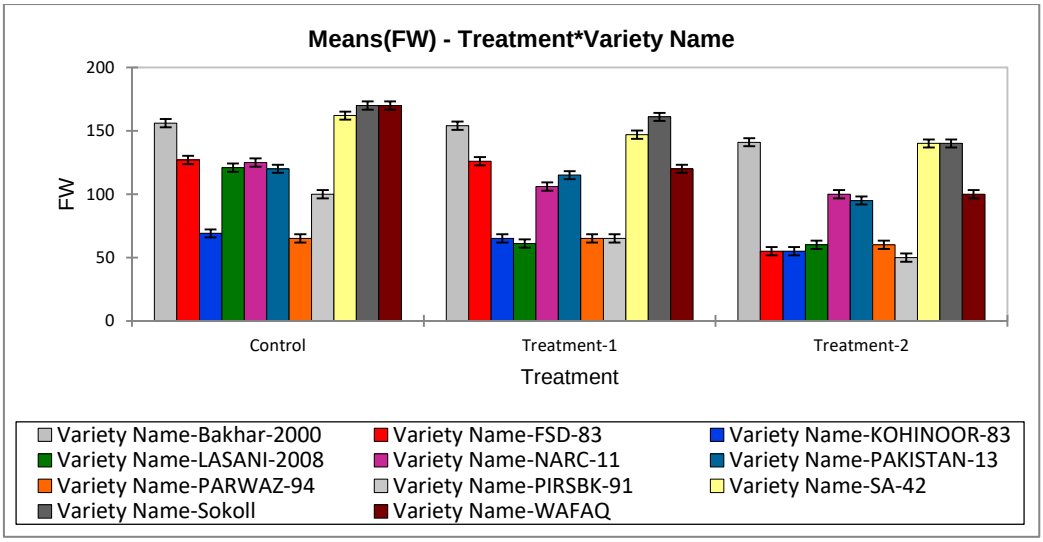

Figure 12 : Graphical representation of Means (FW) of all Varieties.

3.5.4. ANOVA for Shoot Length

The overall mean value for shoot length of the plant was 17.660cm. The maximum value for shoot length was 25.100cm while the minimum value for shoot length was 9.850cm. The mean value in the control group was 18.450 cm while it was reduced to 17.157cm in the Treatment 1 and to 17.373 in the Treatment 2 as shown in the graph.

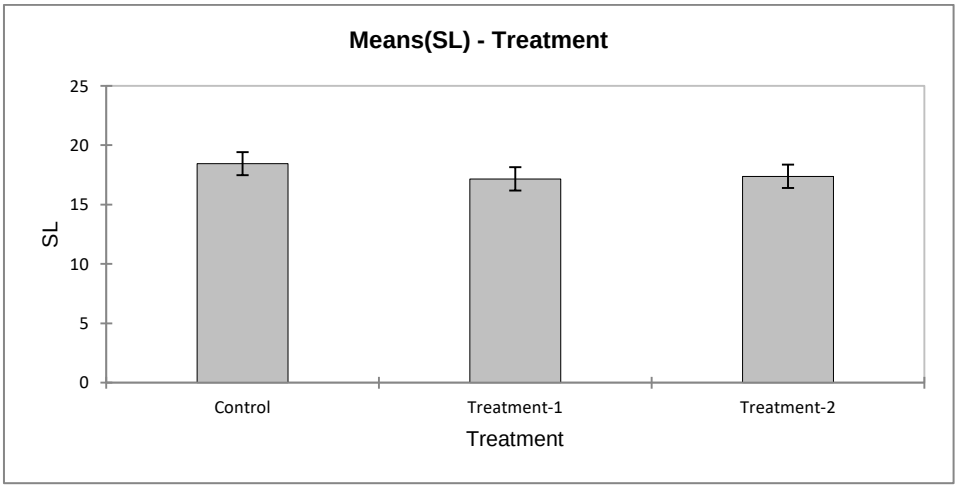

Figure 13 : Graphical representation of Means (SL) of all treatment groups

In controlled group varieties like LSANANI-2008, SA-42 and FSD-83 showed maximum shoot length while other varieties like NARC-11 in treatment 1 and PARWAZ-91 showed minimum shoot length in the treatment 2. In control and treatments SL was found to b highly significant ($p<0.0001$). In varieties the significant difference is shown in graph below.

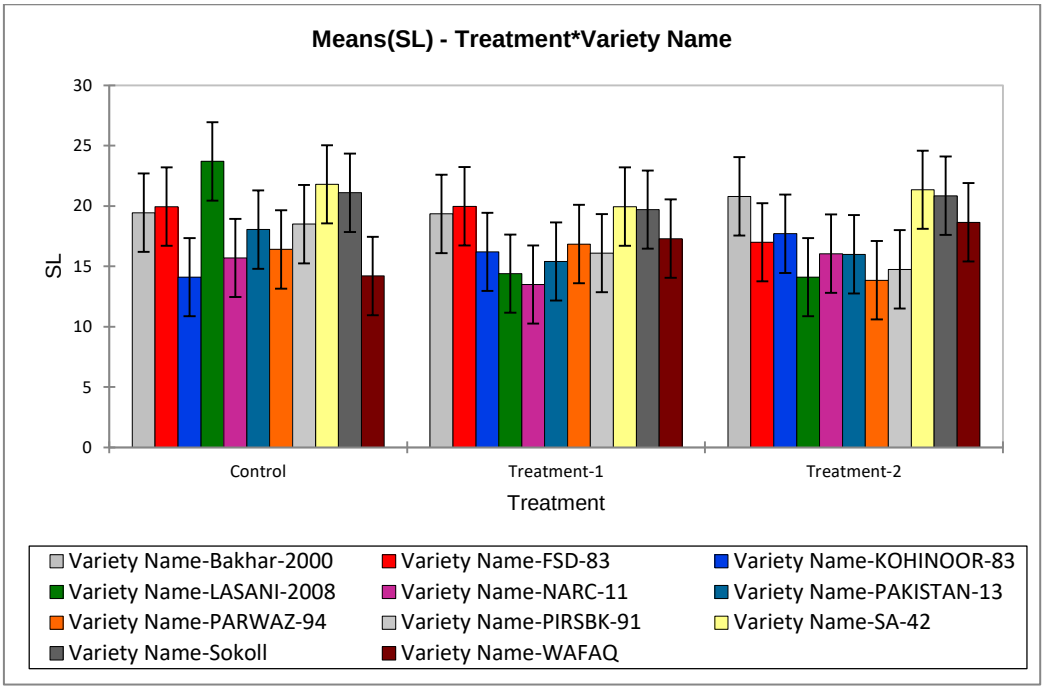

Figure 14 : Graphical representation of Means (SL) of all Varieties.

