A Comparison between Hydroponics and Pots Systems for Growing Wheat on the Speed Breeding Platform

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In dedication to my mother (late), my father (Kirshan Lal), my elder brothers (Suneel Kumar & Santosh Kumar), younger brothers (Pawan Kumar, Ravi Kumar, Jai Kumar) and my younger sister (Nancy).

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KAPEEL KUMAR

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www.wheatgenome.org

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LIST OF ABBREVIATION

S no:	Abbreviation	Full Form
1	ABA	Abscisic acid
2	AD	Anno Domini
3	ANOVA	Analysis of Variance
4	BC	Before Christ
5	BP	Before the Present
6	BY	Biomass Yield
7	CER	Controlled Environment Room
8	Cm	Centimeter
9	CRBD	Complete Randomized Block Design
10	CSI	Crop Science Institute
11	DNA	Deoxyribonucleic acid
12	DtA	Days to Anthesis
13	DtB	Days to Booting
14	DtH	Days to Heading
15	DtM	Days to Maturity
16	DtT	Days to Tillering
17	DW	Dry Weight
18	DWC	Deep Water Culture
19	FW	Fresh Weight
20	GDP	Gross Domestic Product
21	GS	Growth Stage
22	GY	Grain Yield
23	HI	Harvest index
24	IAA	Indole-3-acetic acid
25	IBA	Indole-3-butyric acid
26	KW	Kernel Weight
27	LA	Leaf Area
28	LED	Light Emitting Diode
29	MAS	Marker-Assisted Selection
30	mM	Millimolar

31	MMT	Million Metric Tons
32	NAA	1-Naphthaleneacetic acid
33	NARC	National Agriculture Research Centre
34	NT	Number of Tillers
35	PPFD	Photosynthetic Photon Flux Density
36	RL	Root Length
37	SB	Speed Breeding
38	SL	Shoot Length
39	SPAD	Soil Plant Analysis Development
40	SPD	single pod descent
41	SpL	Spike Length
42	Spl/S	Spikelets per Spike
43	SPS	Single Plant Selection
44	SPS	Spikelets per Spike
45	SSD	Single Seed Descent
46	UK	United Kingdom
47	US	United States
48	USSR	Union of Soviet Socialist Republics

ABSTRACT

Keeping in mind that environmental alterations have affected our agro-based industries very harshly and disturbed the production line of cereal crops, meeting this challenge plant breeders are trying new research techniques. The primary concern of plant breeders is to develop the multi resistant and high production cultivars to feed the growing population that will reach up to 10 billion by 2050. The stream of modern cultivars with remarkable yield are needed to be developed by plant breeders with time and resource efficient manners by using the modern techniques of breeding. One of the crucial bottlenecks in the progress of plant breeding is the duration of life cycle of plants. In this context, plant breeders are exploring more efficient crop improvement strategies. To accelerate the crop research, plant breeding stream line adopted the powerful toolkit ''Speed Breeding'' to shorten the breeding cycle by extending the photoperiod and light intensity during plant growth. SB toolkit is successfully deployed to attain 6 generations of durum wheat (*Triticum. durum*), spring wheat (*T. aestivum*), barley (*Hordeum vulgare*), pea (*Pisum sativum*) and chickpea (*Cicer arietinum*), and 4 generations for canola (*Brassica napus*). This study was aimed at developing an efficient growth medium for wheat (*Triticum aestivum*) to hasten the accelerated cycle and get more than six generation in a year with resilient phenotypic traits by using different easier and cost-effective methodologies and treatments.

Chapter 1:

GENERAL INTRODUCTION

CHAPTER 1: GENERAL INTRODUCTION

Agriculture is and has been the backbone of human civilization. Before agriculture (Neo-lithic era), hunter/gathered lifestyle feed about 4 million people on the globe Cohen (1995). According to World Bank, modern agriculture is serving more than 7.8 billion people directly or indirectly. This giant leap from 4 million to 7.8 billion started from primary crop domestication around 12,000 years ago (Salamini et al., 2002).

Domestication, a gigantic evolutionary step had favored the adaptation and speciation, eventually creating incipient species. The domestication of cereal crops marked a dramatic turn in the development and evolution of human civilization (Darwin, 1905). Man started selection on the species of agronomic interest *i.e.* selecting adaptive genotypes on the basis of agronomical important traits (Brown, 2010). Wheat is successful crop between the latitude of latitudes of 30° and 60°N and 27° and 40°S (Nuttonson, 1955), beyond these limits it can also be grown within the arctic circle to higher elevation near the equator. The wheat production in much warmer area is technological much feasible (Badaruddin et al., 1994).

The optimum temperature for the growth of wheat is 25°C, with the limit of minimum to maximum growth temperature of 3° to 4°C and 30° to 32°C, respectively (Nuttonson, 1955). 95% of the wheat grown throughout the world is hexaploid (Bread wheat) however the remaining 5% is tetraploid (Drum wheat). The drum wheat is more adopted to the dry Mediterranean climate than bread wheat and usually called as pasta wheat. Whereas, it can also be used to bake bread and often used in regional food such as couscous and Bulgar in North Africa. In Spain, Turkey, the Balkans and Indian subcontinent the other species of wheat such as einkorn emmer and spelt are still grown.

In Europe the cultivation of spelt is still observed particularly in alpine areas. The hulled wheats are called as faro in Italy. The recent attention to the spelt and other primitive wheats as nutritious exchange to bread wheat may also result in extensive growth for notable value niche markets in coming time. The members of *Poaceae* family, which are consumed as staple food since their domestication are

called as Cereals (barely, maize, millets, oat, rice, rye, sorghum and wheat). Altogether cereals are cultivated on 65% arable land and provide 60% of daily nutrient to the world population (Varshney et al., 2006).

1.1. Socio-economic Importance of Wheat

Among all cereals, Wheat is the largest cultivated crop of the world *i.e.* 17% of the world crop acreage. 40% of world population consume it as staple food to fulfill 20% of food calories and protein requirements. As a widely grown crop, it also enjoys the status of youngest polyploid species along with the first domesticated crop (Hanson et al., 2021). It is broadly classified in to two types; Bread wheat (hexa-ploid; *Triticum aestivum*) and durum/pasta wheat (tetra-ploid; *Triticum durum*). Bread wheat is used to make breads, cake and chappati, noodles *etc.* while durum wheat is consumed in form of biscuits, noodles, pasta, cereals *etc.* One of the major reasons of the wheat culture establishment comes from the fact of its adaptability to both temperate and tropical climatic regions, unlike other cereal crops, e.g., rice and maize which are specifically adapted to the tropical climatic zone. Bread loaf quality is another advantageous aspect, which makes wheat a best suited crop for human preference. Due to the presence of gluten, a protein that traps carbon dioxide molecules during fermentation, which produces raised bread loaf (Hanson et al., 2021). This makes wheat flour differed and preferred from other cereals with its unique visco-elastic properties (Orth & Shellenberger, 1988). Wheat is a rich source for human nutrition constituting all major macro-molecules like carbohydrate, protein, minerals, vitamins, lipids and higher fiber (Johnson, 1975) and also digestible components such as starch and most proteins (Curtis et al., 2002). Not only for human food, but also for animal feed and as well as for other industrial purposes it serves a leading role from farm to table and to the market.

The top ten wheat producing countries of the world are China, India, Russian Federation, United States of America, France, Australia, Canada, Pakistan, Ukraine and Germany. Global wheat consumption has been predicted to increase to 602 million metric tons (MMT) by 2025, 4% increase than last 5-year average. In last decade, overall increase in global wheat consumption is 90 MMT. In 2019, the

average per capita consumption of wheat was 67.8Kg, accounting 47% of the total cereal consumption. Whereas in Pakistan, per capita consumption for 2019 was 115kg. Making it the largest consumed product, with large impact on the economy of Pakistan.

1.1.1. Worldwide Wheat Production

Wheat occupies 17% of all crop areas (in 2019, 210 million hectares versus 162 million hectares for rice and 177 million for maize). In Pakistan, 80 percent of rabbi season and close to 40% (~9 million hectares) of total cultivated area was used for wheat cultivation in 2020. The export value of wheat exceeds that of any other cereal species, including rice and maize: 45 billion US dollars of world trade in 2020 versus 26 billion US dollars and 37 billion US dollars for rice and maize (*http://faostat.fao.org*).

Figure 1 Global Wheat Production

(*Ref:* *https://www.weforum.org/agenda/2022/08/top-10-countries-produce-most-wheat/*)

1.1.2. Pakistan Wheat Production

Particularly, Pakistan is ranked seventh in the world net wheat production. Total harvested area of wheat in Pakistan is 8 million hectares with an average yield of 25,247,511 tons in 2022 (https://worldpopulationreview.com). The country's food basket is mainly dependent on wheat and enjoys the status of largest grown crop. The contribution of wheat crop to the agriculture sector is 7.8 % whereas 1.8 % to the GDP (https://www.finance.gov.pk). The production of the wheat crop is decreasing since years in Pakistan due to certain limitations of research, policy making, resource shortage and climate change. Pakistan wheat production is declined to 3.9 percent compared to the production of last year.

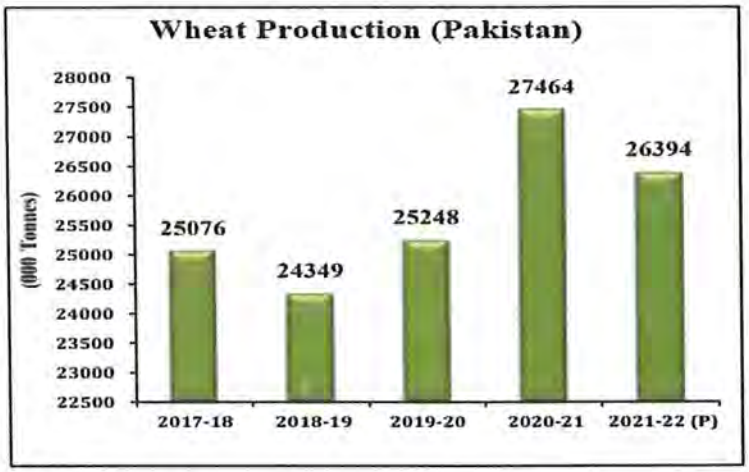

Figure 2. Wheat Production in Pakistan (2017-22)
(*Ref:* *https://www.finance.gov.pk*)

1.1.3. Future Outlook of Wheat

Current exponential growth of world population 7.8 billion people will results in 9.7 billion people on planet earth by 2050. Which in returns means that food security is one of the biggest concerns for the future. Agriculture in general and wheat scientist are facing with this daunting task to increase production to meet our demands of future (www.fao.org). To ensure food security in 2050, an annual 2 percent increase in production is required.

1.1.4. Yield Improvement

Crop yields are contingent on complex interactions between socio-economical, technological, biological, and ecological factors. Wheat breeders and biotechnologist are striving hard with different strategies to increase wheat production. One of the best strategies is to close the yield gap by reducing pre and post-harvest losses. Current research to improve wheat yields covers a broad front and includes further exploring the existing diversity through crossing germplasm, exploring diversity in wild relative through interspecific/inter-generic hybridization crosses, using biotechnology techniques, hybrid wheat, host-plant relationships of various pests/pathogen that attack it and numerous other important research avenues.

1.2. Origin and Evolution of Wheat

The first cultivation of wheat is the part of "Neolithic Revolution" occurred about 10000 years ago, this showed the transition from hunting and gathering of food to settled agriculture. The first cultivated wheat was diploid (genome AA) (einkorn) and tetraploid (genome AABB) (emmer), the genetic relationship showed that they originated from south-eastern part of Turkey. Hexaploid bread wheat appeared when the cultivation spread throughout the east about 9000 year ago.

At the start farmers selected the wheat from the wild populations, because of their characteristics and superior yield, which were essentially landraces; this was considered as an early and nonscientific form of plant breeding! However domestication was also done with the selection of genetic traits which make them different from their wild relatives. The two domestication syndromes are important to discuss here. The first, during the maturity the loss of shattering of spike leads to the seed loss at harvesting, this ensures the seed dispersal in natural population and non-shattering trait is found out by Br (brittle rachis) locus (Nalam et al., 2006). The second vital trait is the modified hulled forms, which allows glumes to stick tightly to the grains, to free threshing naked form. The effect of recessive mutations at the Tg (tenacious glume) locus was modified by the free form which arose by a dominant mutant at Q locus (Nalam et al., 2006).

Apart from the spelt form of bread wheat, the cultivated forms of diploid, tetraploid and hexaploid wheat have a tough rachis. Similarly the advanced forms of

tetraploid and hexaploid wheat are free threshing whereas; the domesticated forms of einkorn, emmer and spelt are hulled. However the domestication of natural population clearly develop the einkorn and emmer, bread wheat only developed through cultivation which results in the hybridization of cultivated emmer with unrelated wild grass *Triticum tauschii* (*Aegilops tauschiiand Aegilops squarosa*). This hybridization possibly went through the process several times autonomously with novel hexaploid (genome AABBDD), which were selected by farmers due to their superior properties. (Figure 3) illustrate the evolution of modern wheat.

The A genome of tetraploid and hexaploid wheat is clearly associated with the wild and cultivated einkorn however the D genome of hexaploid is clearly resulted from that of *T. taushcii*. In actual fact, the little divergence is noticed in the D genome of hexaploid and diploid species because it occurred so recently. In opposition to this, the B genome is probably acquired from the S genome found in the Sitopsis section of Aegilops, with the Ae. Speltoides is the closest extant species.

(Feldman & Kislev, 2007) elegantly described the spread of wheat from its origin across the world and is summarized here. The entrance in Europe was via Anatolia to Greece (8000 BP) after that both northwards through the Balkans to the Danube (7000 BP) and over to Italy, France and Spain (7000 BP), lastly reaching the UK and Scandinavia by about 5000 BP, likewise, wheat roll out via Iran into central Asia landing up to China by 3000 BP and to Africa, at the beginning via Egypt. It was moved from the Spaniards to Mexico in 1529 and to Australia in 1788.

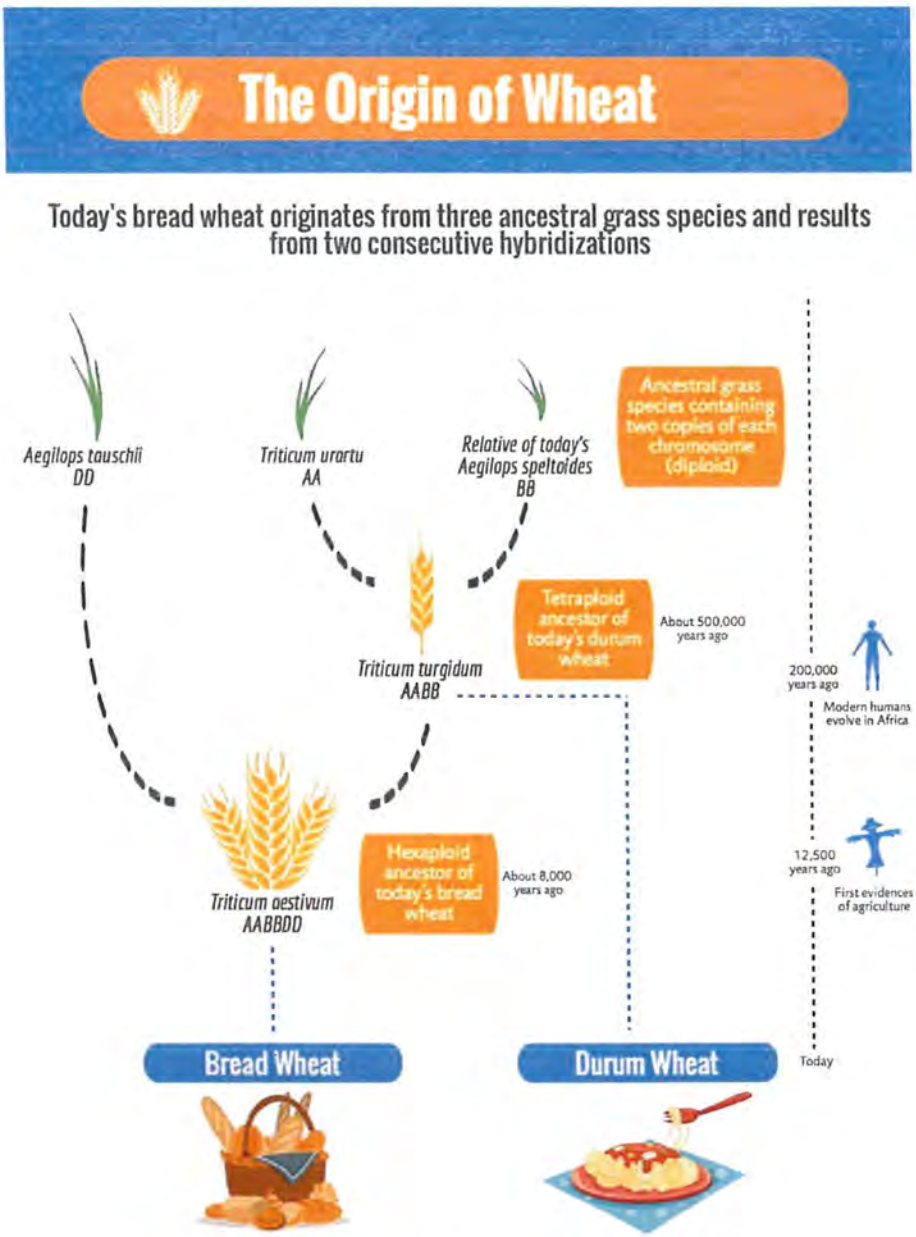

Figure 3. The Origin of Wheat
(*Ref:* www.wheatgenome.org**)**

Until now the studies made confirmed the basic chromosome number of bread wheat is 1x= 7, that is every genome has basic set of seven chromosomes constituting 21 pairs of the hexaploid. These evolutionary events generated wondrous species diversity. This information is being used in intra specific crosses for wheat crop improvement (Charmet, 2011).

1.3. Wheat Growth and Development

Wheat is one of the most adapted cereal crop of the world. It is cultivated in both temperate and tropical agro-climatic zone as compare to tropical agro-climatic conditions for maize and rice. This acclimatization is due to its complex genome having great plasticity. In addition, wheat can withstand broad ranges of osmotic conditions i.e. xerophytic to littoral conditions (Curtis et al., 2002). Due to adaptation in different agro-climatic conditions and different utilities, bread wheat is classified into different groups i.e. spring or winter wheat based on growth habit; soft, medium hard or hard based on grain hardiness; amber, white, red based on grain color etc.

Growth and development of wheat plant is dependent upon its type. In winter wheat, exposure of cold temperature (-5° to 5°C) is required for heading. Therefore, it is planted in between September to November, germinates and is subjected to cold treatment of winter. Whereas, spring wheat is planted in early spring (November/ December) and harvested in summer (April- May). Spring wheat is also cultivated in areas of mild winters such as in North Africa, South Asia, Middle East and the lower latitudes.