3.5.5. ANOVA for Leaf Area

The overall mean value of leaf area was 3.957m^2. The maximum value of the leaf area was 6.660m^2, and minimum value was 0.325m^2.The mean value of leaf area in control was 4.186 that reduced to the 4.094m^2 in the treatment 1 and significantly to the 3.592m^2 in treatment 2 as shown in graph.

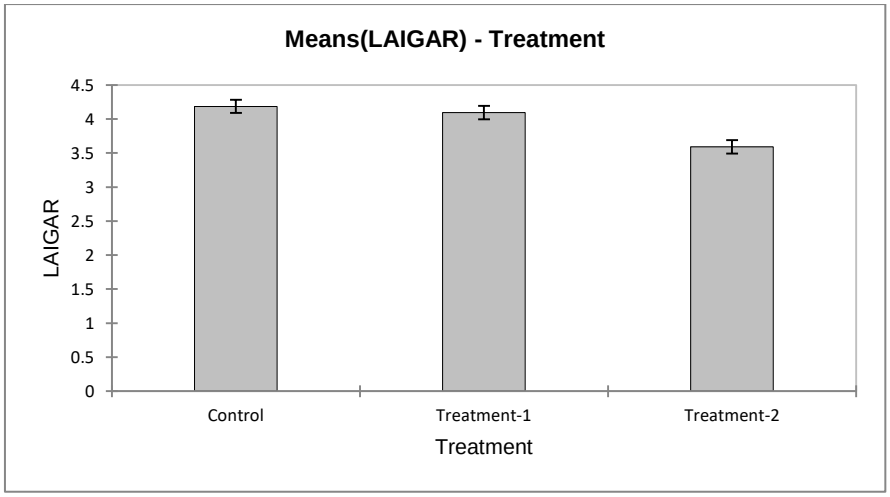

Figure 15 : Graphical representation of Means (LAIGAR) of all treatment groups.

In control group the varieties like WAFAQ showed minimum leaf area 0.425 cm as compared to the variety SA-42 6.460 cm that showed maximum leaf area value. The varieties i.e. Sokoll and SA-42 showed overall maximum value of the leaf area in all of the groups. In treatments and varieties LA was found to be highly significant ($p<0.0001$).

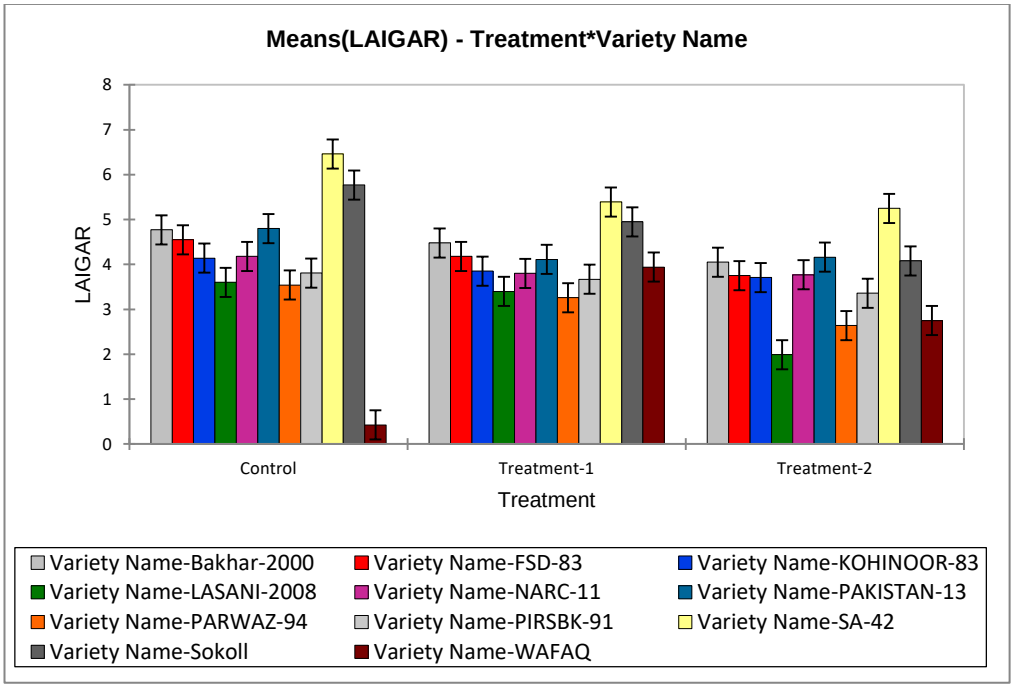

Figure 16 : Graphical representation of Means (LAIGAR) of all Varieties.

3.5.6. ANOVA for RWC

The overall means value of relative water content (RWC) was 73.714. The maximum value of the relative water content was 94.580, while the minimum value of the relative water content (RWC) in the experiment was 54.912. The mean value of relative water content (RWC) in control group was 76.178 that significantly reduced to the 73.785 in the treatment 1 and to the 71.178 in the treatment 2 as shown in the graph.

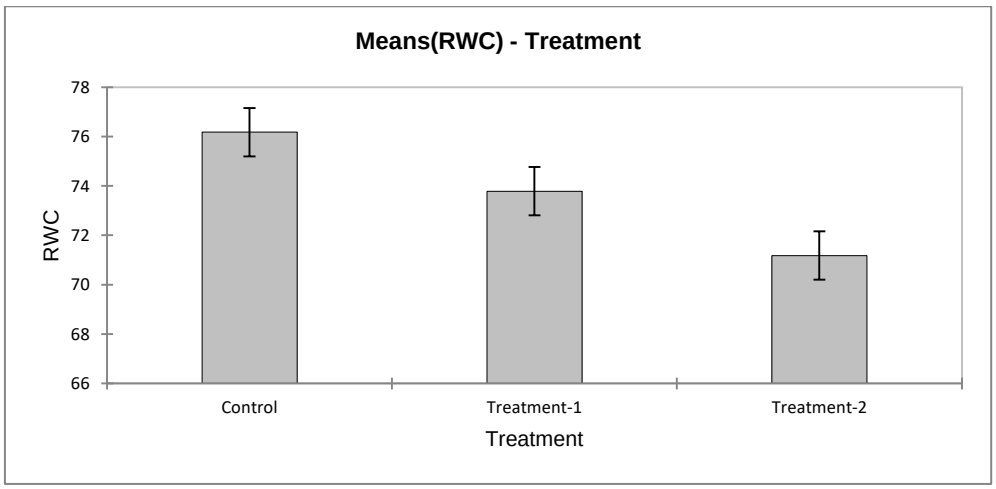

Figure 17 : Graphical representation of Means (RWC) of all treatment groups.

Varieties like PARWAZ-94 showed maximum value of relative water content (RWC) in the treatment 2 as compared to the LASANI-2008 that showed minimum value of the relative water content (RWC) in the treatment 2. NARC-11 showed minimum value out of all plants in the treatment 1. Bakhar-2000 showed maximum value of relative water content (RWC) in control whereas LASANI-2008 showed minimum value of relative water content (RWC) as shown in graph below.

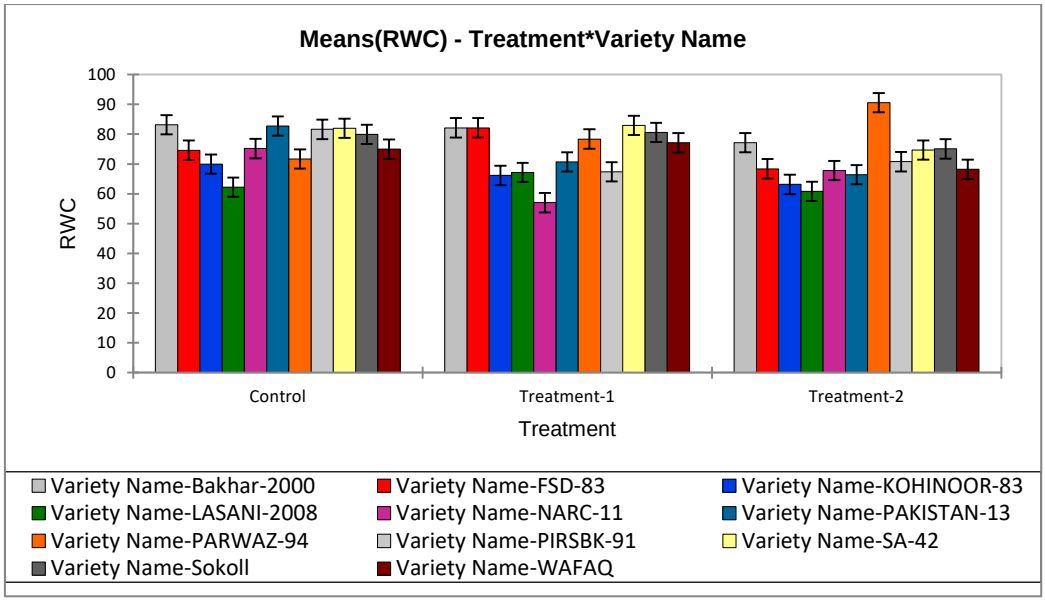

Figure 18 : Graphical representation of Means (RWC) of all Varieties.

3.5.7. ANOVA for Root Width

Overall mean value for the root width was 5.277cm however the maximum and minimum values of the root width were 7.600 cm and 2.910 cm respectively. The mean value for root width in control group was 5.170cm that was increased significantly to 5.353cm in treatment 1 and then reduced to the 5.306cm in the treatment 2 as shown in graph.

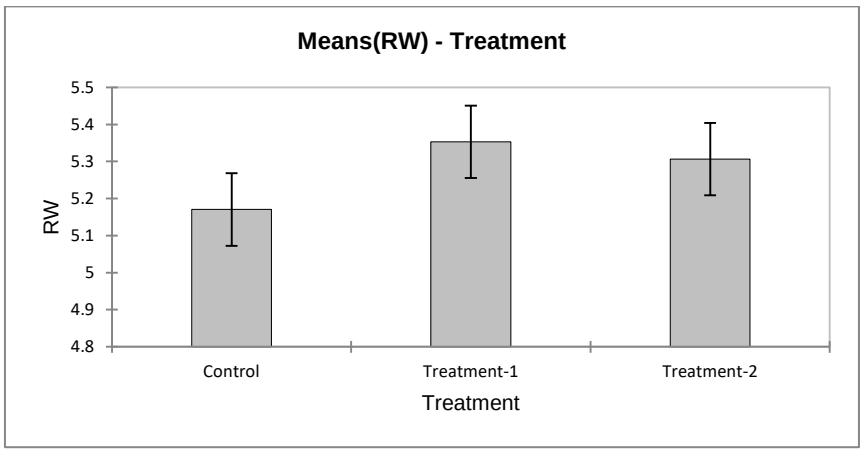

Figure 19: Graphical representation of Means (RW) of all treatment groups.

The variety BAKHAR-2000 showed maximum value of the root width in the treatment 1 group while the variety FSD-83 showed the minimum value of root width in the treatment 2 group. FSD-83 showed minimum value of root width in overall treatments as compare to other varieties. In treatments RW was found to be highly significant ($p<0.0001$). This shows a huge variation between the values obtained from the experiment as represented in the graph.