Table 1. Days from Emergence to Physiological Maturity of Wheat

DEVELOPMENT STAGE	**TIME**	
	Spring	Winter
Emergence	0	0
Floral initiation	20	35
Terminal spikelet	45	60
First node	60	80
Heading	90	120
Anthesis	100	130
Physiological maturity	140	170

Unlike maize, wheat and rice are C3 plants. But wheat is only crop that is planted in rabbi season meaning it needs cool environment to initial vegetative stage. Growth and development of wheat plant is divided into different stages based on it morphology and physiology. The eventual grain yield depends on each of these developmental stages. The stages and their influence on grain yield is briefly described below.

1.3.1. Wheat Development Phases

Wheat plant life cycles can be morphologically classified into these key stages; germination, emergence, tillering, floral initiation, spikelet emergence, stem elongation, booting, heading, anthesis and physiological maturity. These stages can be grouped into: Germination to Emergence (E); Growth Stage 1 (GS1) from Emergence to Double Ridge or Heading; Growth Stage 2 (GS 2) from Double ridge to Anthesis; and Growth Stage 3 (GS3), which includes the Grain Filling period, from Anthesis to Maturity (Hanft & Wych, 1982). The time of these stages is dependent upon genotype and environmental condition. Different stressors (both biotic and abiotic) can reduce or increase time span of each cycle. Different gradation systems for developmental stages have been used for numerical identification of wheat. The simplest classification of wheat plant development divides plant cycle into two stages i.e. vegetative and seed developmental phase (Hanft & Wych, 1982).

1.3.1.1 Wheat Vegetative Growth Phase

The vegetative growth of wheat plant is divided into 7 morphological phases from Germination to Flowering: Wheat life cycle is initiated with seed imbibition, which results in the emergence of radical and coleoptile. The radical is converted into three seminal roots, while coleoptile elongates. The seedling phase is marked with the appearance of first leaf and ends at tillering. From auxiliary bud of primary tiller second crown tiller is formed. Tillering is one of the most important stages of growth and has significant impact on eventual growth and yield of each plant. The end of tillering stage means that production of new leaf and tiller on main tillers are stopped. In other terms, vegetative growth of the plant is stopped, and growth of reproductive parts is initiated. After tillering phase, the intermodal region of each tiller elongates. The nodes and internodes move upward from crown to produce stem.

The head becomes visible under the sheath of flag leaf, this phase is also called as booting phase. Then comes heading phase in which the first awn and then complete spike comes out of the flag leaf. Just after the heading, anthesis is triggered. Which starts in the middle spikelet and continues to apical and basal side of the spike (Peterson, 1965). All spikes of same genotype planted at same time go through anthesis within a few days.

1.3.1.2. Wheat Seed Developmental Phase

Wheat plant is often self-pollinated crop. After fertilization and initial cellular divisions, endosperm cells and amyloplasts are formed. The initial phase just after fertilization is called as Lag phase. After lag phase grain filling is initiated and last for about 20-30 days. The first phase of filling is known as milky or water ripe phase. In this phase endosperm is developed and storage of starch and protein is initiated. Then comes dough developmental phase, linear grain growth and starch deposition in the endosperm is continued (Jones Jr, 2016). This is that stage of grain development where it gains most of its weight and all the food/vitamins stored during vegetative phase are translocated to seed. At physiological maturity, the seed dough loss the water and gets hardened and final grain weight is achieved. This phase is called as ripening phase. And has direct impact on eventual yield of the crop.

1.4. Wheat Yield and Related Traits

As expounded earlier, wheat is cultivated and preferred around the world because of its agronomic adaptability, grain storage and flour production *etc.* It is preferred because it is used to produce interesting, palatable, and satisfying foods adapted to different culture of the world. Wheat is also the source of essential amino acids, vitamin, minerals, phytochemicals, and dietary fiber components in our daily diet. On the other hand, wheat and its products are reported to have various adverse impacts on human health, including allergies (food and respiratory) and intolerance (like coeliac disease). Sustaining production of wheat is one of the biggest challenges of this century, its quality while reducing inputs of agrochemicals and developing lines with enhanced quality for specific end-uses, notably for biofuel and human nutrition. Thus, improvement of bread wheat with all of these traits is a daunting task for the breeders.

The annual rate of return of a crop is called as yield. The basic aim of all breeders is the grain yield per unit area, as it is the eventual target of every breeding program. Crop yield is a complex trait influenced by combination of several physiological, morphological genetically controlled traits. To improve crop yield we need to understand morphological and physiological attributes contributing to grain yield (Gupta et al., 2008). The extent of divergence of grain yield was first explained by Slafer and Rawson (1994), by the following equation.

$$GY = BY \times HI$$

Where, *GY*= grain yield; *BY*= biomass yield; *HI* =Harvest index.

They further explained the contributors to grain yield by following equation

$$GY = Till\ No. \times SPS \times KW$$

Where, *GY*= grain yield; *Till No.* = tiller number; SPS =spikelet per spike; KW= kernel weight.

As explained earlier, wheat goes through vegetative phase first and then grain filling phase. Each phase of plant growth has an eventual impact on different component of grain yield e.g. as early as emergence from seed will lead a positive significant impact on plant/m^2 and as late as physiological maturity which will determine thousand kernel weight.

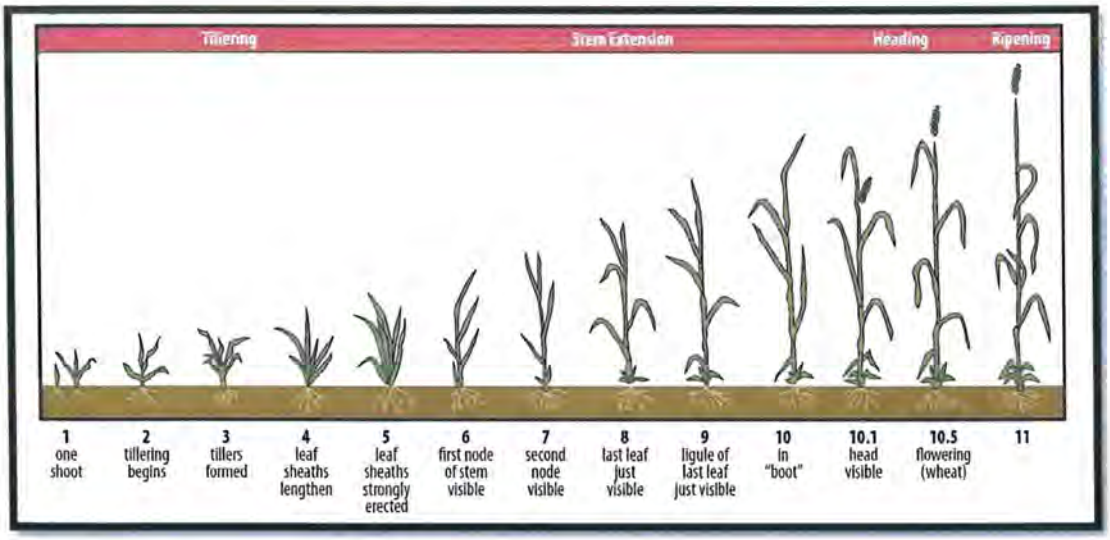

Figure 4. Wheat Growth Stages
(*Ref:* https://warrencountyagriculture.com/wheat-freeze-damage-information/wheat-growth-stages/
)

1.5. Role of Wheat Genetic Diversity

In order to broaden the basis of wheat rust resistance, it is crucial to discover and utilize resistant-genes from all sort of wheat genetic resources. Today's modern hexaploid wheat varieties have less genomic variability compared with wild wheat relatives because of less diverse germplasm cultivated by cultivar adopting and moreover affected by crop selection and genetic erosion (Doebley et al., 2006; Rasheed et al., 2018; Reif et al., 2005). Recently wheat stem rust epidemics occurred in Africa and in another places confirmed that stem rust is a re-evolving pathogen becoming a big threat to crop yields globally. Wheat varietal germplasm has a narrow genomic bases for resistance to virulent pathotypes, like pathotypes in the Ug-99 race lineage. One of the best reasonable way to support national food security is to utilize the genomic diversity through hybridization from wild relative resources of wheat.

Aegilops species have been established as great genetic reserves for broadening the genetic diversity of potential wheat cultivars against biotic and abiotic factors. Wheat wild relatives are a potential source of rust-resistant genes and *Aegilops* germplasm have not been used to any exciting level as a genetic solution for wheat rust resistance. Actually, many of resistant-genes transferred from wheat

relatives performed to be more resilient against the Ug-99 lineage than other wheat derived stem rust gene (Jin et al., 2007; Singh et al., 2016). *Aegilops* species are very close relatives of *Triticum* species and these includes twenty three species that comprise hexaploid, tetraploid and diploid plants (Jiang et al., 1994; Olivera et al., 2018). These species are well-known to be a great basis of rust resistance, and many resistance-genes transferred to wheat varieties (Liu et al., 2011; Olivera et al., 2018; Olson et al., 2013). Easy breeding and decreased linkage drag make hybridization from other species in the basic genetic resources desired by scientists to combine new allelic diversity in their wheat germplasm (Feuillet et al., 2008). Yet, species in gene-pools like the secondary and tertiary contributes to a significant pool of genomic diversity (Qi et al., 2007).

1.6. Advanced Toolkit to Accelerate Wheat Cycle (Speed breeding)

Quickly growing human population and climate change have upraised very important concern for world food security, the present development rate of many food crops is insufficient to meet upcoming food demands (Ray *et al.* 2012; Ray *et al.* 2013). By using different breeding techniques, distinctive recombinant lines were developed to widen the existing genetic diversity in hexaploid wheat and the desired characteristics has been combined from synthetic hexaploid wheat donors (Trethowan & van Ginkel, 2009; van Ginkel & Ogbonnaya, 2007). Novel alleles which were categorized better were identified utilizing non-cultivated germplasm from wild wheat relatives, which collectively broaden genetic diversity in bread wheat cultivars. The key prerequisite to exploit SHWs in hybridization is to escape from F1 plant necrosis. So, it is desired to utilize a number of good wheat cultivars for hybridization with SHWs to avoid the occurrence of F1 plant necrosis, the hybridization failure and to rise the possibility of the production of better cross combinations.

Genetic resistance for biotic factors like wheat rusts can be transferred from wheat cultivars, landraces, and synthetic wheats and also from wild relative of wheat through pre-breeding and breeding. After crossing of two parent genotypes, four to six breeding cycles are normally essential to attain homozygosity for evaluation of grain yield and agronomic traits. Field-grown crops like wheat are usually limited to

harvest only one or two generations/year and this method is generally time consuming. To address the slow wheat generation development rate, most common method is "shuttle breeding", presented by N. E. Borlaug in 1950s at the International Centre for Maize and Wheat Improvement, Mexico, which is capable to produce two generations/year by planting wheat progenies at field stations unlike in latitude, altitude and other climatic conditions (Ortiz et al., 2007).

(Watson et al., 2018) from The University of Queensland, Australia presented a breeding method known as "speed breeding" that have potential of significantly shorten the plant generation period and fast-tracks crop hybridization and crop development methods. The unique method was initially defined and employed for hexaploid wheat at the University of Queensland, Australia to gain rapid plant selection and generation advancement (Hickey et al., 2009). (Hickey et al., 2017) demonstrated distinctions of this technique to be an effective scheme for fast evaluation of wheat cultivars for disease resistance at the adult plant stage in speed breeding glasshouse (Dinglasan et al., 2016; Riaz et al., 2016).

1.7. Role of Nanoparticles in Plant Growth

In this last decade the nanomaterial and nanotechnology has got big fame and widely be used throughout the world. It provide the platform for the study and transformation of the biological system (Monica & Cremonini, 2009). Nanoparticles (NPs) are the molecular aggregates with the dimension between 1 to 100 nm (Ball, 2002). These NPs can modify their physio-chemical properties drastically compared to their bulk aggregates (Nel et al., 2006). The efficiency of the NPs is totally dependent on the chemical composition and the size/shape of the particles (Brunner et al., 2006). Nanoparticles are proved as '' magic bullets'' that contain Nano-pesticides fertilizers and herbicides by which specific cellular organelles are targeted to release their content. Nanoparticles are sometimes said to be nano-fertilizers for plants that possess some properties that are effective to plant growth and can enhance the target activity (DeRosa et al., 2010). Nanoparticles have potential to increase the metabolic activity of plants by different physiochemical properties (Brew & Strano, 2014). Few highly engineered nanoparticles have the ability to enter the plant cell and transport chemicals and DNA in it (Galbraith, 2007; Torney et al., 2007). Now

plants can also harvest the more light energy by the help of carbon nanotubes in their chloroplast that could act as artificial antennae by which the chloroplast is able to capture the wavelengths of lights that are not in their normal range for example near-infrared, green and ultraviolet (Cossins, 2014; SM & AA, 2014). Seed germination, growth and development of plants can also be boosted by engineered carbon nanotubes (Lahiani et al., 2013; Siddiqui et al., 2012). The impact of engineered nanoparticles (ENPs) depends on the concentration, composition, size, chemical and physical attributes of ENPs as well as plant (Ma et al., 2010).

1.8. Plant Growth Regulators (PGRs)

Plant growth regulators are the organic compounds that can affect the physiological process of plants when used in very small concentrations. They can be applied directly to the plants. These are able to enhance yield, facilitate yield and improve the quality of plants (Rameau & Beveridge, 2010). These PGRs have vital role in quality, earliness, sex modification, increasing the yield and regulation of the growth. Growth regulating substances, phytohormones or simply plant hormones when produced artificially these are known as plant growth regulators (Rademacher, 2015). There are few main plant growth regulator such as Abscisic acid (ABA), Gibberellin, Auxin, Cytokinin, Jasmonic acid, Brassinosteroids and Ethylene etc. These all growth regulators have multiple and specific function and can perform synergistically (Gibberellin and Auxin) and antagonistically (Auxin and Abscisic Acid).

Table 2. PGRs and their Classes (Thakur, 2022)

PGRs	Classes
Auxin	NAA (1-Naphthaleneacetic acid), IAA (Indole-3-acetic acid), IBA (Indole-3-butyric acid), 2-4D (2,4-Dichlorophenoxyacetic acid) and 4-CPA (4-Chlorophenoyacetic acid)
Gibberellin	Gibberellic acid (GA_3)
Cytokinin	Kinetin, Zeatin
Ethylene	Etheral
Abscisic Acid	Dormins, Phaseic acid

Table 3. Site of Production and Function of PGRs (Thakur, 2022)

PGRs	Site of Production	Functions
Auxin	Young expanding leaves, Embryo of seed, Meristem of apical buds,	(i) Involved in Apical dominance (ii) stimulates Cell division and enlargement (iii) Shoot and root growth (iv) Plant growth movement (v) Parthenocarpy (vi) Abscission (vii) root induction (viii) control fruits drops
Gibberellin	Immature seeds	(i) Prevent genetical dwarfism (ii) Regulation in bolting and flowering (iii) Production of parthenocarpic fruit (iv) Germination (v) Increase flower and fruit size
Cytokinin	Endosperm of seeds, Young fruits and Root apex	(i) Cell and organ enlargement (ii) Seed germination (iii) Development of bud and shoot growth (iv) Flower induction (v) delay senescence
Ethylene	Ripe fruits, flowers and Leaves and nodes of stem.	(i) Ripening of fruit (ii) Seedling growth and emergence (iii) Abscission of leaf
Abscisic acid	Roots and Terminal Buds	(i) Abscission (ii) Maintaining Dormancy (ii) Inhibit seed germination and development (iv) stimulate stomatal closure

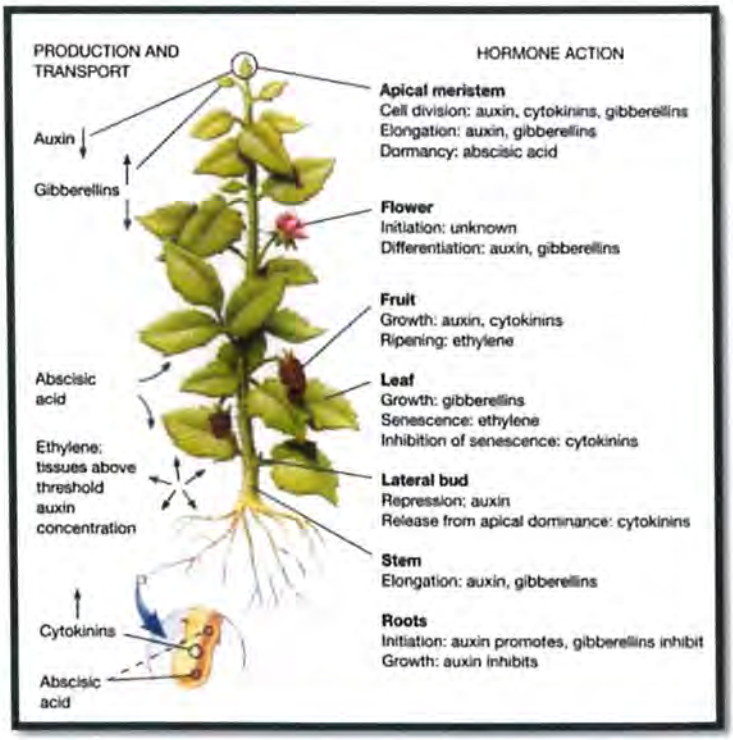

Figure 5. Production, Transport & Production of Hormones
(*Ref:* https://www.ugaoo.com/knowledge-center/wp-content/uploads/2016/11/Hormoneaction.jpg)

1.9. Hydroponics

The word hydroponics is the combination of two words, hydro means water and pones means labor. The real meaning of hydroponics is "working water (Hollmann, 2017). It is the method for growing plants without soil. Plants are allowed to contact directly with the nutrients in soilless medium. It replaces the soil with growing media and soil, the growing media can be Rockwool, sand, perlite etc., Their main role is to make the roots oxygenated and to transfer nutrients to water. The water pump is usually used to add the nutrients in growing media which moves throughout the roots (Jensen, 1997). (Figure 6) show the basic concept of hydroponic.

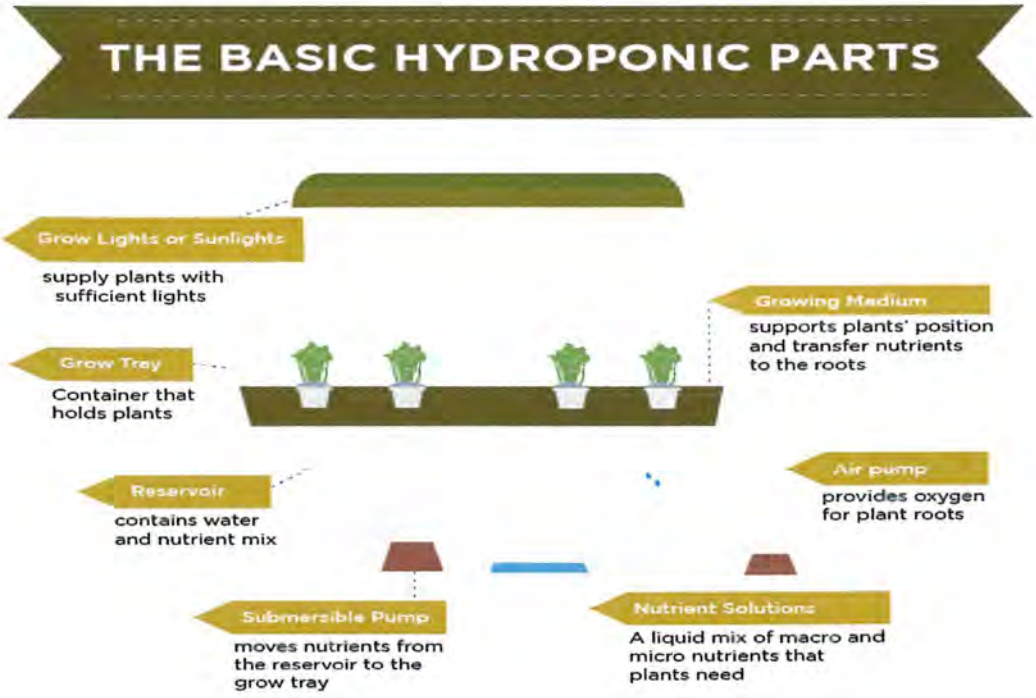

Figure 6. The Basic Hydroponics Parts

(Ref: https://philprimeventures.com/hydroponics-101/types-of-hydroponics/)

1.9.1. History of Hydroponics

Hydroponics does not evolved over night but it has undergone many scientific researches by scientists (Resh, 2022). The detailed history (Figure 7) of hydroponics is given as follows:

- **The 600 BC:** The Euphrastes River and the hanging gardens of Babylon in Babylonia are the ancient examples of hydroponics.
- **The 1000 to 1100 AD:** "Chinampas" floating gardens developed by Aztecs in the Island city of Tenochtitlan.
- **The late 1200s:** Floating gardens were discovered by Marco polo during his trip to china.
- **The 1600s:** The first experience was performed by Balgian Jan Van Helmont on plant growth and constituents.
- **1699**: John Woodward an English man grew in mixture of different soil particles in water, he came to know that plants absorb nutrient from minerals and certain substances in water, obtained from the soil. This is incorrect statement.
- **The 1860s:** Julius von Sachs and Willhelm Knop (German Scientists) derived the first standard formula for plant mineral nutrients dissolved in water.
- **The 1920s and 1930s:** The terms "Hydroponics" coined by W.F Gericke (U.C. Berkley). He practiced to grow plants in a water solution and also performed many experiments regarding hydroponics.
- **The 1940s:-** Hydroponics was used to supply the troops stationed with fresh vegetables on the isolated, non-arable Wake Island.
- **The 1950s:** Globally, Hydroponics was used for commercial farms and greenhouses. It gained much popularity in many countries such as Spain, England, France, Germany, Italy, the USSR, Israel, etc.
- **The 1960s to now:** Several Hydroponic systems are evolved and are put into use, including the, the Drip System, Ebb & Flow, Nutrient Film Technique and Aeroponics. In the recent two decades, there is a keen interest of farmer into Hydroponics when it is applied to large-scale greenhouse farms to provide foods for millions of people around the world.

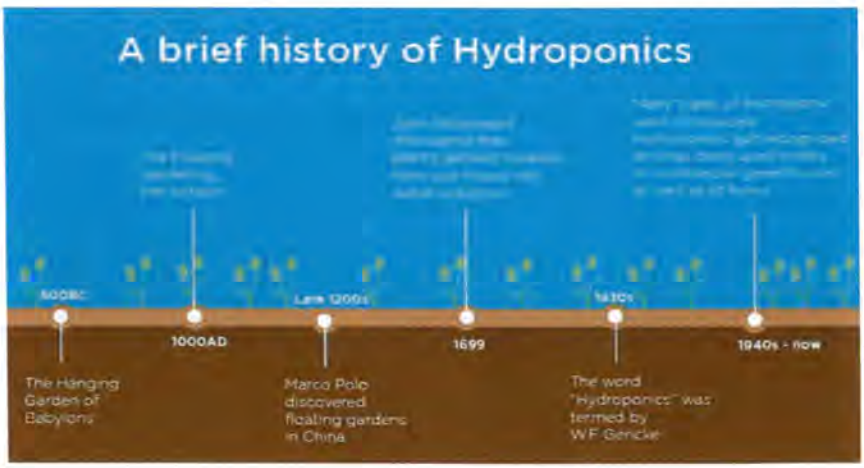

Figure 7. A Brief History of Hydroponics

(Ref: https://homehydroponicsindia.blogspot.com/2018/11/history-of-hydroponics.html)

1.9.2. Scope of Hydroponics

Hydroponics has more advantages than soil and it cannot be compared with the soil. To prove that few important points are discussed as bellow:

1.9.2.1. Better Growth Rate

The plants which are grown hydroponically are enjoying 20-30% more growth rate than the plants which are grown in soil (Ehret et al., 2001). This is because the energy used in searching the nutrients from the soil is saved and utilized in direct uptake of minerals and nutrients from the provided media and water, thus the plant growth and production of fruit is enhanced. The grower can operate the whole crowing system such as temperature, nutrients and light etc. by doing this plants will be provided with ideal conditions that they require (Jones Jr, 2016).

1.9.2.2. Hydroponics Preserves Water

Hydroponics is the method that is very useful to save the water because it consume only 10% water in comparison to the soil agriculture (Putra & Yuliando, 2015). This is due to its convenient system. The plants grown hydroponically absorb the enough water while the run-off ones are picked up and get back to the scheme.

1.9.2.3. No Need of Soil

This leads to the two great advantages:

i. There is a great choice to grow plants anywhere whether in arable or contaminated places. Land is saved by growing plants in any locations like your apartment or in large-scale indoor greenhouses.

ii. Hydroponics system is efficient to eliminate all the diseases, weeds and soil related pests.

1.9.2.4. Direct Use of Nutrients

The nutrients are added with correct amount required by the plants and no nutrient is lost in this system as in soil. The nutrients are added in the media by 100% control (Seawright et al., 1998).

Besides all the advantages there are also few disadvantages or drawbacks of hydroponic system such as the lack of technical knowledge can lead to the disturbance in your system, system failure and power shortage results in the death of your plants as the plant roots could not get the water.

1.10. Hoagland Solution

Hoagland solution was developed by Hoagland and Snyder in 1933 and used as hydroponic nutrient solution (Hoagiand, 1933) and it was refined by (Hoagland & Arnon, 1950) The water-culture method for growing plants without soil (Hoagland & Arnon, 1950) For growing plants it is one of the most popular solutions with large amount (15000) of citation listed by Google scholar (Zhao et al., 2012). Hoagland solution contains necessary nutrients which are required by a plant to support its growth (Metali et al., 2012) The solution developed by Hoagland was modified several times mainly to change the concentration and number of micronutrients by adding the Ferric EDTAs. In the comparison of 1938 to 1950 there was only one changing in the concentration (Mo 0.01 ppm) however, the composition and concentration of macronutrient remained the same since the time of development (1933) hence, the modified and original concentration are given below:

N 210 ppm, K 235 ppm, Ca 200 ppm, P 31 ppm, S 64 ppm, Cl 0.65 ppm, Na 1.2 ppm, Mg 48.6 ppm, B 0.5 ppm, Fe 2.9 ppm, Mn 0.5 ppm, Zn 0.05 ppm, Cu 0.02 ppm, Mo 0.05 ppm.

Hoagland solution is more advantageous for the growth of large plant such as tomato and bell pepper due to the presence of high concentration of N and K (He et al., 2019) Because of relatively high concentrations in the stock solutions the solution is efficient for the developments of plants with smaller nutrient requirement as well, such as lettuce and aquatic plants, with the more dilution of the preparation to 1/4 or 1/5 of the modified altered solution (Shimul et al., 2014). Hoagland solution required following salts and acids:

1. Potassium nitrate, KNO_3
2. Calcium nitrate tetrahydrate, $Ca(NO_3)_2 \cdot 4H_2O$
3. Magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$
4. Potassium dihydrogen phosphate, KH_2PO_4 or

5. Ammonium dihydrogen phosphate, $(NH_4)\ H_2PO_4$
6. Iron (III)-EDTA or Iron chelate, Fe-EDTA or Fe-EDDHA
7. Boric acid, H_3BO_3
8. Copper sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$
9. Zinc sulfate heptahydrate, $ZnSO_4 \cdot 7H_2O$
10. Manganese chloride tetrahydrate, $MnCl_2 \cdot 4H_2O$
11. Molybdic acid monohydrate, $H_2MoO_4 \cdot H_2O$ or
12. Sodium molybdate dihydrate, $Na_2MoO_4 \cdot 2H_2O$.

The Hoagland solution formation depends on $(NH_4)\ H_2PO_4$ in place of KH_2PO_4 must be managed according to a different protocol, which is described in the circulars of 1938 and 1950. Sprint 138 iron chelate is formed as sodium Fe-EDDHA, whereas, Hoagland's original solution formulation (1933) additionally contains ferric or ferrous tartrate but not any ion of Na. Other micronutrients (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 (Murrashige & Skoog, 1962). As in confidence, there is clue that, for example, some algae need cobalt for the production of vitamin B12. On the other side, it is proved that the altered Hoagland solutions of 1938 and beyond are balanced solutions of the nutrients that answer the question how to concentrate and prepare the solutions which is best for the growth of plants (Hoagland, 1920).

1.11. Aims and Objectives of the Study

Crop research alterations and improvements are helping us to meet the challenge of global food demand in the alarming situation of growing world population. The breeders are adopting new and advance technologies to breed better. Speed breeding or rapid plant breeding is prominent strategy among plant breeders to develop resilient cultivars of high productivity in short span of time. Now days almost 6 generations of wheat can be produced through SB. The aim of this study was to reduce the length of breeding cycle of wheat and get more than 6 generations at SB platform.

Chapter 2:

MATERIAL AND METHODS

CHAPTER 2: MATERIAL AND METHODS

The experiment was performed at **"Speed Breeding Facility"** of Crop Science Institute CSI (Wheat Program) **"National Agriculture Research Centre (NARC) Islamabad"**. The proper guidance regarding speed breeding protocol was taken from Dr. Zahid Mahmood (Senior Scientific Officer NARC).

2.1. Experiment Design

The experiment was adopted as Complete Randomized Block Design (CRBD) with three replications of eight treatments and control. Each replication had three plants. Two systems were built to carry out the experiment i.e. Hydroponics system and Pots system. The whole experiment was conducted in Speed Breeding Facility at NARC Islamabad.

2.1.1 Material Required

As the experiment was based on two different systems Hydroponics and Pots System hence different materials for both systems were required.

2.1.1.1 Hydroponics Material and Setup Design

Hydroponics setup was constructed following different basic setups of the system. It was initially adopted as trial after trial it was properly adopted in experiment. The following material (Table 4) (Figure 8) was used for the setup of hydroponics system.

Table 4. Required Material for Hydroponics Setup

S No:	MATERIAL	QUANTITY
1	Rolling Storage Box (24 L)	3
2	Silicone pipe	10 ft. long
3	Thermopore Sheet (15 x 11 inch)	3
4	Aluminum Foil Roll	1
5	Cutter	1
6	Air pump	3
7	Foam Sheet (1 Inch Thickens)	½ Meter
8	Air stone Bar (1 feet)	6

Figure 8 Material of Hydroponics System

The hydroponics setup was built with a very simple and modified way to fulfill the requirement of experiment and the desired results of growth of plants. For hydroponics experiment the Deep Water Culture (DWC) system was built which consist of a floating platform and a container filled of nutrient and water.

a) **Floating Platform:** A 1 inch thick thermopore sheet (Figure 9) of 15 x 11.5 inches size was taken and 31 holes of 12.7 mm were made by using a hot rod with 1.5 inches space between each hole. These holes were made to adjust the germinated seeds covered by foam. Foam provided the support and held the plant from seedling stage up to the maturity.

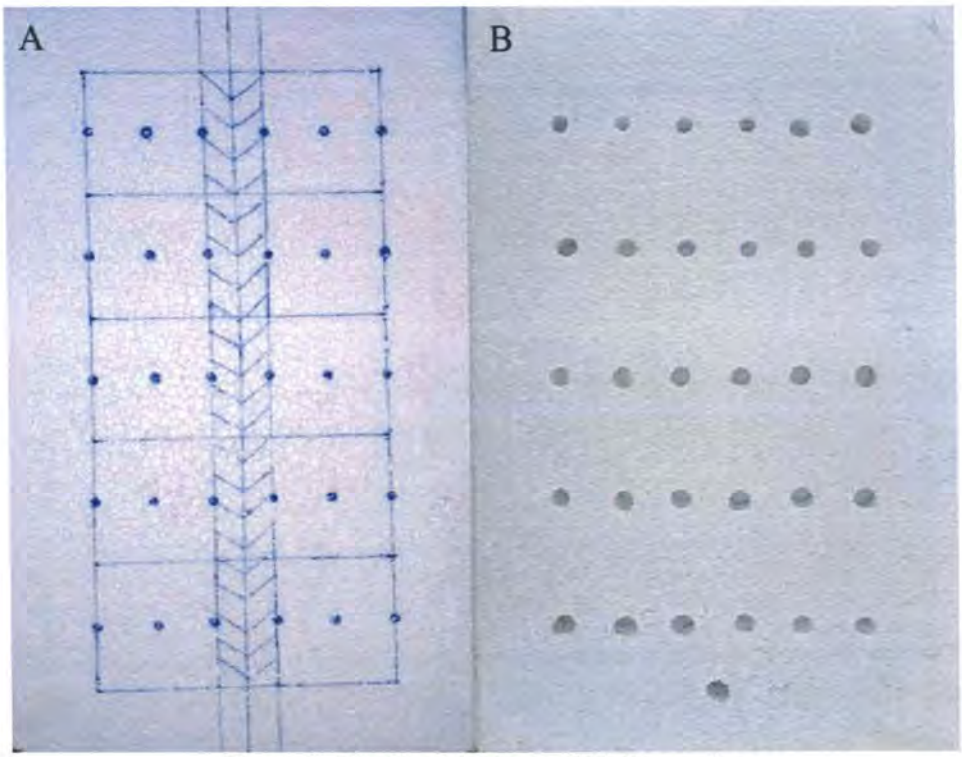

Figure 9. Floating Platform of Hydroponics

(*A = Thermopore sheet marked for making holes at distance of 1.5 inches, B = Thermopore sheet with holes*)

b) **Container/Reservoir:** A 24 liters transparent rolling storage box (Figure 10) was used for the nutrient and water storage. The container was built with air stone that was connected with the air pump by the help of plastic pipe.

Figure 10. Container/ Reservoir of Hydroponics

c) **Hydroponics Nutrient Solution:** Hoagland solution was used in hydroponics as a nutrient solution. The standardized recipe (Table 5) of Hoagland stock solution was followed to prepare the final nutrient solution.

Table 5. Hoagland Stock Solution Recipe

MACRONUTRIENTS		
S#:	**Chemicals**	**Conc. Per Liter**
1	2M KNO_3	202g/L
2	2M $Ca(NO_3)_2.4H_2O$	236g/L
3	Fe EDTA	1.5g/L
4	2M $MgSO_4.7H_2O$	493g/L
MICRONUTRIENTS		
S#:	**Chemicals**	**Conc.: Per Liter**
1	H_3BO_3	2.86g/L
2	$ZnSO_4.7H_2O$	1.81g/L
3	MnCl2.$4H_20$	0.22g/L
4	$CuSO_4.7H_2O$	0.08g/L
5	$Na_2MoO_4.2H_2O$	0.12g/L
6	1M KH_2PO_4	136g/L

Table 6. Stock Solution Required for 20 L and 60 L Nutrient Solution

MACRONUTRIENTS					
S#:	**Chemicals**	**Conc. Per Liter**	**Stock per 1 Liter**	**Stock per 20 Liter**	**Stock per 60 Liter**
1	KNO_3	202g/L	5 ml	100 ml	300 ml
2	$Ca(NO_3)_2.4H_2O$	236g/L	5 ml	100 ml	300 ml
3	Fe EDTA	1.5g/L	1.5 ml	30 ml	90 ml
4	$MgSO_4.7H_2O$	246g/L	2 ml	40 ml	120 ml
MICRONUTRIENTS					
S#:	**Chemicals**	**Conc.: Per Liter**	**Stock per 1 Liter**	**Stock per 20 Liter**	**Stock per 60 Liter**
1	H_3BO_3	2.86g/L	1 ml	20 ml	60 ml
2	$ZnSO_4.7H_2O$	1.81g/L	1 ml	20 ml	60 ml
3	MnCl2.4H20	0.22g/L	1 ml	20 ml	60 ml
4	$CuSO_4.7H_2O$	0.08g/L	1 ml	20 ml	60 ml
5	$Na_2MoO_4.2H_2O$	0.12g/L	1 ml	20 ml	60 ml
6	KH_2PO_4	136g/L	1ml	20ml	60 ml

Table 7.Concentration required for 20 L and 60 L Nutrient Solution

MACRONUTRIENTS					
S#:	**Chemicals**	**Conc. For 20 Liters**	**DW for 20 liter**	**Conc. For 60 Liters**	**DW for 60 liter**
1	KNO_3	10.1 g	100 ml	30.3 g	300 ml
2	$Ca(NO_3)_2.4H_2O$	23.6 g	100 ml	70.8 g	300 ml
3	Fe EDTA	45 mg	30 ml	135 mg	90 ml
4	$MgSO_4.7H_2O$	9,84 g	40 ml	29.52 g	120 ml
MICRONUTRIENTS					
S#:	**Chemicals**	**Conc. For 20 Liters**	**DW for 20 liter**	**Conc. For 60 Liters**	**DW for 60 liter**
1	H_3BO_3	57.2 mg	20 ml	171.6 mg	60 ml
2	$ZnSO_4.7H_2O$	4.4 mg	20 ml	13.2 mg	60 ml
3	MnCl2.4H20	36.4 mg	20 ml	109.2 mg	60 ml
4	$CuSO_4.7H_2O$	1.8 mg	20 ml	5.4 mg	60 ml
5	$Na_2MoO_4.2H_2O$	24 mg	20 ml	0.72 mg	60 ml
6	KH_2PO_4	2.72 g	20 ml	8.16 g	60 ml

d) **Setup Design:** As the Deep Water Culture (DWC) system of hydroponics consists of floating platform and container/reservoir. The floating platform was made up of thermopore sheet (15 x 11.5 inches) having 31 holes of 12.7 mm. These holes were made for the fixing of germinated seeds covered with the foam of half Inc. /sq. The 30 holes were filled with the germinated seedlings and 1 hole was used for the aerations pipes (Figure 11).