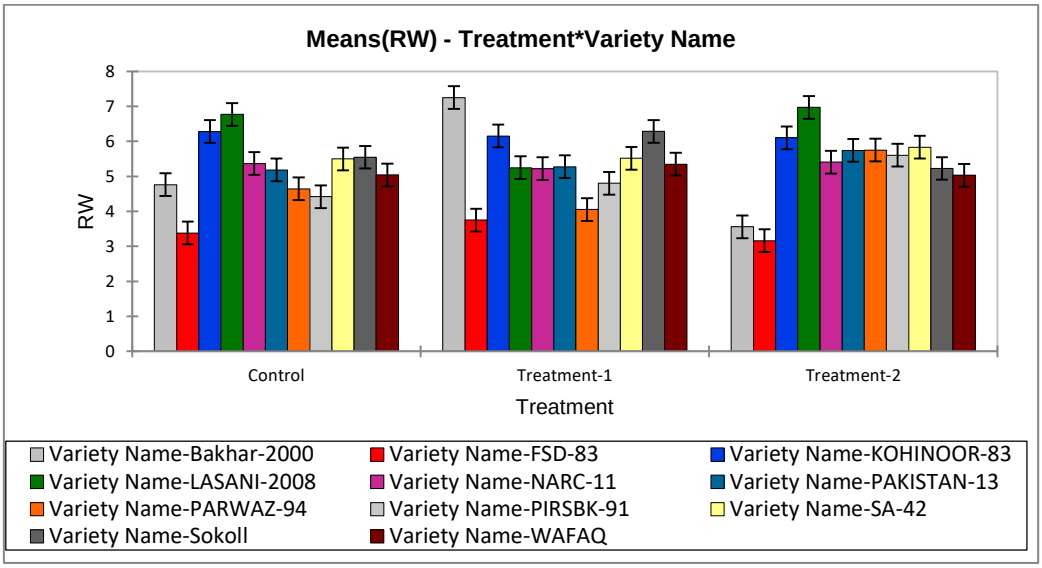

Figure 20: Graphical representation of Means (RW) of all Varieties

3.5.8. ANOVA for Nitrogen

The mean value of Nitrogen in control was 0.227. The maximum and minimum values for the nitrogen were 0.035 and 0.085 respectively. However, the mean value for the nitrogen in the control group was 0.285 which was significantly reduced to the 0.230 in the treatment 1 and 0.168 in the treatment 2. This showed a significance difference among the values obtained from experiment, as shown in graph below.

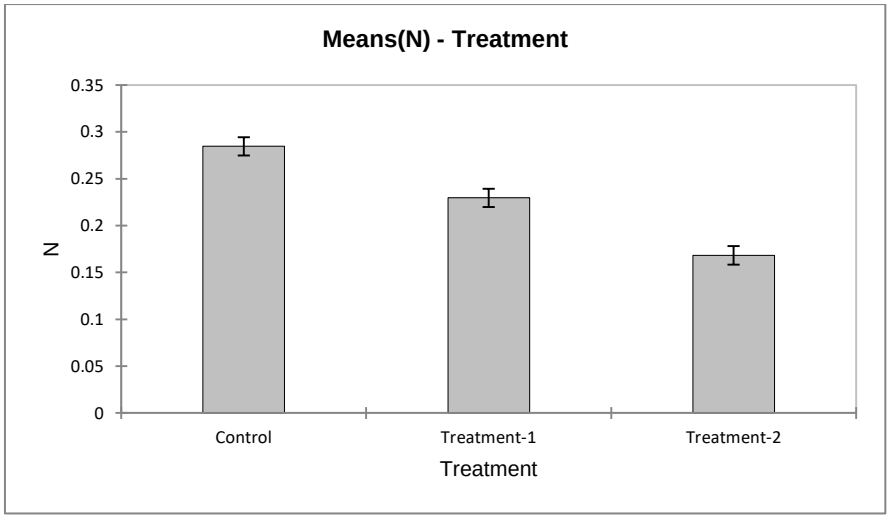

Figure 21: Graphical representation of Means (N) of all treatment groups.

The variety FSD-83 showed the maximum value of nitrogen 0.360 in the controlled group. PAK-13 showed minimum value of nitrogen 0.200 in the control group while in the treatment 1, PAK-13 showed minimum value of nitrogen 0.134 and FSD-83 showed maximum value of nitrogen 0.320. In the treatment 2 PIRSBK-91, PAKISTAN-13 and KOHINOOR-83 showed minimum value of nitrogen 0.120 as shown in graph. In treatments and varieties N was found to be highly significant ($p<0.0001$). This graph shows the significant change in nitrogen in different varieties.