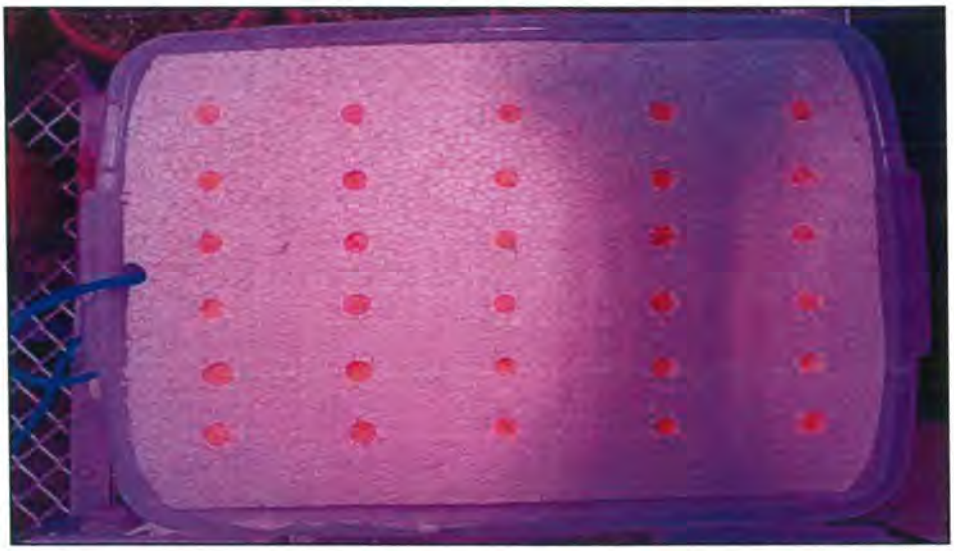

Figure 11 Floating Platform Showing Seedlings Filling

The container/reservoir was made of transparent rolling storage box (24 L) (Figure 10) covered with aluminum foil. The aluminum foil was used as the opaque layer that do not allow the light to pass through the container. Two air stones (1 Feet) were tightly fixed at the bottom of container for the purpose of aeration and mixing of nutrients. These air stones were connected to the air pumps by the help of silicon pipes. The 20 liter nutrient solution was filled in the container and the air pump was regularly run.

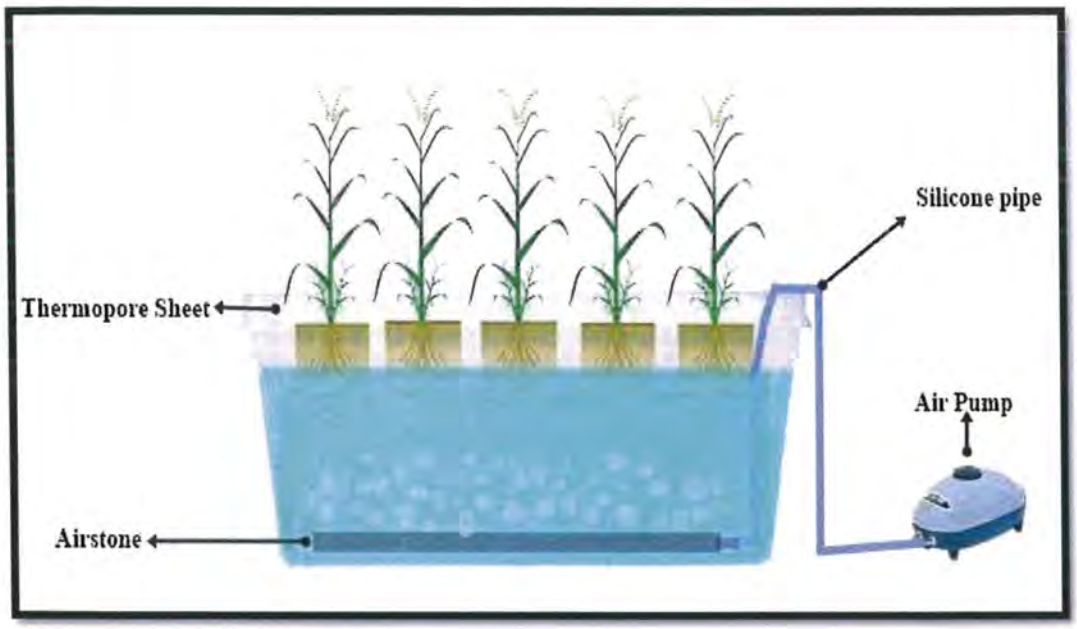

Figure 12. Hydroponics Setup Design

(*Illustration made by using PhotoScape software*)

e) **Setup Working:** When the seeds sprouted, they were transferred to the hydroponics setup (floating platform). The sprouted seeds covered with foam and were fixed in 27 holes (3 replicates of control and 8 treatments). The container was filled with 20 liters distill water and Hoagland stock solution of required volume. The pH of stock solution was maintained at 5.8 by using the buffers (NaOH and HCl). The air pump was kept functional regularly to avoid the nutrient settlement at the bottom of the container. The nutrient solution was changed after every 15 days until the tillering stage, after tillering stage the solution was changed weekly up to the maturity stage. After the ten days of post anthesis, water was completely removed from the container to accelerate the ripening of seeds.

2.1.1.2 Pots System Material and Setup

For Pots system almost 27 pots (8 x 14.5 inches) of good quality were purchased. These pots were washed properly and filled with 550 grams of potting mixture. The potting mixture was prepared with 2: 1 proportion. Two parts of peat moss and one part of soil were mixed and added to the pots (Figure 13). The following (Table 8) material was required for Pots System setup.

Table 8. Required Material for Pots Setup

S no:	Material	Quantity
1	Plastic Pot (Dia 8 and Height 14.5 inches)	27
2	Peat Moss (Pinstrub)	10 KG
3	Soil	5 KG

Figure 13. Material of Pots System

Table 9. Chemical Composition of Pindstrub Peat moss.

(Ref: https://www.pindstrup.com/professional/general-potting)

Declaration	
Screening	0-6 mm 0-10 mm 0-20 mm
Ph	5.5 (For plants that demand low pH) 6.0 (For most crops)
Dry matter content	55 – 57 grams/liter
NPK Fertilizer per m^3	1.0 KG per m^3
Micro fertilizer per m^3	50 gram per m^3
E.C Dutch standard	App. 1.0
Wetting agent	100 ml per m^3

All 27 pots were filled with growth medium and irrigated with tape water. After 2 hours the sprouted seeds were transferred from petri plates to the pots. The five sprouted seeds of PAK-13 cultivar were sown in to the pots. When the plants reached at three leaf stage the two plants from each pot were removed and only three plants were grown until the maturity. The pots were watered two times a day regularly. At the last week the pots were not watered in order to gain ripened seeds earlier.

2.2. Plant Material

Only one wheat cultivar (PAK-13) of bread wheat was used during the experiment. The seeds of the cultivar were attained from NARC wheat department in 2022. Before the start of experiment the seeds were sterilized properly.

2.2.1 Seed Sterilization

The seed were washed twice with distilled water. After this seeds were sterilized with 70% ethanol for 10 minutes on shaker. Later sterilized seeds were again washed with sterile 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 for germination.

2.2.2. Seed Germination

Four petri plates were washed and surface sterilized properly, the filter paper was cut in the size of petri plates. The filter paper was placed in the petri plates and wetted with the autoclave distill water. Almost 90 seeds were imbibed in each petri plate (Figure 14) and the lid was sealed with parafilm. These closed petri plates were kept in envelope to maintain the darkness for stratification of seeds. The petri plates were placed in SB glass house for two days. When the seeds were sprouted the lids were removed, the seeds were placed in open air in the SB room for germination. After 24 hours the seeds were germinated at 85 percent.

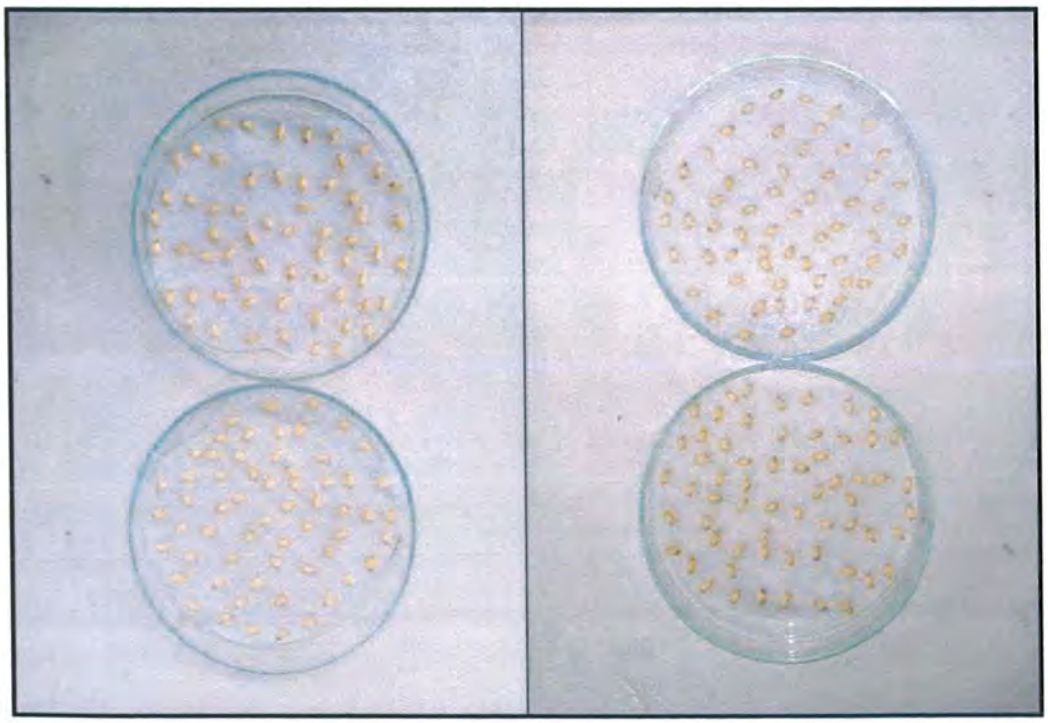

Figure 14. Seed Germination

2.3. Speed Breeding Glass House Protocol

For the setup of SB plant growth chamber or controlled environment room (CER) the following parameters should be maintained.

2.3.1. Lights

The lights that produces spectrum that come under the photosynthetically active radiation (PAR) i.e. 400 – 700 nm with particularly having the blue, red and far red ranges can be used for SB. The required spectral range can be achieved through LEDs or the combination of LEDs and other sources of light such as halogen lamps. After the quality of light, it is also recommended that photosynthetic photon flux density (PPFD) should be 450 – 500 µmol. m^{-2}. s^{-1} at the height of plant canopy (Watson et al., 2018). The Heliospectra E602G lights (Figure 15) having above mentioned properties are used at SB Facility CSI at NARC.

Figure 15 Heliospectra E602G Lights

2.3.2. Photoperiod

The photoperiod of 22 hours with 2 hours dark through a 24 hour diurnal cycle is recommended. The continuous light is beneficial for plant but the dark period also improves the plant health. It is also suggested by some studies that only 18 hours photoperiod are enough for the proper growth of oat, triticale, barley and wheat (Ghosh et al., 2018). At SB facility CSI of NARC the wheat plants were grown under the 22 hours of photoperiod with 2 hours darkness.

2.3.3. Temperature

In SB the optimal temperature regime with minimum and maximum should be applied for each crop. The high temperature can be maintained in photoperiod while the fall in temperature can be achieved in dark that is helpful in the stress recovery. At University of Queensland and John Innes Centre UK, 12 hour 22 354 °C / 17 °C temperature cycling regime with the 2 hours of darkness occurring within the 12 hours of 17 °C and 22 °C / 17 °C for 22 hours light and 2 hours dark, respectively proved successful (Watson et al., 2018). Throughout the experiment the temperature was maintained at 22 °C / 17 °C for 22 hours light and 2 hours dark in SB facility CSI NARC.

2.3.4. Humidity

Most SB growth chamber have limited control over humidity but range of 60 to 70 percent is ideal. A lower humidity level may be advisable for crops that are more resistant to drier conditions. The humidity throughout the experiment was maintained at the range of 60 to 70 percent.

The Speed Breeding Facility of CSI NARC Islamabad is shown in some shots (Figure 16) and the protocol details are also illustrated in the lay out (Figure 17).

Figure 16. Speed Breeding Facility CSI NARC Islamabad

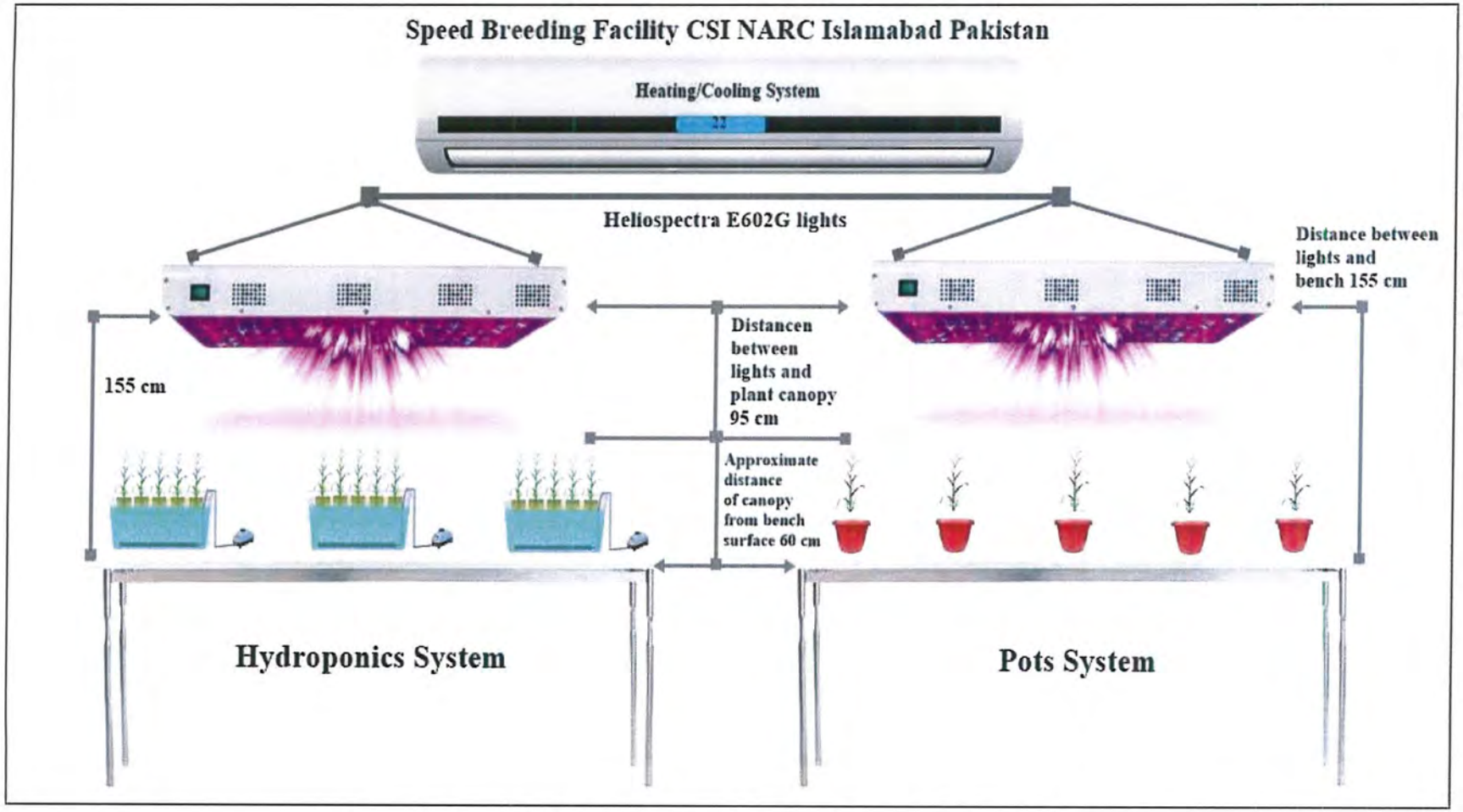

Figure 17. Lay out of Glass house used for Speed Breeding at CSI NARC Islamabad.

2.4. Treatments Applied During Experiment

The experiment was obtained as the complete randomized block design (CRBD) with one control and eight treatments as shown in table. Two concentrations (50 and 100 mg) of each Auxin (Indole-3-butyric acid) and Gibberellin (Gibberellic acid (GA3)), three concentrations of MnO nanoparticles (100, 250, and 500 ppm) and one concentration of $MnCl_2$ (100 mM) were prepared and applied as foliar spray at different developmental stages of the plant growth mentioned in (Table 11).

Table 10. List of Treatments

S #:	Treatments	Explanation
1	T_0	Control
2	T_1	Auxin 50 mg
3	T_2	Auxin 100 mg
4	T_3	Gibberellin 50 mg
5	T_4	Gibberellin 100 mg
6	T_5	MnO_2 NPs 100 ppm
7	T_6	MnO_2 NPs 250 ppm
8	T_7	MnO_2 NPs 500 ppm
9	T_8	$MnCl_2$ 100 mM

Table 11. List of Foliar Spray and Stages.

S #:	Foliar Spray	Developmental Stage
1	1st Spray	Three leaves stage
2	2nd Spray	Tillering Stage
3	3rd Spray	Booting Stage
4	4th Spray	Heading Stage

2.5 Phenotypic Traits

The phenotypic traits evaluated during the research work are as follow:

2.5.1 Chlorophyll Content

The chlorophyll content of leaves was carefully, and non-destructively measured (Figure 18) using a hand-held battery portable optical meter (Minolta SPAD-502 chlorophyll meter). The readings were recorded after 15 days of germination with interval of seven days.

Figure 18. Collection of Chlorophyll Data

2.5.2. Shoot Length (SL)

After the 20 days of germination the Shoot length was measured (Figure 19) in centimeters (cm) by the help of steel meter tap at the intervals of seven days. It was recorded from crown.

Figure 19. Measuring Shoot Length by Meter Tape

2.5.3. Fresh Weight (FW)

After harvesting the plants they were weighed in grams (g) by using electronic balance.

2.5.4. Dry Weight (SDW) or Biomass

When the fresh weight was measured the plants were subjected to the oven at 60 °C for 48 hours. Shoot dry weight was measured by using electric balance in grams (g). It was the dry shoot weight and is also known as biological yield (BY) or plant biomass.

$$Biomass = Spike\ weight + Straw\ weight$$

2.5.5. Leaf Area (LA)

Leaf length and leaf width of flag leaf was measured in centimeter (cm) by using meter tape the these two factors was put in the following formula (Yoshida, 1976).