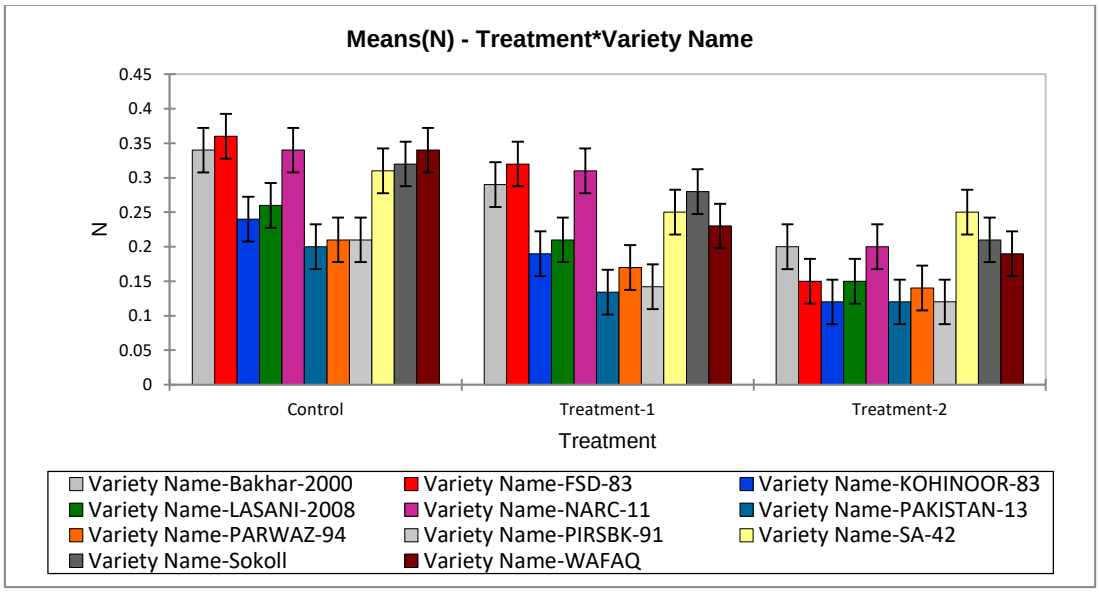

Figure 22: Graphical representation of Means (N) of all Varieties.

3.5.9. ANOVA for Nitrogen Uptake Efficiency

Overall mean value of nitrogen uptake efficiency was 0.081. The maximum and minimum value of the nitrogen uptake efficiency was 0.386 and 0.078 respectively. In control group the mean value of the nitrogen uptake efficiency was 0.135 that was significantly increased to 0.166 in treatment 1 and 0.240 in the treatment 2, as sown in graph below.

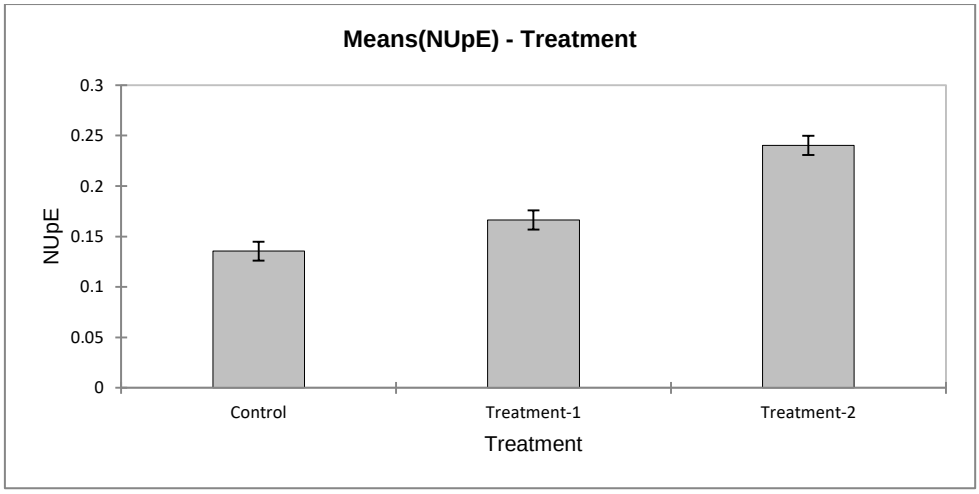

Figure 23: Graphical representation of Means (NUpE) of all treatment groups.

In the treatment 2, the varieties SA-42 and Sokoll showed maximum values for nitrogen uptake efficiency 0.357 and 0.300 respectively. In the control group, the varieties PARWAZ-94, PIRSBK-91 and PAK-13 showed minimum values for nitrogen uptake efficiency 0.100, 0.100 and 0.095 respectively. In treatment 1, the varieties FSD-83 and NARC-11 showed maximum value of NUpE and PAK-13 showed minimum value. In the varieties as well as in treatments NUtE was found to be highly significant ($p<0.0001$), as shown in graph below.

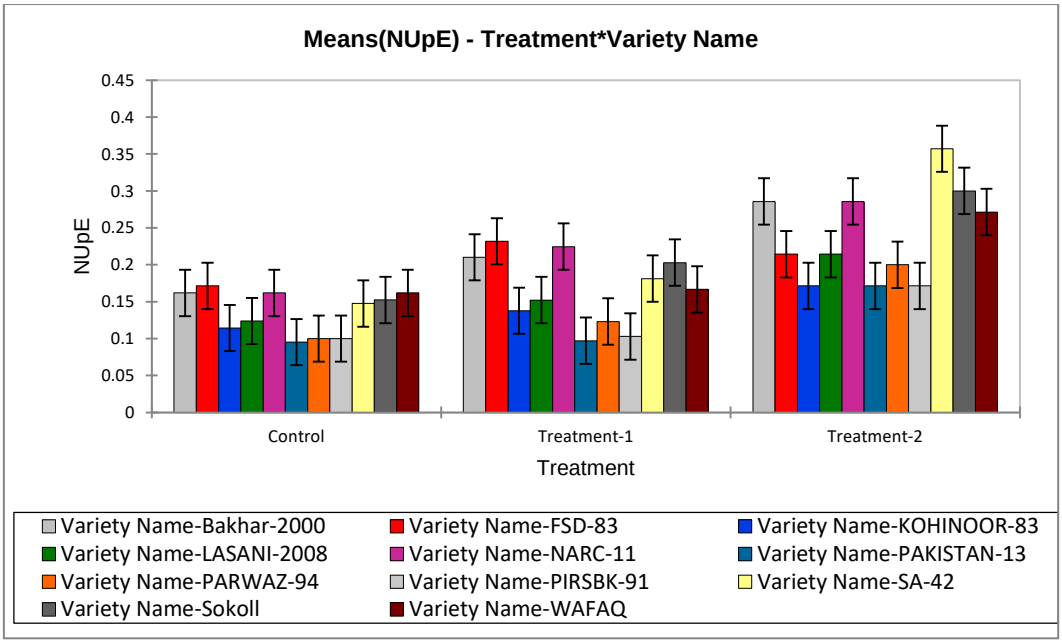

Figure 24: Graphical representation of Means (NUtE) of all Varieties.