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

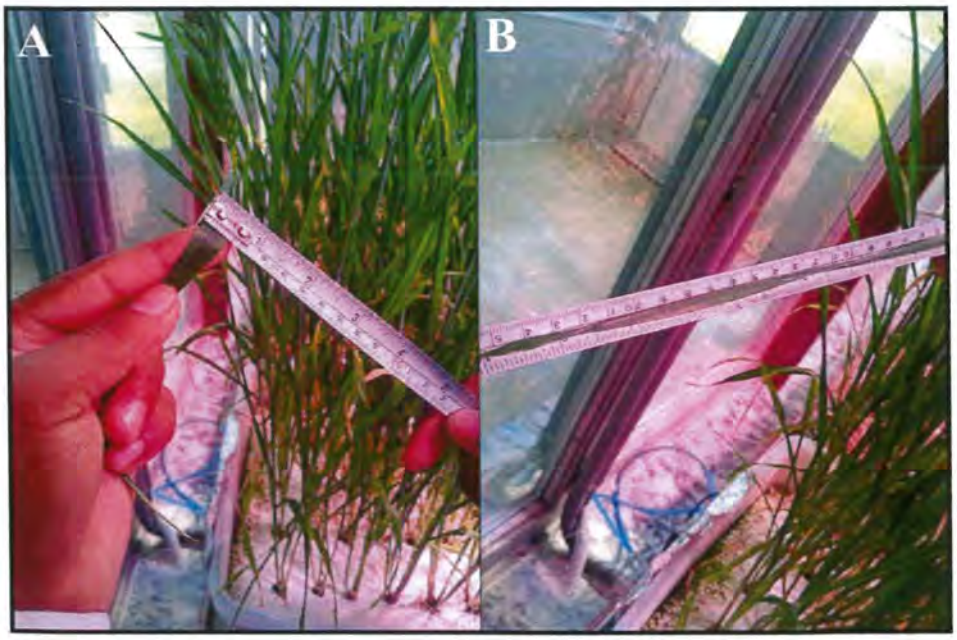

Figure 20. Leaf Area Data Collecting

(A = Leaf Area, B = Leaf Length)

2.5.6. Days to Tillering (DtT)

A shoot that is originated from the coleoptilar node and sharing the same root mass of main tiller that stage of growth is known as tillering. The plants were observed from the germination to the tillering stage and days were recorded.

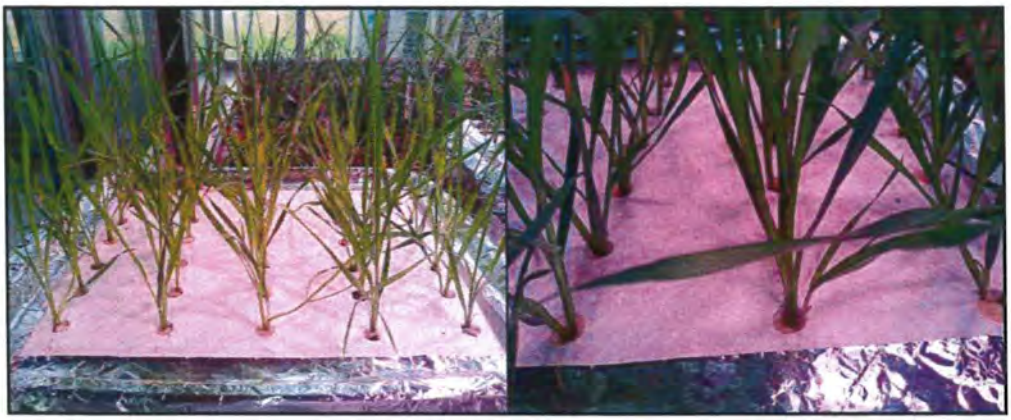

Figure 21. Tillering Stage

2.5.7. Days to Booting (DtB)

A developmental stage where a fully developed covered with leaf sheath below the flag leaf can be easily seen is called as boot stage or booting stage. The days were recorded from germination to the boot stage.

Figure 22. Booting Stage

2.5.8. Days to Heading (DtH)

When the wheat head emergence from the leaf sheath of flag leaf occurs that stage is marked as heading (Figure 23). The days of heading were recorded from the germination in this study.

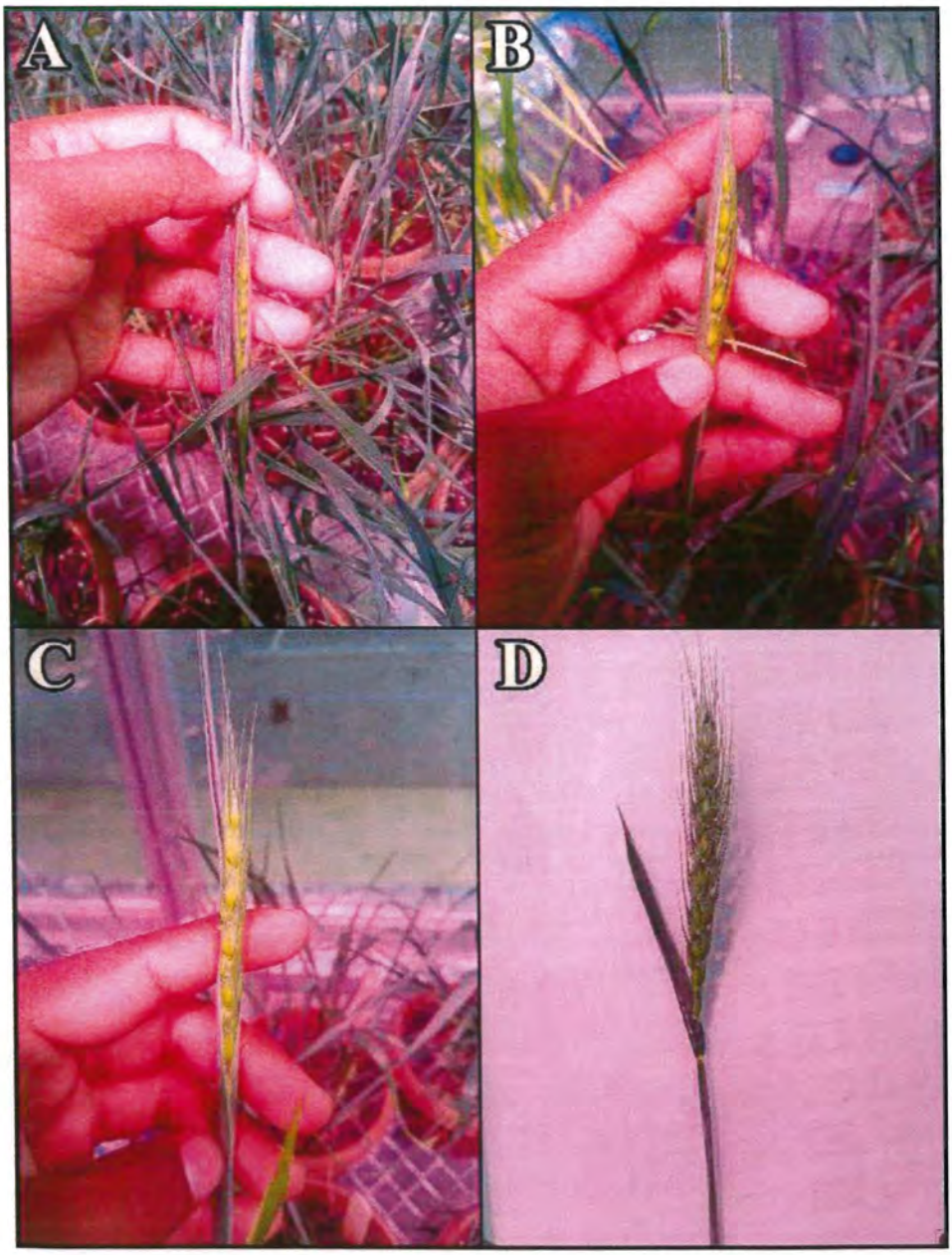

Figure 23. Heading Stage

(A, B=Emergence of Heading, C= partially emerged head C= Full emerged head)

2.5.9. Days to Anthesis (DtA)

When the extrusion of anther from florets is clearly observed in the center of the spike that developmental stage is marked as Anthesis or Flowering (Figure 24). The days from the germination to the anthesis were recorded for all 27 replicates.

Figure 24. Anthesis Stage

2.5.10. No of Tillers (NT)

No of tillers were counted for all replicates at the boot stage (Figure 25).

Figure 25. Data Collecting for No: of Tillers

2.5.11. Spike Length (SpL)

Spike length was measured manually in centimeter (cm) from base to the tip of spike by using the meter tape (Figure 26).

Figure 26. Data Collecting for Spike Length

2.5.12. Spikelets per Spike (SPS)

After anthesis the spikes of each replicate were observed for the counting of spikelets.

Figure 27. Collecting Data for Spikelets per Spike

2.5.13. Root Analysis

The roots of each replicate of treatments from both Pots and Hydroponics system were collected after harvesting the plants. The roots obtained from pots were washed carefully to remove dust. After washing they were left for 1 hour to dry. After drying the were scanned and their analysis for root length (RL), root network area (RtNA) and root tips (RtT) was done by using the RhizoVision Explorer-2.0.3 software (Seethepalli et al., 2021).

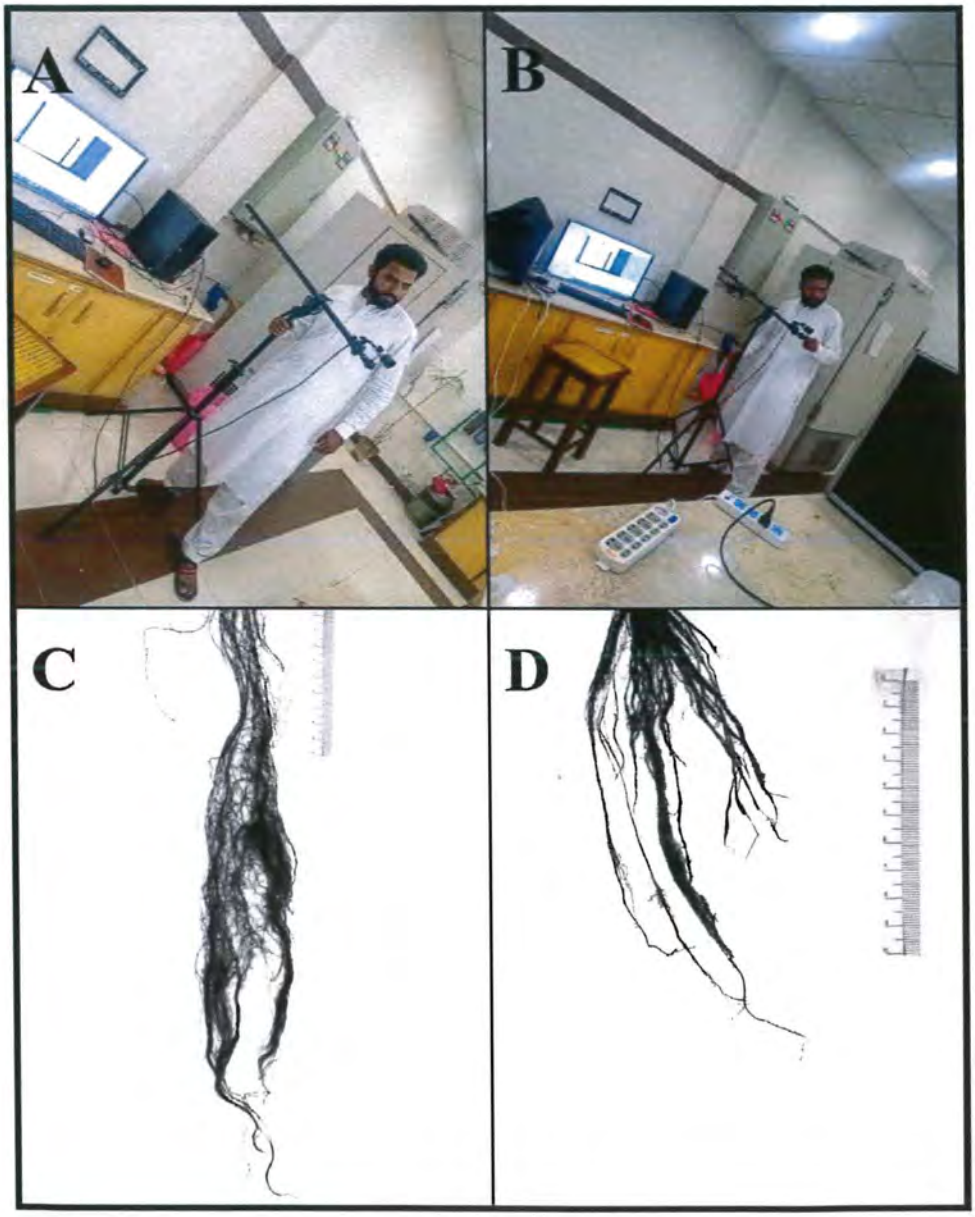

Figure 28. Root Analysis

(A, B= Capturing Root Photos for scanning, C=Hydroponics Root Scan and D= Pots Root Scan)

Chapter 3:

RESULTS AND DISCUSSION

CHAPTER 3: RESULTS AND DISCUSSION

3.1. ANOVA Results

3.1.1. ANOVA for Chlorophyll Content

The chlorophyll content was measured by using Minolta SPAD 502 chlorophyll meter. It was measured after 15 days of germination at the intervals of seven days.

3.1.1.1. ANOVA for Chlorophyll at 15th Day after Germination (Chl_15DAG)

Overall, in Pots experiment, the average value of Chl_15DAG was found to be 45.407 SPAD counts with a minimum value of 40.0 SPAD counts and maximum value of 50.40 SPAD Counts. P-value was found to be non-significant i.e., **p< 0.320** (Table 12). In Hydroponics experiment, the mean value was found to be 40.62 SPAD counts with a minimum value of 35.0 SPAD Counts and maximum value of 44.0 SPAD counts. P-value was found to be highly significant i.e., **p< 0.0001** (Table 12).

In Pots experiment, the minimum average value in replicates of treatments was observed as 44.33 SPAD counts in T_3 and maximum average was observed as 48.1 SPAD counts in T_7 (Figure 29). In Hydroponics experiment the minimum average value in replicates of treatments was observed as 39.2 SPAD counts in T_1 and maximum average was observed as 43.4 SPAD counts in T_8 (Figure 30). There was an increase in CHL_15DAG with comparison of respective control (Hydroponics: 35.6 SPAD counts, Pots: 41.6 SPAD counts) during overall experiment however, the highest mean values were recorded in Pots experiment as compared to Hydroponics experiment (Figure 31).

3.1.1.2. ANOVA for Chlorophyll at 22nd Day after Germination (Chl_22DAG)

The overall mean of Chl_22DAG in Pots experiment was 52.73 SPAD counts, the minimum value was 43.40 SPAD counts and maximum value was 58.20 SPAD counts. The p value of Chl_22DAG in Pots experiment was non- significant (**p< 0.167**) (Table 12) whereas, in Hydroponics experiment the overall mean of Chl_22DAG was 51.96 SPAD counts, the minimum value was 44.3 SPAD counts

and maximum value was 58.2 SPAD counts. The p value of Chl_22DAG in Hydroponics experiment was non-significant (**p< 0.372**) (Table 12).

In Pots experiment the minimum average of Chl_22DAG in replicates of treatments was observed as 49.7 SPAD counts in **T1** and maximum average was observed as 56.66 SPAD counts in **T6** (Figure 29). In Hydroponics experiment the minimum average of Chl_22DAG in replicates of treatments was observed as 49.23 SPAD counts in **T1** and maximum average was observed as 56.6 SPAD counts in **T6** (Figure 30). There was an increase in Chl_22DAG with comparison of respective control (Hydroponics: 51.1 SPAD counts, Pots: 49.4 SPAD counts) during overall experiment however, the highest mean values were recorded in Pots experiment as compared to Hydroponics experiment (Figure 31).

3.1.1.3. ANOVA for Chlorophyll at 29th Day after Germination (Chl_29DAG)

The overall mean of Chl_29DAG in Pots experiment was 55.25 SPAD counts, the minimum value was 47.40 SPAD counts and maximum value was 59.60 SPAD counts. The p value of Chl_29DAG in Pots experiment was significant (**p< 0.036**) whereas, in Hydroponics experiment the overall mean of Chl_29DAG was 55.26 SPAD counts, the minimum value was 48.4 SPAD counts and maximum value was 59.6 SPAD counts. The p value of Chl_29DAG in Hydroponics was non-significant (**p< 0.365**) (Table 12).

In Pots experiment the minimum average of Chl_29DAG in replicates of treatments was observed as 53.63 SPAD counts in **T3** and maximum average was observed as 57.66 SPAD counts in **T6** (Figure 29). In Hydroponics experiment the minimum average of Chl_29DAG in replicates of treatments was observed as 52.46 SPAD counts in **T4** and maximum average was observed as 57.6 SPAD counts in **T6** (Figure 30). There was an increase in Chl_29DAG with comparison of respective control (Hydroponics: 53.00 SPAD counts, Pots: 51.033 SPAD counts) during overall experiment however, the highest mean values were recorded in Hydroponics experiment as compared to Pots experiment (Figure 31).

3.1.1.4. ANOVA for Chlorophyll at 35th Day after Germination (Chl_35DAG)

The overall mean of Chl_35DAG in Pots experiment was 57.28 SPAD counts, the minimum value was 52.00 SPAD counts and maximum value was 63.20 SPAD counts. The p value of Chl_35DAG in Pots experiment was significant (**p< 0.006**) whereas, in Hydroponics experiment the overall mean of Chl_35DAG was 58.24 SPAD counts, the minimum value was 52.6 SPAD counts and maximum value was 62.6 SPAD counts. The p value of Chl_35DAG in Hydroponics was non-significant (**p< 0.096**) (Table 12).

In Pots experiment the minimum average of Chl_35DAG in replicates of treatments was observed as 54.96 SPAD counts in **T_3** and maximum average was observed as 60.56 SPAD counts in **T_6** (Figure 29). In Hydroponics experiment the minimum average of Chl_35DAG in replicates of treatments was observed as 56.06 SPAD counts in **T_4** and maximum average was observed as 60.66 SPAD counts in **T_8** (Figure 30). There was an increase in Chl_35DAG with comparison of respective control (Hydroponics: 55.43 SPAD counts, Pots: 53.2 SPAD counts) during overall experiment however, the highest mean values were recorded in Hydroponics experiment as compared to Pots experiment (Figure 31).

3.1.1.5. ANOVA for Chlorophyll at 42nd Day after Germination (Chl_42DAG)

The overall mean of Chl_42DAG in Pots experiment was 59.41 SPAD counts, the minimum value was 53.30 SPAD counts and maximum value was 65.2 SPAD counts. The p value of Chl_42DAG in Pots experiment was significant (**p< 0.001**) whereas, in Hydroponics experiment the overall mean of Chl_42DAG was 60.37 SPAD counts, the minimum value was 54.6 SPAD counts and maximum value was 66.7 SPAD counts. The p value of Chl_42DAG in Hydroponics was significant (**p< 0.016**) (Table 12).

In Pots experiment the minimum average of Chl_42DAG in replicates of treatments was observed as 57.1 SPAD counts in **T_4** and maximum average was observed as 63.06 SPAD counts in **T_8** (Figure 29). In Hydroponics experiment the minimum average of Chl_42DAG in replicates of treatments was observed as 57.8 SPAD counts in **T_4** and maximum average was observed as 62.66 SPAD counts in **T_6** (Figure 30). There was an increase in Chl_42DAG with comparison of respective

control (Hydroponics: 56.26 SPAD counts, Pots: 54.36 SPAD counts) during overall experiment however, the highest mean values were recorded in Hydroponics experiment as compared to Pots experiment (Figure 31).

3.1.1.6. ANOVA for Chlorophyll at 49th Day after Germination (Chl_49DAG)

The overall mean of Chl_49DAG in Pots experiment was 60.55 SPAD counts, the minimum value was 55.10 SPAD counts and maximum value was 67.00 SPAD counts. The p value of Chl_49DAG in Pots experiment was highly significant (**p< 0.000**) whereas, in Hydroponics experiment the overall mean of Chl_49DAG was 62.1 SPAD counts, the minimum value was 56.7 SPAD counts and maximum value was 68.9 SPAD counts. The p value of Chl_49DAG in Hydroponics was non-significant (**p< 0.032**) (Table 12).

In Pots experiment the minimum average of Chl_49DAG in replicates of treatments was observed as 58.1 SPAD counts in T_4 and maximum average was observed as 64.43 SPAD counts in T_8 (Figure 29). In Hydroponics experiment the minimum average of Chl_49DAG in replicates of treatments was observed as 59.3 SPAD counts in T_4 and maximum average was observed as 65.1 SPAD counts in T_8 (Figure 30). There was an increase in Chl_49DAG with comparison of respective control (Hydroponics: 58.16 SPAD counts, Pots: 55.4 SPAD counts) during overall experiment however, the highest mean values were recorded in Hydroponics experiment as compared to Pots experiment (Figure 31).

3.1.2. ANOVA for Shoot Length (SL)

The Shoot length was measured at 20th day after germination at the interval of seven days.

3.1.2.1. ANOVA for Shoot length at 20th Day after Germination (SL_20DAG)

The mean value for SL_20DAG in Pots experiment was calculated as 36.83 cm whereas, the minimum SL_20DAG was observed as 34.2 cm and the maximum was 40.1 cm. The p value of SL_20DAG was non-significant (**p <0.329**). In Hydroponics experiment the mean value for SL_20DAG in was calculated as 25.37 cm whereas, the minimum SL_20DAG was observed as 23 cm and the maximum was 29.5 cm. The p value of SL_20DAG was non-significant (**p <0.960**) (Table 12).

In Pots experiment the decrease in the average of SL_20DAG was observed in T_1, T_2, T_3, T_4, T_5 and T_7 however, the increase the average of SL_20DAG was observed in T_6 and T_8 as compared to the control (Figure 32). The minimum average of SL-20DAG in replicates of treatments was observed as 35.533 cm in T_3 and maximum average was observed as 38.23 cm in T_6. In Hydroponics experiment the decrease in SL_20DAG was observed in T_2, T_3, T_4, T_6, T_7 and T_8 however, the increase in SL_20DAG was observed in T_1 and T_5 as compared to the control. The minimum average of SL-20DAG in replicates of treatments was observed as 24.5 cm in T_7 and maximum average was observed as 26.26 cm in T_5 (Figure 30). There was a decrease in SL-20DAG with comparison of respective control during overall experiment however, the highest mean values were recorded in Pots experiment as compared to Hydroponics experiment (Figure 34).

3.1.2.2. ANOVA for Shoot length at 27th Day after Germination (SL_27DAG)

The mean value for SL_27DAG in Pots experiment was calculated as 40 cm whereas, the minimum SL_27DAG was observed as 36.7 cm and the maximum was 45.5 cm. The p value of SL_27DAG was non-significant (**p <0.265**). In Hydroponics experiment the mean value for SL_27DAG was calculated as 33.57 cm whereas, the minimum SL_27DAG was observed as 29.2 cm and the maximum was 38 cm. The p value of SL_27DAG was non-significant (**p <1.000**) (Table 12).

In Pots experiment the decrease in the average of SL_27DAG was observed in T_3, T_4 and T_7 however, the increase in the average of SL_27DAG was observed in T_1, T_2, T_5, T_6 and T_8 as compared to the control (Figure 32). The minimum average of SL_27DAG in replicates of treatments was observed as 37.96 cm in T_4 and maximum average was observed as 42.33 cm in T_6. In Hydroponics experiment the decrease in SL_27DAG was observed in T_1, T_2, T_4, T_6 T_7 and T_8 however, the increase in SL_27DAG was observed in T_5 as compared to the control. The minimum average of SL_27DAG in replicates of treatments was observed as 33.03 cm in T_8 and maximum average was observed as 34.03 cm in T_5 (Figure 30). There was a decrease in SL_27DAG with comparison of respective control during overall experiment in few treatments as mentioned above however, the highest mean values

were recorded in Pots experiment as compared to Hydroponics experiment (Figure 34).

3.1.2.3. ANOVA for Shoot length at 34th Day after Germination (SL_34DAG)

The mean value for SL_34DAG in Pots experiment was calculated as 41.63 cm whereas, the minimum SL_34DAG was observed as 38 cm and the maximum was 47.6 cm. The p value of SL_34DAG was non-significant (**p <0.153**). In Hydroponics experiment the mean value for SL_34DAG was calculated as 39.39 cm whereas, the minimum SL_34DAG was observed as 34.4 cm and the maximum was 43 cm. The p value of SL_34DAG was non-significant (**p <0.972**) (Table 12).

In Pots experiment the decrease in the average of SL_34DAG of replicates in treatments was observed in **T_2**, **T_3**, **T_4** and **T_7** however, the increase in the average of SL_34DAG of replicates in treatments was observed in **T_1**, **T_5**, **T_6** and **T_8** as compared to the control (Figure 32). The minimum average of SL_34DAG in replicates of treatments was observed as 39.46 cm in **T_4** and maximum average was observed as 43.86 cm in **T_8**. In Hydroponics experiment the decrease in SL_34DAG was observed in **T_1**, **T_2**, and **T_3** however, the increase in SL_34DAG was observed in **T4, T_5 T_6 T_7** and **T_8** as compared to the control. The minimum average of SL_34DAG in replicates of treatments was observed as 38.5 cm in **T_3** and maximum average was observed as 40.53 cm in **T_5** (Figure 30). There was a decrease in SL_34DAG of above mentioned treatments with comparison of respective control during overall experiment however, the highest mean values were recorded in Pots experiment as compared to Hydroponics experiment (Figure 34).

3.1.2.4. ANOVA for Shoot length at 41th Day after Germination (SL_41DAG)

The mean value for SL_41DAG in Pots experiment was calculated as 43.607 cm whereas, the minimum SL_41DAG was observed as 39.1 cm and the maximum was 50 cm. The p value of SL_41DAG was significant (**p <0.014**). In Hydroponics experiment the mean value for SL_41DAG was calculated as 45.967 cm whereas, the minimum SL_41DAG was observed as 39 cm and the maximum was 51 cm. The p value of SL_41DAG was non-significant (**p <0.820**) (Table 12).

In Pots experiment the decrease in the average of SL_41DAG of replicates in treatments was observed in **T2**, **T3**, **T4** and **T8** however, the increase in the average of SL_41DAG in replicates of treatments was observed in **T1**, **T5**, **T6** and **T7** as compared to the control (Figure 32). The minimum average of SL_41DAG in replicates of treatments was observed as 40.867 cm in **T4** and maximum average was observed as 47.567 cm in **T6**. In Hydroponics experiment the decrease in SL_41DAG was observed in only **T7** however, the increase in SL_41DAG was observed in **T1**, **T2**, **T3**, **T4**, **T5** and **T8** as compared to the **T6** and control. The minimum average of SL_41DAG in replicates of treatments was observed as 45.167 cm in **T6** and maximum average was observed as 48.733 cm in **T1** (Figure 30). There was a decrease in SL_41DAG of above mentioned treatments with comparison of respective control during overall experiment however, the highest mean values were recorded in Hydroponics experiment as compared to Pots experiment (Figure 34).

3.1.2.5. ANOVA for Shoot length at 47th Day after Germination (SL_47DAG)

In Pots experiment the mean value for SL_41DAG was calculated as 49.407 cm whereas, the minimum SL_41DAG was observed as 41.5 cm and the maximum was 58 cm. The p value of SL_41DAG was significant (**p <0.022**). In Hydroponics experiment the mean value for SL_41DAG was calculated as 57.767 cm whereas, the minimum SL_41DAG was observed as 50.5 cm and the maximum was 64.5 cm. The p value of SL_41DAG was non-significant (**p <0.350**) (Table 12).

In Pots experiment the decrease in the average of SL_41DAG of replicates in treatments was observed in **T3**, and **T4** however, the increase in the average of SL_41DAG in replicates of treatments was observed in **T1**, **T5**, **T6**, **T7** and **T8** as compared to the **T2** and control (Figure 32). The minimum average of SL_41DAG in replicates of treatments was observed as 45.167 cm in **T4** and maximum average was observed as 55.33 cm in **T1**. In Hydroponics experiment the decrease in SL_41DAG was observed in only **T2** **T7** and **T8** however, the increase in SL_41DAG was observed in **T1**, **T3**, **T4**, **T5** and **T6** as compared to the control. The minimum average of SL_41DAG in replicates of treatments was observed as 53.5 cm in **T7** and maximum average was observed as 61.267 cm in **T1** (Figure 30). There was a decrease in SL_41DAG of above mentioned treatments with comparison of

respective control during overall experiment however, the highest mean values were recorded in Hydroponics experiment as compared to Pots experiment (Figure 34).

3.1.3. ANOVA for Flag Leaf Area (LA)

Flag leaf areas was measured at the booting heading and anthesis stage in overall experiment. The measurement was done by the using the meter tape.

3.1.3.1. ANOVA for Flag Leaf Area at Boot Stage (LA_Boot)

In Pot experiment the mean value was 9.142 with 6.75 cm minimum and 12.4 cm maximum LA, p value was observed as non-significant (**p <0.337**). In Hydroponics experiment the mean value was observed as 10.86 with 8.1 cm minimum and 13.5 cm maximum LA, p value was non-significant (**p <0.406**) (Table 12).

The LA was observed increased with comparison of control in overall experiment. The largest LA was observed as 10.8 cm in T_7 of Pots experiment (Figure 35) and 11.96 cm in T_1 of Hydroponics experiment (Figure 36). In overall experiment the highest values for LA were observed in Hydroponics experiment as compared to Pots experiment (Figure 37).

3.1.3.2. ANOVA for Flag Leaf Area at Heading Stage (LA_Head)

The mean value was 10.467 with 8.1 cm minimum and 12.9 cm maximum LA in Pots experiment, p value was observed as non-significant (**p <0.221**). In Hydroponics experiment the mean value was observed as 19.496 with 15.2 cm minimum and 22 cm maximum LA, p value was significant (**p <0.003**) (Table 12).

The LA was observed increased with comparison of control in overall experiment. The largest LA was observed as 11.867 cm in T_7 of Pots experiment (Figure 35) and 21.667 cm in T_6 of Hydroponics experiment (Figure 36). In overall experiment the highest values for LA were observed in Hydroponics experiment as compared to Pots experiment (Figure 37).

3.1.3.3. ANOVA for Flag Leaf Area at Anthesis Stage (LA_Anth)

In Pot experiment the mean value was 11.852 with 8.9 cm minimum and 14.3 cm maximum LA, p value was observed as non-significant (**p <0.129**). In

Hydroponics experiment the mean value was observed as 10.8624.619 with 20 cm minimum and 27.5 cm maximum LA, p value was non-significant (**p <0.000**) (Table 12).

The LA was observed increased with comparison of control in overall experiment. The largest LA was observed as 10.8 cm in T_7 of Pots experiment (Figure 35) and 11.96 cm in T_1 of Hydroponics experiment (Figure 36). In overall experiment the highest values for LA were observed in Hydroponics experiment as compared to Pots experiment (Figure 37).

3.1.4. ANOVA for No of Tillers per Plant (T/P)

The mean value in Pot experiment was 3.556 with 2 minimum and 5 maximum T/P and the value was non-significant (**p <0.171**) in Pots experiment. In hydroponics the mean value was 6.667 with minimum 3 and maximum 11 T/P, the p value was significant i.e. (**p <0.021**) (Table 12).

In Pots experiment the highest number of T/P was marked as 5 in T_2 and T_4 and lowest number of T/P was marked as 2 in control (Figure 38). In Hydroponics experiment the highest number of T/P was marked as 11 in T_5 and lowest number of T/P was marked as 3 in T_4. (Figure 39) The highest number of T/P was observed in Hydroponics and compared to Pots experiment (Figure 40).

3.1.5. ANOVA for Spike Length (SpL)

The mean value for SpL was 10.544 with 9.5 cm minimum 11.2 cm maximum in Pots experiment, the p value was significant (**p <0.001**). In Hydroponics experiment the mean value was 10.8 with 9.5 cm minimum and 11.5 cm maximum SpL, the p value was significant (**p <0.001**) (Table 12).

In Pots the largest SpL (11.2 cm) was observed in T_3, T_5 and the smallest SpL was observed in 9.5 in control (Figure 41). In Hydroponics the largest SpL (11 cm) was observed in T_3, T_5, and T_7 and the smallest SpL (9.5 cm) in T_8 (Figure 42). In overall experiment the largest spike was observed in Hydroponics experiment as compared to the Pots experiment (Figure 43).

3.1.6. ANOVA for Spikelets per Spike (Spl/S)

The mean value in Pots experiment was 16.333 with minimum 15 and maximum 17 Spl/S, the p value was significant (**p <0.005**). In Hydroponics the mean value was 17.592 with minimum 15 and maximum 19 Spl/S, the p value was significant (**p <0.001**) (Table 12).

In Pots experiment the largest number of Spl/S was observed as 17 in **T_1**, **T_2**, **T_3**, **T_4**, **T_5**, **T_6**, and **T_7** whereas, the smallest number of Spl/S was observed as 15 in control and **T_8** (Figure 44). The highest number of Spl/S in Hydroponics experiment was observed as 19 in **T_1**, **T_2**, **T_3**, **T_4**, **T_5**, **T_6**, and **T_7** however the lowest number of Spl/S was observed as 15 in **T_8** (Figure 45). In overall experiment the highest values of Spl/S were observed in Hydroponics experiment as compared to the Pots experiment (Figure 46).

3.1.7. ANOVA for Fresh Weight (FW)

The mean value in Pots experiment was marked as 15.89 with minimum 12.29 g and maximum 18.71 g FW, the p value was significant (**p <0.007**). In Hydroponics the mean value was observed as 32.22 and the minimum 18.45 g and maximum 55.7 g FW was noted, the p value was non-significant (**p<0.415**) (Table 12).

The lowest average value of FW in Pots experiment was observed 12.98 g in control and 13.453 g **T_8** and the highest average value for experiment was observed 17.393 g in **T_5** (Figure 47). In Hydroponics experiments the lowest average value for FW was observed 23.17 g in **T_4** and the highest average value was observed 39.83 g in **T_6**. (Figure 48). In overall experiment the highest values of FW were observed in Hydroponics experiment as compared to the Pots experiment (Figure 49).

3.1.8. ANOVA for Dry Weight (DW)

The mean value in Pots experiment was marked as 8.734 with minimum 5.41 g and maximum 10.6 g DW, the p value was non-significant (**p <0.053**). In Hydroponics the mean value was observed as 18.34 and the minimum 10.5 g and maximum 29.6 g DW was noted, the p value was non-significant (**p<0.510**) (Table 12).

The lowest average value of DW in Pots experiment was observed 6.57 g in control and 8.03 g **T_8** and the highest average value for experiment was observed 9.7 g in **T_6** (Figure 50). In Hydroponics experiments the lowest average value for DW was observed 13.2 g in **T_4** and the highest average value was observed 21.8 g in **T_6** (Figure 51). In overall experiment the highest values of DW were observed in Hydroponics experiment as compared to the Pots experiment (Figure 52).

3.1.9. ANOVA for Root Parameter

The root analysis was done by using the RhizoVision Explorer-2.0.3 for the measurement of root length, root network area and number of root tips (Seethepalli et al., 2021).

3.1.9.1. ANOVA for Root Length (RL)

The mean value in Pots experiment was observed as 19.39 with minimum 13.34 cm and maximum 29.44 cm root length, the p value was highly significant (**$p<0.0001$**). In Hydroponics the mean value was marked as 45.28 with minimum 30.13 cm and maximum 65.87 cm RL, the p value was highly significant (**$p<0.0001$**) (Table 12).

Between the treatments of Pots experiment the highest average of RL was noted in **T_2** (27.57 cm) and the lowest average was observed in **T_8** (16.84 cm) (Figure 53). In Hydroponics experiment the highest average value for RL was observed in **T_6** (64.38 cm) and the lowest average value was observed in **T_4** (35.357 cm) (Figure 54). The overall highest values of RL were observed in Hydroponics as compared to the Pots experiment (Figure 55).

3.1.9.2. ANOVA for Root Tips (RtT)

The mean value in Pots experiment was observed as 912.33 with minimum 337.45 and maximum 1319.2 root tips, the p value was significant (**$p<0.013$**). In Hydroponics the mean value was marked as 2125.51 with minimum 684.32 and maximum 3361.9 RtT, the p value was significant (**$p<0.003$**) (Table 12).

Between the treatments of Pots experiment the highest average RtT was noted in **T_2** (1198.783) and the lowest average was observed in **T_8** (646.850) (Figure

56). In Hydroponics experiment the highest average value for RtT was observed in T_1 (3134.483) and the lowest average value was observed in T_4 (1300.563) (Figure 57). The overall highest values of RtT were observed in Hydroponics as compared to the Pots experiment (Figure 58).

3.1.9.3. ANOVA for Root Network Area (RtNA)

The mean value in Pots experiment was observed as 25.732 with minimum 11.721 and maximum 37.01 root tips, the p value was non-significant (**p<0.094**). In Hydroponics the mean value was marked as 134.674 with minimum 89.61 and maximum 195.91 RtNA, the p value highly significant (**p<0.0001**) (Table 12).

Between the treatments of Pots experiment the highest average RtNA was noted in T_3 (30.935) and the lowest average was observed in T_8 (16.649) (Figure 59). In Hydroponics experiment the highest average value for RtNA was observed in T_1 (191.483) and the lowest average value was observed in T_4 (105.160) (Figure 60). The overall highest values of RtNA were observed in Hydroponics as compared to the Pots experiment (Figure 61).

3.1.10. ANOVA for Days to Tillering (DtT)

The mean value for DtT was observed 14.889 with minimum 14 and maximum 18 days in Pots experiment and the p value was significant (**p<0.001**), while in hydroponics the mean value was observed 12.852 with minimum 12 and maximum 14 days and the p value was significant (**p<0.036**) (Table 12).