3.5.10. ANOVA for Nitrogen Utilization Efficiency

The mean value of the NUtE was 0.661. However, the maximum and minimum values for NUtE were 1.351 and 0.230 respectively. The mean value for the control group was 0.935 that was reduced significantly to 0.669 in treatment 1 and in treatment 2 it was further significantly reduced to the 0.377 that showed a significant variation among the different groups/treatments.

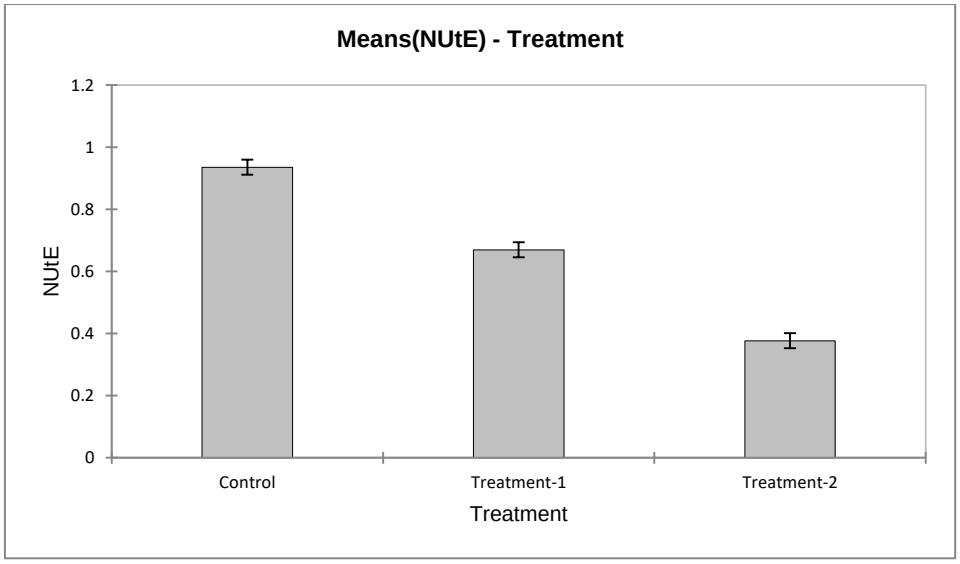

Figure 25: Graphical representation of Means (NUtE) of all treatment groups.

In control group, the varieties PAKISTAN-13 and Sokoll showed maximum value of the nitrogen utilization efficiency 1.265 and 1.125 respectively while KOHINOOR-83 and PARWAZ-94 showed minimum values 0.608 and 0.661 respectively. In the treatment 1, the variety PAK-13 showed maximum value 1.194 of NUtE and LASANI-2008 showed minimum value 0.401 that represented a significant change in the values. In treatment 2, PAK-13 showed maximum value 0.560 of NUtE and FSD-83 showed minimum value 0.260 of NUtE. A significant difference was observed in the 3 groups/ treatments. In treatments and varieties NUtE was found to be highly significant ($p<0.0001$). The significant difference between varieties is shown in graph below.

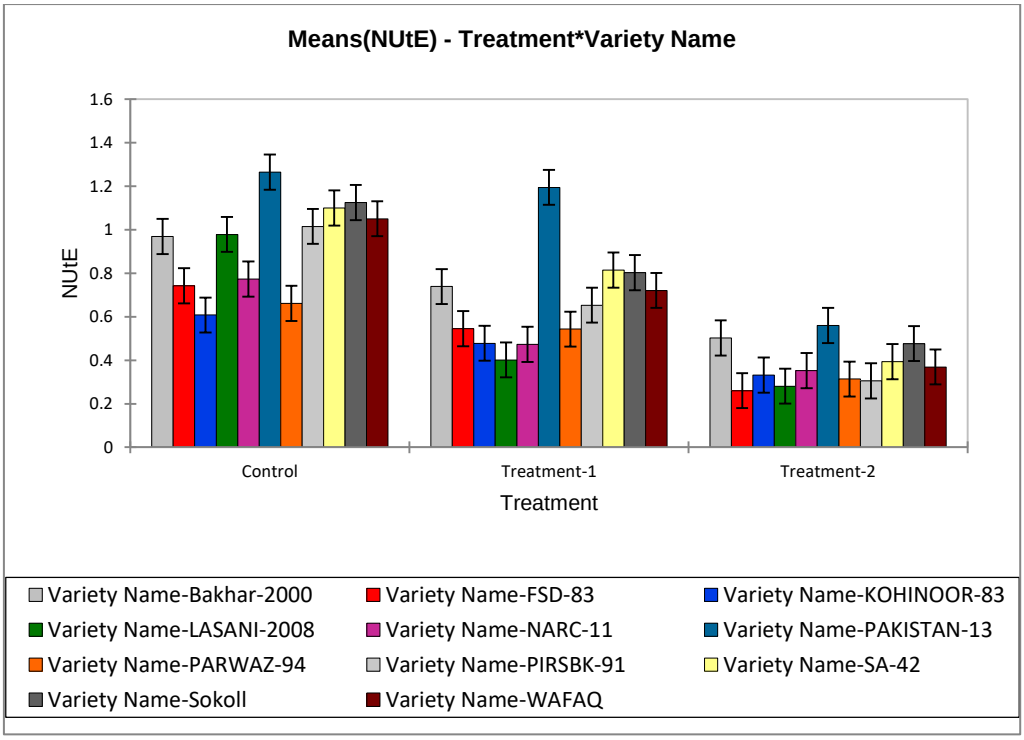

Figure 26: Graphical representation of Means (NUtE) of all varieties

3.5.11. ANOVA for Nitrogen Use Efficiency

The overall mean value for nitrogen use efficiency was 0.888. The overall maximum and minimum values of nitrogen use efficiency were 2.064 and 0.290 respectively. The mean of nitrogen use efficiency was 0.600 in control group and it increases significantly to the 0.796 in treatment 1 and to the 1.294 in the treatment 2 that represented a significant difference among the groups/treatments.

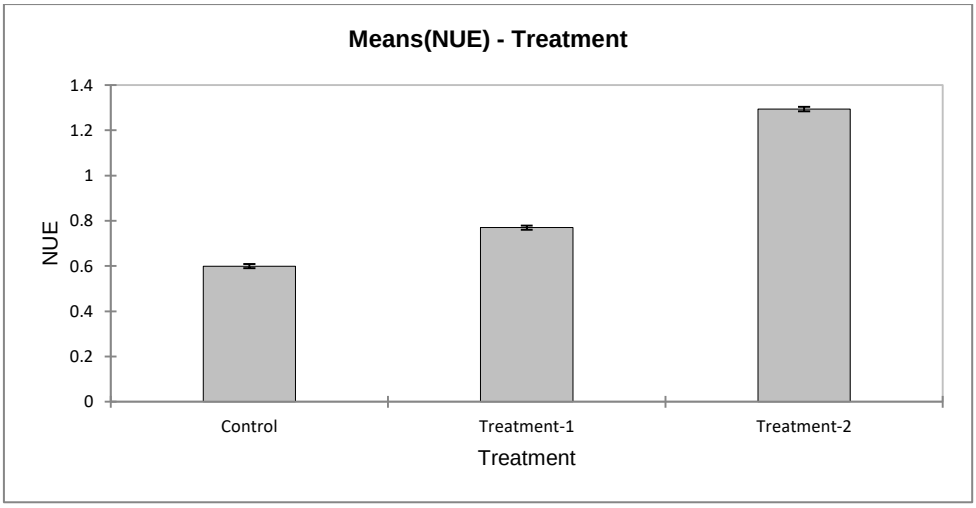

Figure 27: Graphical representation of Means (NUE) of all treatment groups.

In treatment 2, the varieties Bakhar-2000, SA-42 and Sokoll showed maximum values of NUE 2.014, 2.000 and 2.000 respectively while the other susceptible varieties like PIRSBK-91 showed minimum values of NUE. In the treatment 1, Bakhar-2000, Sokoll and SA-42 showed maximum value of NUE and variety LASANI -2008 showed minimum value. The control group, the varieties Sokoll, SA-42, WAFAQ and Bakhar-2000 showed maximum value of NUE and KOHINOOR-83 and PARWAZ-94 showed minimum value of NUE. The significant difference in the varieties ($p<0.0001$) is represented by graph below.

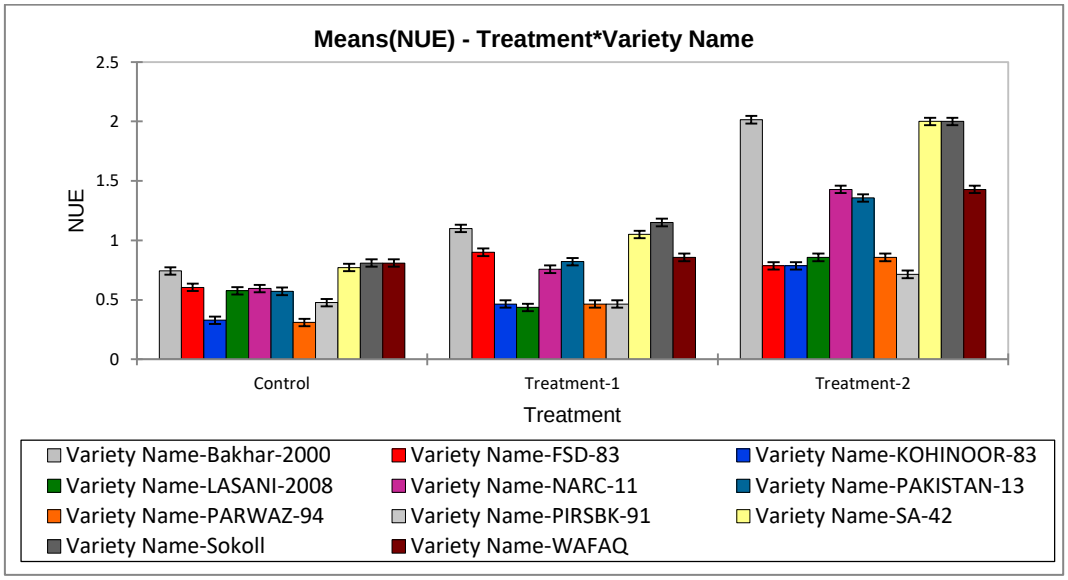

Figure 28: Graphical representation of Means (NUE) of all Varieties.

4. Discussions

Crop productivity has recently reached to a peak and to feed the rapidly growing world population, increase in our crop production in limited land resources is a challenging task for wheat breeder. Nitrogen is very important and needed for plant growth. Nitrogen in a form that plants can use is necessary to the production of crop. The use of nitrogen fertilizers, which are developed in industrial level by chemical reduction of atmospheric (gaseous) nitrogen, has allowed exponential growth in the human population and food supply(Bacon,1995; Lloyd T Evans *et al.*,1998). Increased nitrogen availability is most likely a result of population-driven technical advancements, in a dynamic and poorly known relationship.(Lloyd T Evans *et al.*,1998).

Nitrogen absorption plays an essential role in growth of plant. However, unnecessary use of nitrogen fertilizers is not economical(Ju *et al.*,2009) and cause hazards to the environment with excess nitrogen lost by leaching into aquifers (Conley *et al.*,2009).Identifying wheat genotypes that can effectively use nitrogen is essential to maintain economic balance and prevent contamination caused by nitrates. This effectively used nitrogen is needed in wheat to maintain quality and increase yield while reducing negative environmental effects.(Foulkes *et al.*,2009).On the basis of phenomics and genomics of a historical collection of bread wheat, nitrogen usage effective lines are chosen for potential breeding programs in the present research.

In our study nutrient concentration was adjusted for the proper plant growth in completely sterilized hydroponic system(Kawasaki *et al.*,2018).The varieties of wheat were grown under the controlled condition to study different parameters of plants such as Chl content, SL, SW, LA, RW and RWC, NUE, NUtE, NUpE. The results of variance (ANOVA) revealed that above phenotypic traits shows significant difference between control, treatment 1 and treatment 2.