In pots experiment the minimum average days were marked in **T_4** (14 days) and maximum average days were marked in **T_3** and **T_5** (15 days), in control the average days to tillering were 17.33 (Figure 62). In Hydroponics experiment the minimum average days were marked in and **T_2** (12 days) and maximum average days were marked in **T_6** and **T_7** (13.33), in control the average days to tillering were 13.667 (Figure 63). In overall experiment the tillering was observed earlier in Hydroponics than Pots experiment at the difference of almost 2 days (Figure 64).

3.1.11. ANOVA for Days to Booting (DtB)

The mean value for DtB was observed 34.852 with minimum 31 and maximum 38 days in Pots experiment and the p value was highly significant (**p<0.0001**), while in hydroponics the mean value was observed 31.889 with minimum 29 and maximum 35 days and the p value was highly significant (**p<0.0001**) (Table 12).

In pots experiment the minimum average days were marked in **T6** (32 days) and maximum average days were marked in **T8** (37.333 days), in control the average days to booting were 36.667 (Figure 62). In Hydroponics experiment the minimum average days were marked in **T6** (29.667 days) and maximum average days were marked in **T8** (34.333), in control the average days to booting were 33.333 (Figure 63). In overall experiment the booting was observed earlier in Hydroponics experiment as compared to the Pots experiment (Figure 64).

3.1.12. ANOVA for Days to Heading (DtH)

The mean value for DtH was observed 39.556 with minimum 36 and maximum 46 days in Pots experiment and the p value was highly significant (**p=0.0001**), while in hydroponics the mean value was observed 35.37 with minimum 31 and maximum 42 days and the p value was highly significant (**p<0.0001**) (Table 12).

In pots experiment the minimum average days were marked in **T6** (36.333 days) and maximum average days were marked in **T8** (44.667 days), in control the average days to heading were 41.667 (Figure 62). In Hydroponics experiment the minimum average days were marked in and **T6** (31.667 days) and maximum average days were marked in **T8** (41 days), in control the average days to heading were 37 (Figure 63). In overall experiment the heading was observed earlier in Hydroponics experiment as compared to the Pots experiment (Figure 64).

3.1.13. Days to Anthesis (DtA)

The mean value for DtA was observed 46.963 with minimum 45 and maximum 52 days in Pots experiment and the p value was significant (**p=0.002**),

while in hydroponics the mean value was observed 39.185 with minimum 35 and maximum 46 days and the p value was highly significant (**p<0.0001**) (Table 12).

In pots experiment the minimum average days were marked in T_6 (45.333 days) and maximum average days were marked in T_8 and control (49.667 days) (Figure 62). In Hydroponics experiment the minimum average days were marked in and T_6 (35.333 days) and maximum average days were marked in T_8 (45.667), in control the average days to anthesis were 43 (Figure 63). In overall experiment the anthesis was observed 10 days earlier in Hydroponics experiment as compared to the Pots experiment (Figure 64).

3.1.14. ANOVA for Days to Maturity (DtM)

The mean value for DtA was observed 62.667 with minimum 57 and maximum 69 days in Pots experiment and the p value was significant (**p<0.001**), while in hydroponics the mean value was observed 53.704 with minimum 48 and maximum 62 days and the p value was highly significant (**p<0.0001**) (Table 12).

In pots experiment the minimum average days were marked in T_6 (58.33 days) and maximum average days were marked in T_8 (66.667 days) and in control the average value was (66 days) (Figure 62). In Hydroponics experiment the minimum average days were marked in and T_6 (49 days) and maximum average days were marked in T_8 (66.333), in control the average days to maturity were 58 (Figure 63). In overall experiment the maturity was observed 9 days earlier in Hydroponics experiment as compared to the Pots experiment (Figure 64).

3.2. Correlation Results

Correlation test provide information about significance of any trait. It was calculated for determining traits. The results of correlation are as follow.

Chlorophyll showed positive correlation with shoot length, leaf area, spike length, spikelet per spike, root length and fresh weight at r values 0.375, 0.313, 0.241, 0.151, 0.381 and 0.317. It showed negative correlation with days to maturity at r value 0.233. It sowed highly significance shoot length and root length (Table 13).

Shoot length showed positive correlation with chlorophyll, leaf area, spike length, spikelets per spike, and fresh weight at r value 0.375, 0.746, 0.139, 0.420,

0.650 and 0.597. The days to maturity were observed negatively correlated at r= 0.651. Shoot length significantly correlated with chlorophyll, leaf area, and spikelets per spike, root length and fresh weight (Table 13).

Leaf area showed positive correlation with chlorophyll, shoot length, spike length, spikelets per spike, root length and fresh weight at r value 0.313, 0.746, 0.340, 0.543, 0.857 and 0.782 respectively. It showed negative correlation with days to maturity at r= 0.881. Leaf area was significantly correlated with, shoot length, spikelets per spike, root length and fresh weight (Table 13).

Spike length showed positive correlation with chlorophyll, shoot length, leaf area, spikelets per spike, root length and fresh weight at r value 0.241, 0.139, 0.340, 0.749, 0.273, 0.294. It showed negative correlation with days to maturity at r = 0.531. It was significantly correlated with spikelets per spike (Table 13).

Spikelets per spike showed positive correlation with chlorophyll, shoot length, leaf area, spike length, root length and fresh weight at r value 0.151, 0.420, 0.543, 0.749, 0.474 and 0.353. It showed negative correlation with days to maturity at r = 0.655. It is significantly correlated with shoot length, leaf area, spike length, root length and fresh weight (Table 13).

Days to maturity are negatively correlated with chlorophyll, shoot length, leaf area, spike length, spikelets per spike, root length and fresh weight at r value 0.233, 0.651, 0.881, 0.531, 0.655, 0.819 and 718. It is correlated significantly with shoot length, leaf area, spike length, spikelets per spike, root length and fresh weight (Table 13).

Root length is positively correlated with chlorophyll, shoot length, leaf area, spike length, spikelets per spike and fresh weight at r value 0.381, 0.650, 0.856, 0.273, 0.474 and 0.753. It showed negative correlation with days to maturity at r = 0.818. It showed highly significant correlation with chlorophyll, shoot length, leaf area, spike length, and fresh weight (Table 13).

Fresh weight is positively correlated with chlorophyll, shoot length, leaf area, spike length, spikelets per spike and root length at r value 0.317, 0.597, 0.782, 0.294, 0.353 and 0.753 and it is negatively correlated with days to maturity at r =

0.718. It showed highly significant correlation with shoot length, leaf area, spikelets per spike and root length (Table 13).

3.3. Discussion

Rapidly increasing human population and climate change have upraised important concern for world food security, the present development rate of many food crops is insufficient to meet upcoming food demands (Ray et al., 2013). According to (Hovmøller et al., 2016) in recent past, very aggressive pathotypes with adaptation to little higher temperatures have evolved from the Himalaya areas in Asia, which threaten wheat crop production in Indus-Ganga plains. Higher rust disease pressures can cause 50 to 70% grain yield losses in both bread and durum wheats. The 80 percent of global wheat cultivars are extensively affected by different pathotypes (Sharma-Poudyal et al., 2014; Yu et al., 2012). In this context, the new cultivars of wheat with high resistance and tolerance are needed to be developed rapidly. Development of cultivars combining valuable traits for rapidly emerging environmental changes and disease pathogens is essential to withstand food production demands.

Newly established speed breeding technique is capable of fast generation development under controlled and light-emitting diode (LED) supplemented glasshouse. The rapid development of wheat is achieved under speed breeding glass house at Speed breeding facility CSI NARC Islamabad. At the speed breeding platform the wheat is matured in 64 days and almost six generations are developed in a year. The main focus of ours study was to achieve more than six generations by adopting the different growth medium techniques i.e. Hydroponics and Pots System along with hormonal and nanoparticle treatments. Two systems were developed for the growth of wheat under the treatment of Auxin, gibberellin, MnO_2 NPs and $MnCl_2$ with two concentration of each (Table 10).

In pots system the soil and peat moss was as used as growth medium however, in hydroponics system the nutrient solution was used by following Hoagland recipe that is mentioned in Table 5. The treatments were applied as foliar spray on four developmental stages as listed in Table 11. Both Systems performed effectively to grow faster and healthier plants under SB platform.

Plants were observed regularly for the collection of data mainly the counting of days from germination to different developmental stages. As our main focus was to compare the pots and hydroponics system under speed breeding, we observed a huge difference in both mediums growing the wheat. A distinct difference was noted in each trait analyzed during this study as discussed in the chapter of results. The plants that were grown hydroponically having the high chlorophyll content at maturity, plant height, leaf area, tiller per plant, spike length, spikelets per spike, root length, root tips, root network area, fresh weight and dry weight also having greater numbers than plants grown in pots.

The development stages were also noted at key differences. In hydroponics the tillering was observed two days earlier, the booting was also noted earlier at the difference of three days, the heading was marked five day before the plants growing in pots, the anthesis was decreased to 10 days as compared to the pots system, the plants were fully matured nine days earlier in hydroponics system that is remarkable achievement.

Chapter 4:

CONCLUSION AND FUTURE PERSPECTIVE

CHAPTER 4: CONCLUSION AND FUTURE PERSPECTIVE

Any crop breeding program needs several plant generations to be generated and assessed before a variety is registered. This prolonged and extensive procedure is prerequisite to evaluate and observe various crop traits of our choice. The speed breeding method saves breeding time and resources through rapid generation advancement. Various selection methods can be integrated into speed breeding, such as the single seed descent (SSD), single pod descent (SPD), single plant selection (SPS), clonal selection and marker-assisted selection (MAS) to shorten the breeding cycle and for efficient resource use.

Time in breeding programs is a key factor. Shortening plant cycles allows to increase the efficiency of the programs. The speed breeding approach was executed under LED supplemented light and controlled temperature at Speed breeding facility of CSI NARC Islamabad obtaining almost 6 generations in a year. The wheat crop is matured in almost 60-64 days by growing in pots using specialized peat moss. This research work was established to adopt hydroponics system and pots system to evaluate the speedy growth of wheat under some foliar application of hormones and nanoparticles (Table 10). Our results through SB glasshouse following hydroponics protocol, depicted fast growth of wheat as compared to the pots system. We found that the plants that are grown hydroponically got matured in 48 days however the plants that are grown in pots got matured in 57 under SB conditions. Not only days were decreased for maturity in our study but also we noted extensive improvement in different traits i.e. chlorophyll content, shoot length, leaf area, number of tillers, spike length, spikelets per spike etc.

Many researchers have found that hydroponic plants grow 30% to 50% faster than a plant grown in soil. The ample amount of oxygen in a hydroponics system allows the roots to absorb nutrients much faster, stimulating root and shoot growth. The results of our study shows that hydroponics can be proved and other tool that can hasten the generation cycle.

SUPPLIMENTARY MATERIAL

ANOVA P-Table

Table 12. P-Table of Phenotypic Traits

SERIAL No & TRAITS		POTS				HYDROPONICS			
S No:	Variable	Min	Max	Mean±SD	Pr > F(T)	Min	Max	Mean±SD	Pr > F(T)
1	Chl_15DAG	40	50.4	45.407±3.527	0.32	35	44	40.62±2.31	**<0.0001**
2	Chl_22DAG	43.4	58.2	52.73±3.585	0.167	44.3	58.2	51.96±3.66	0.372
3	Chl_29DAG	47.4	59.6	55.259±3.031	**0.036**	48.4	59.6	55.26±3.13	0.365
4	Chl_35DAG	52	63.2	57.285±3.227	**0.006**	52.6	62.6	58.24±2.81	0.096
5	Chl_42DAG	53.5	65.2	59.415±3.023	**0.001**	54.6	66.7	60.37±2.75	**0.016**
6	Chl_49DAG	55.1	67	60.556±2.929	**0**	56.7	68.9	62.1±2.8	**0.032**
7	SL_Day20	34.2	40.1	36.83±1.536	0.329	23	29.5	25.37±1.723	0.96
8	SL_Day27	36.7	45.5	40.059±2.16	0.265	29.2	38	33.57±1.723	1
9	SL_Day34	38	47.6	41.63±2.16	**0.153**	34.4	43	39.393±1.824	**0.972**
10	SL_Day41	39.1	50	43.607±2.637	**0.014**	39	51	45.967±3.218	0.82
11	SL_Day48	41.5	58	49.407±4.215	**0.022**	50.5	64.5	57.767±3.401	0.35
12	LA_Boot	6.75	12.4	9.142±1.46	0.337	8.1	13.5	10.861±1.538	0.406
13	LA_Head	8.1	12.9	10.467±1.43	0.221	15.2	22	19.496±1.881	**0.003**
14	LA_Anth	8.9	14.3	11.852±1.773	0.129	20	27.5	24.619±1.93	**0**
15	T/P	2	5	3.556±0.751	0.171	3	11	6.667±1.941	**0.021**
16	SpL	9.5	11.2	10.544±0.537	**0.001**	9.5	11.5	10.8±0.542	**0.001**
17	Spl/S	15	17	16.333±0.961	**0.005**	15	19	17.592±1.359	**0.001**
18	FW	12.29	18.71	15.89±1.886	**0.007**	18.45	55.7	32.22±10.198	0.415
19	DW	5.41	10.6	8.734±1.253	0.053	10.5	29.6	18.38±5.172	0.51
20	RL (cm)	13.34	29.44	19.39±3.67	**<0.0001**	30.13	65.87	45.28±11.965	**<0.0001**
21	RtTips	337.45	1319.2	912.33±263.64	**0.013**	684.32	3361.9	2125.51±263.64	**0.003**
22	RtNA (cm^2)	11.721	37.01	25.732±6.899	0.094	89.61	195.91	134.674±6.899	**<0.0001**
23	DtT	14	18	14.889±1.086	**0.001**	12	14	12.885±0.864	**0.069**
24	DtB	31	38	34.852±1.657	**<0.0001**	29	35	31.962±1.612	**0**
25	DtH	36	46	39.556±2.764	**<0.0001**	31	42	35.423±2.701	**<0.0001**
26	DtA	45	52	46.963±1.629	**0.002**	35	46	39.231±3.266	**<0.0001**
27	DtM	57	69	62.667±2.869	**0.001**	48	62	53.731±3.505	**<0.0001**

Pearson Correlation Results Table

Table 13. Correlation Table

		Chl_DAG49	SL_Day48	LA_Anth	SpL	Spl/S	DtM	RL (cm)	FW
Chl_DAG49	Pearson Correlation	1	.375**	.313*	0.241	0.151	-0.233	.381**	.317*
	Sig. (2-tailed)		0.005	0.021	0.079	0.275	0.090	0.004	0.019
	N	54	54	54	54	54	54	54	54
SL_Day48	Pearson Correlation	.375**	1	.746**	0.139	.420**	-.651**	.650**	.597**
	Sig. (2-tailed)	0.005		0.000	0.317	0.002	0.000	0.000	0.000
	N	54	54	54	54	54	54	54	54
LA_Anth	Pearson Correlation	.313*	.746**	1	.340*	.543**	-.881**	.857**	.782**
	Sig. (2-tailed)	0.021	0.000		0.012	0.000	0.000	0.000	0.000
	N	54	54	54	54	54	54	54	54
SpL	Pearson Correlation	0.241	0.139	.340*	1	.749**	-.531**	.273*	.294*
	Sig. (2-tailed)	0.079	0.317	0.012		0.000	0.000	0.046	0.031
	N	54	54	54	54	54	54	54	54
Spl/S	Pearson Correlation	0.151	.420**	.543**	.749**	1	-.655**	.474**	.353**
	Sig. (2-tailed)	0.275	0.002	0.000	0.000		0.000	0.000	0.009
	N	54	54	54	54	54	54	54	54
DtM	Pearson Correlation	-0.233	-.651**	-.881**	-.531**	-.655**	1	-.819**	-.718**
	Sig. (2-tailed)	0.090	0.000	0.000	0.000	0.000		0.000	0.000
	N	54	54	54	54	54	54	54	54
RL (cm)	Pearson Correlation	.381**	.650**	.857**	.273*	.474**	-.819**	1	.753**
	Sig. (2-tailed)	0.004	0.000	0.000	0.046	0.000	0.000		0.000
	N	54	54	54	54	54	54	54	54
FW	Pearson Correlation	.317*	.597**	.782**	.294*	.353**	-.718**	.753**	1
	Sig. (2-tailed)	0.019	0.000	0.000	0.031	0.009	0.000	0.000	
	N	54	54	54	54	54	54	54	54