4.1. Phenotypic analysis

In phenotypic analysis, yield and yield-related traits, especially the chlorophyll trait, which has a direct impact on NUE, are determined. Variance analysis showed that yield-related traits are highly significant ($p>0.0001$) among treatments. The correlation test also showed a positive correlation of yield related traits with chlorophyll trait e.g Chlorophyll traits positively correlates to N contents for all 3 sets having r values 0.873, 0.491 and 0.938 respectively(Gáborčík,2003).The analysis for the phenotypic traits was performed during present study. The correlation between traits was recorded (positive and negative). The important difference was observed in 11 varieties of Pakistan Wheat in the different treatments of Nitrogen. The significant results were showed by the selected traits in control as well as in treatments. In present study, leaf nitrogen content is directly related with leaf chlorophyll content, so higher SPAD values means higher nitrogen content in plants(Yuan *et al.*,2016).

In this research work, Bakhar-2000, FSD-83, KOHINOOR-83 and LASANI-2008 are genotypes best NUE and NUpE in control, T1 and T2. This 3 depicts that these lines can be used under nitrogen limited condition. The cultivar NARC11 and Pak-13 has significant difference in FW between control and T2. But the FW of these in T2 was better than FW of T1 in FSD-83, KOHINOOR-83 and LASANI-2008. There was a highly significant relationship between cultivars and treatments ($p<0.0001$). Also, there was a positive and linear relationship between Chl traits and Nitrogen content (NUpE) ($r=0.653$ in control, $r=0.564$ in treatment 1 and $r=0.618$ in treatment 2) which is already observed by (Bojović & Marković,2009).

In present studies, ANOVA results for SL trait showed negative trend among control and treatments i.e. minimum values in C while maximum values in T2. Same trend was reported by (Gaju *et al.*,2016; Guttieri *et al.*,2017), according to him shorter genotypes of wheat tended to be more nitrogen use efficient. In the present work, the phenotypic traits like CHL, SL, FW, RW and N contents showed maximum values in control (100% N) while minimum values were observed in treatment-2 (33% N). Same trend was reported earlier by (Gaju *et al.*,2011).It means, increased N application have significant effect on the parameter. This indicated that Control is leading both T1 and T2 in growth trend.

4.2. Morpho-physiological Traits

The morpho-physiological traits show wide variations, the chlorophyll content ranges from 30.15 to 46.15 in control and 30.15 to 51.15 in Treatment(Ranjitha *et al*.,2017; Sathisha & Desai,2016). In present research work, 11 wheat cultivars show variations in chlorophyll ranges from 20.35 to 35.35 in control and 15.55 to 25.55 in T2. The average reduction of Chl content in cultivar KOHNOOR-83, LASANI-2008, PAK-13, PARWAZ-94, PISBK-91 and WAFAQ of T2 was 19.83, 19.620.61 and 20.97 respectively. An average reduction in cultivars PIRSBK-91 and PAK-13 in T2 0.11 and 0.11 was estimated in N concentration respectively. Both cultivars also showed reduction in C and T1. N concentration also reduced in cultivar PARWAZ-94 in C 0.18.However, the reported 11 cultivars that performed efficiently had a better N uptake under lower supply levels. Enhancement of NUpE, NUE by N in wheat has also been reported by (Sathisha & Desai,2016).

5. Conclusions

Food security is becoming more problematic in both developed and emerging countries. The researcher's aim is to boost the yield of food crops. Different methods, such as molecular breeding techniques, are used to increase the ability of plants to produce more. The poor yield of cash crops is caused by a lack of nutrients. To solve this problem, the right amount of fertilizers for plant growth is needed. The different strategies applied for the increase of grain yield faces different problem as in hydroponic system it faces a lot of contaminations of microorganisms. To prevent contaminations, a completely sterilized hydroponic system was designed to grow bread wheat in Hoagland solution under nitrogen treatment to study bread wheat physiology. Plants grown in this sterile hydroponic system responded in a number of ways, depending on the variety's tolerance capacity.

The primary objective of our study was to identify N-deficiency tolerant wheat which could be recommended for sowing in N-deficient cropping systems. After giving different treatments of nitrogen 6 varieties were observed that show tolerance to nitrogen limiting conditions. These are; Bakhar-2000, NARC-11, PAK-13, WAFAQ, SA-42 and SOKOLL .These showed efficient N uptake and utilization under a limited nitrogen circumstance. KOHINOOR, LASANI-2008 and PARI-73 were susceptible under limited N regime. There was a highly significant relationship between cultivars and treatments ($p<0.0001$). SA-42 and SOKOLL are cultivars from post-green revolution era and have the potential to be used in breeding programs to enhance NUE. Bakhar-2000, NARC-11,PAK-13, WAFAQ, SA-42 and SOKOLL were efficiently perform under reduced nitrogen level which indicates that these varieties can be use in future breeding programs with further confirmation.

6. Appendix A

Table 9: List of cultivars of wheat used in experiment along with their pedigree

S.No.	Variety Names	Pedigree
1	BAKHAR-2000	P20102/PIMA/SKA/3/TTR'S'/BOW'S'
2	FSD-83	MAYA/MON//KVZ/TRM
3	KOHINOOR-83	PT'S'/3/TOB/LFN//BB/4/BB/HD-832-5//ON/5/G-V/ALD'S'//HPO
4	LASANI-2008	PAVON MUTANT-3
5	NARC-11	CNO67/8156//TOB66/CNO67/4/NO/3/12300//LR64A/8156/5/PVN or CNO67/8156//TOB66/CNO67/4/NOROESTEF66/3/12300//LR64A/8156/5/PVN
6	PAKISTAN-13	CMH84.3379/CMH78.578//MILAN
7	PARWAZ-94	OASIS/SKAUZ//4*BCN/3/2*PASTOR
8	PIRSBK-91	KAUZ//ALTAR84/AOS
9	SA-42	C 209 X C 591
10	SOKOLL	Synthetic Derivative Variety
11	WAFAQ	Kauz/Yaco//Kauz

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