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

ANOVA Result's Charts

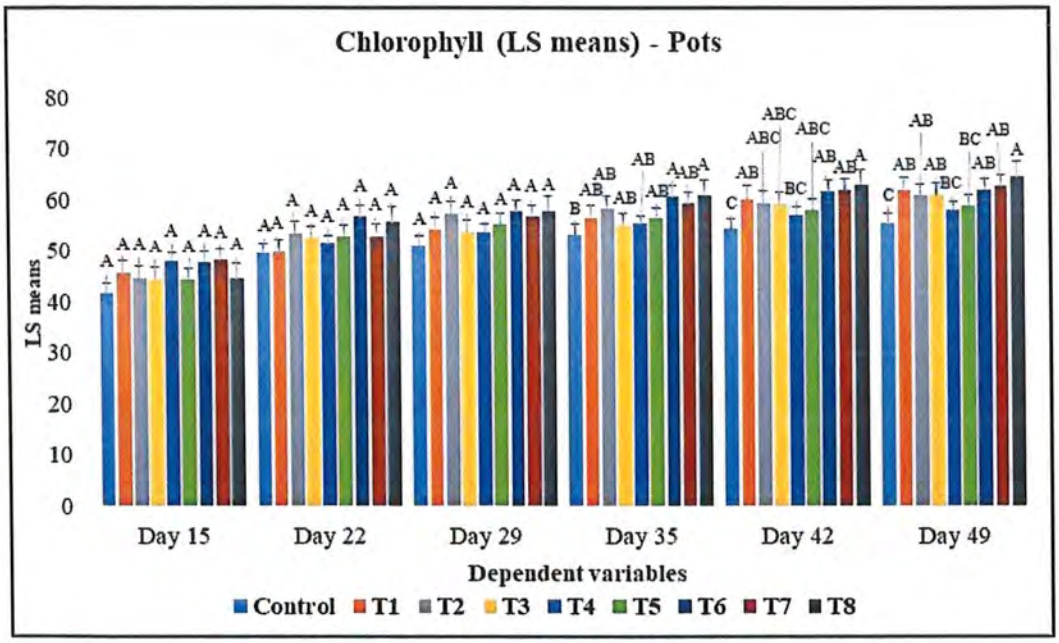

Figure 29. ANOVA for Chlorophyll – Pots

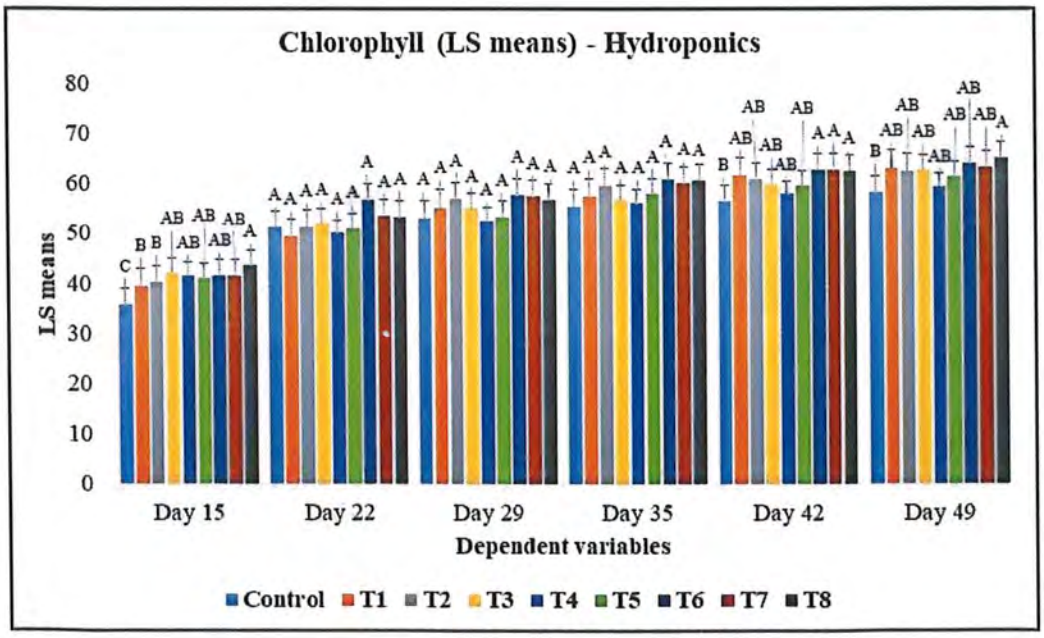

Figure 30. ANOVA for Chlorophyll – Hydroponics

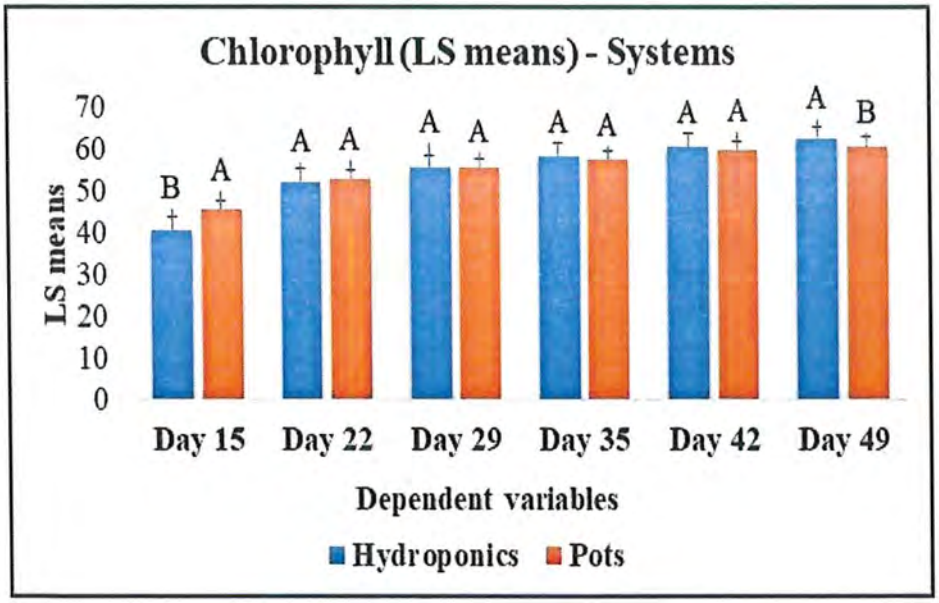

Figure 31. ANOVA for Chlorophyll - Systems

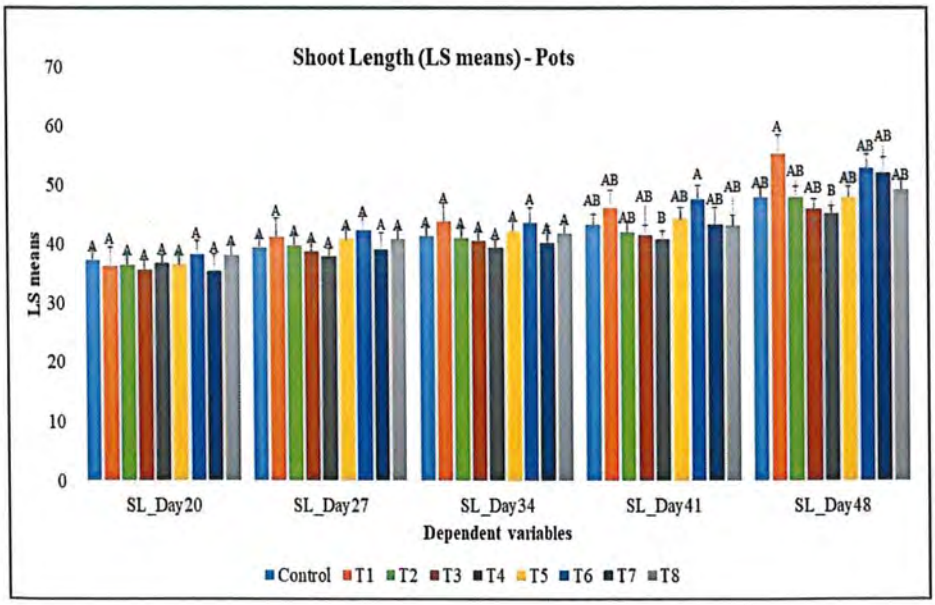

Figure 32. ANOVA for Shoot Length - Pots

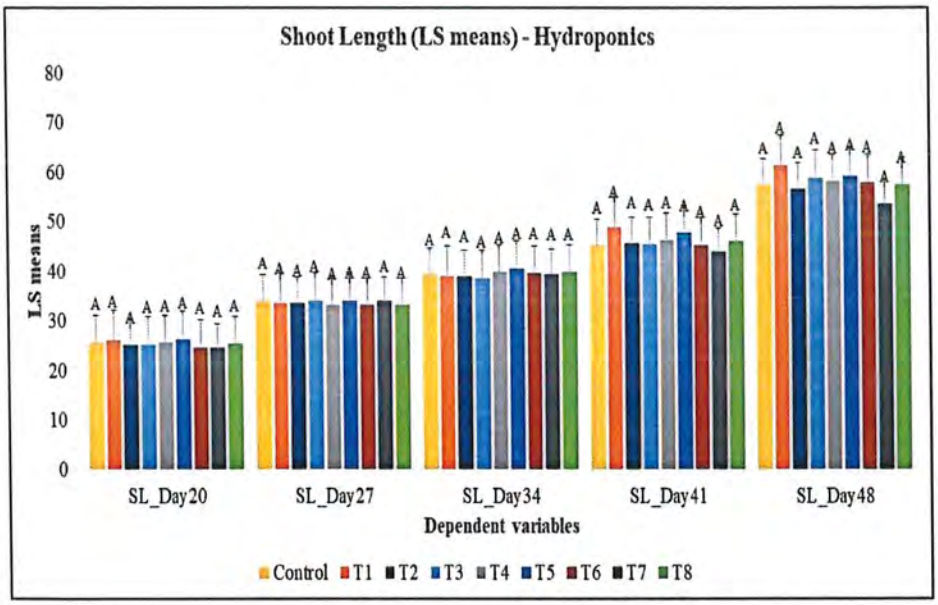

Figure 33. ANOVA for Shoot Length - Hydroponics

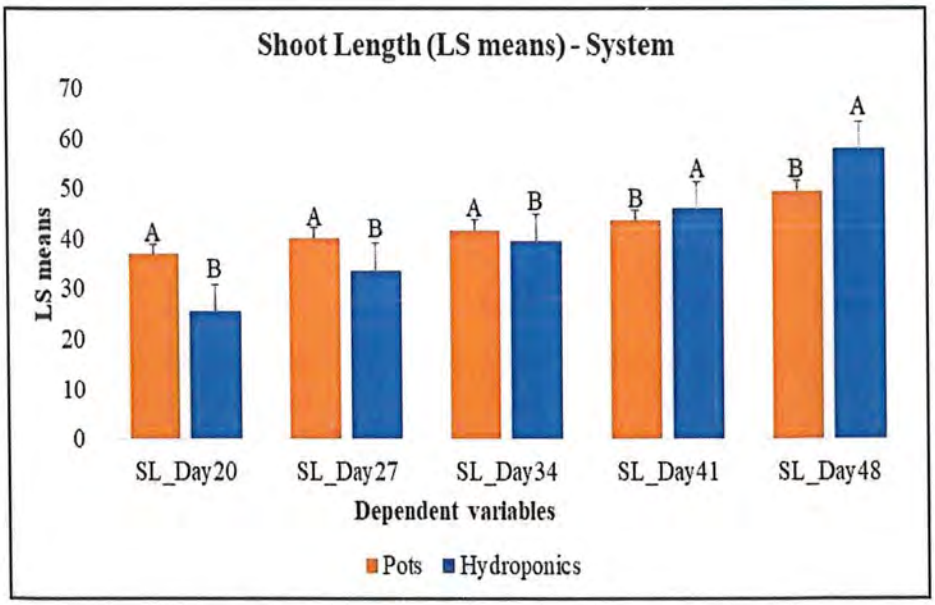

Figure 34. ANOVA for Shoot Length - System

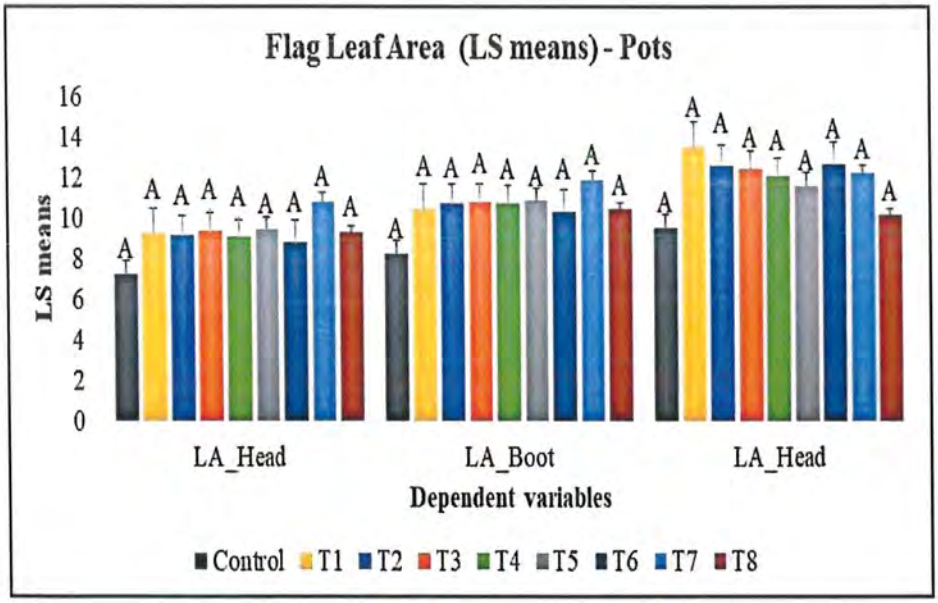

Figure 35. ANOVA for Flag Leaf Area - Pots

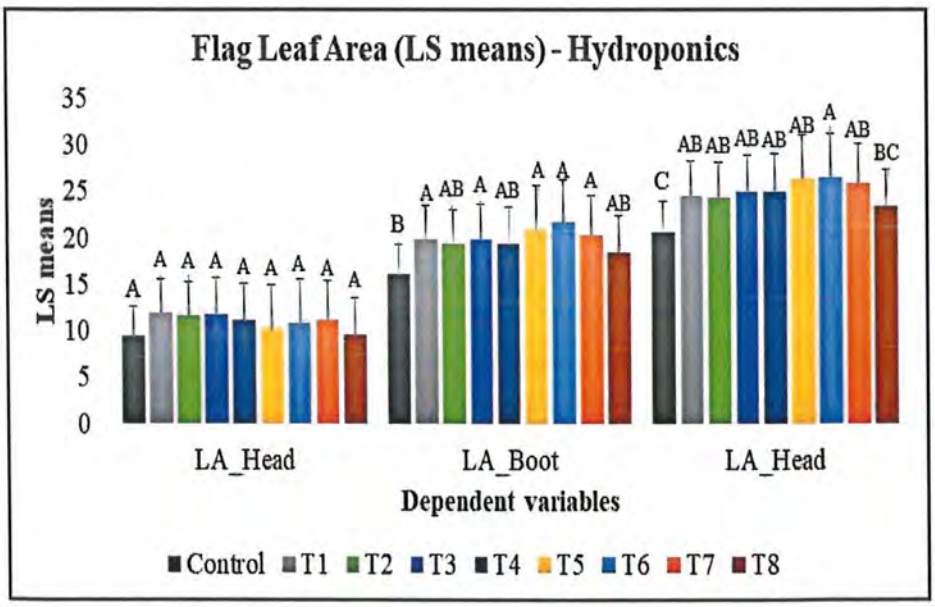

Figure 36. ANOVA for Flag Leaf Area - Hydroponics

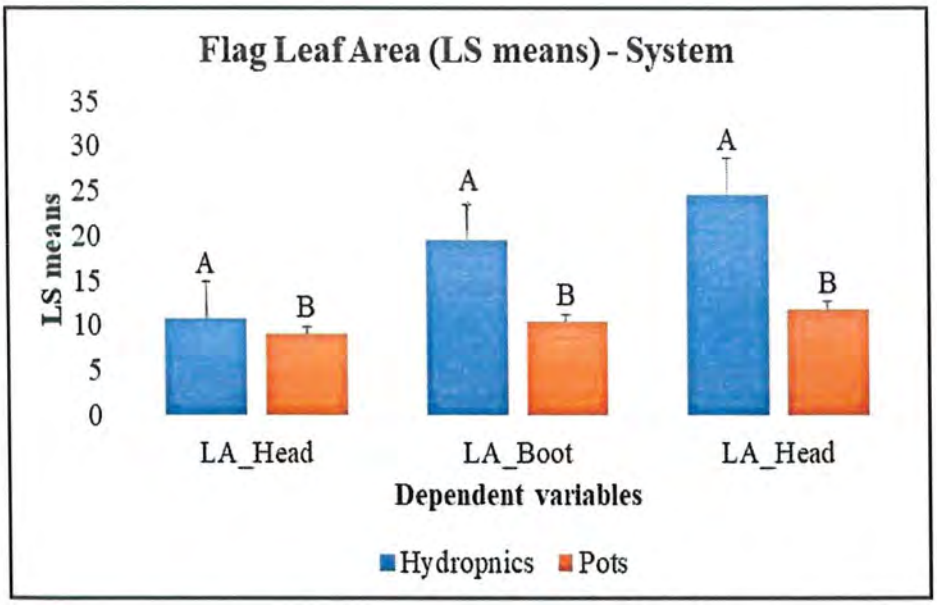

Figure 37. ANOVA for Flag Leaf Area - System

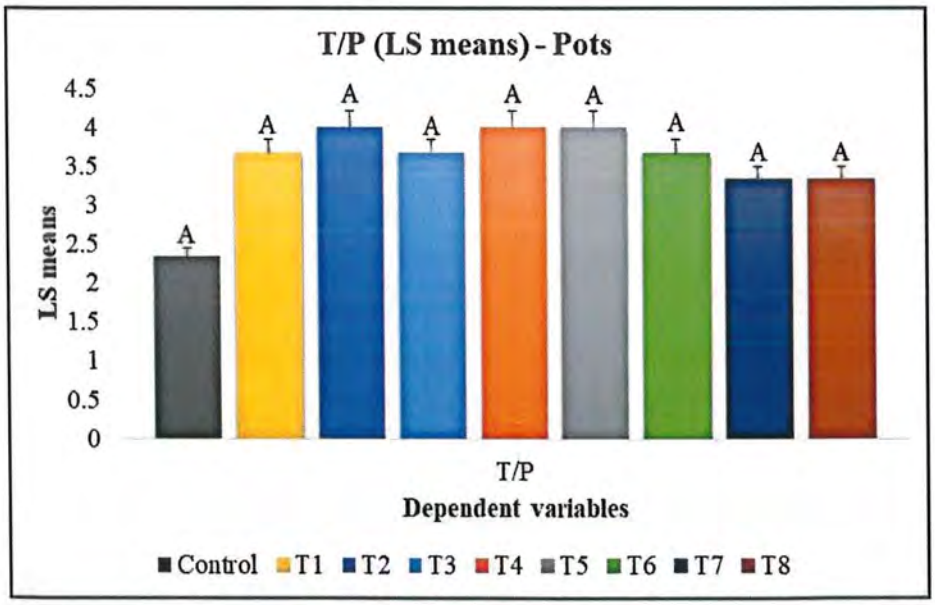

Figure 38. ANOVA for Tiller per Plant - Pots

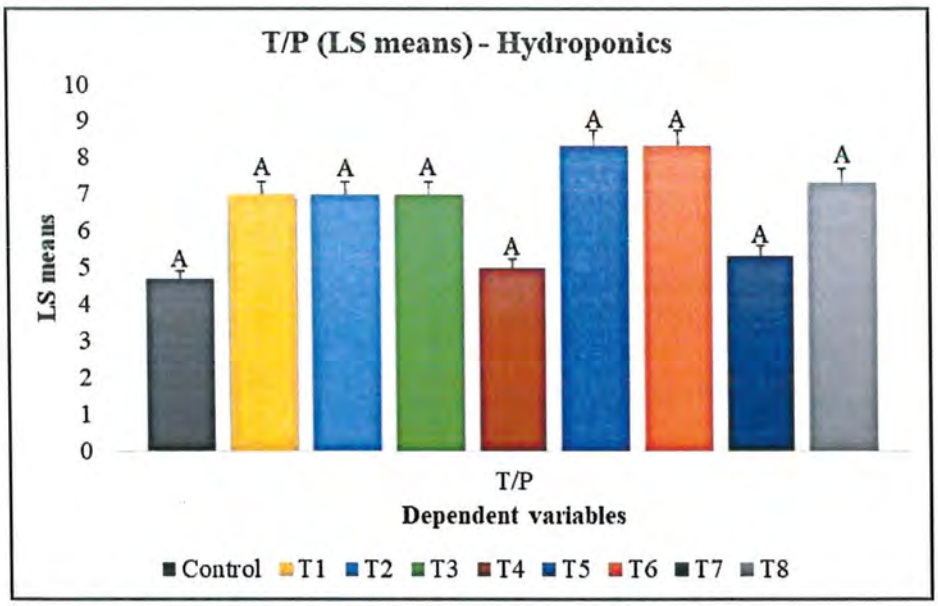

Figure 39. ANOVA for Tiller per Plant - Hydroponics

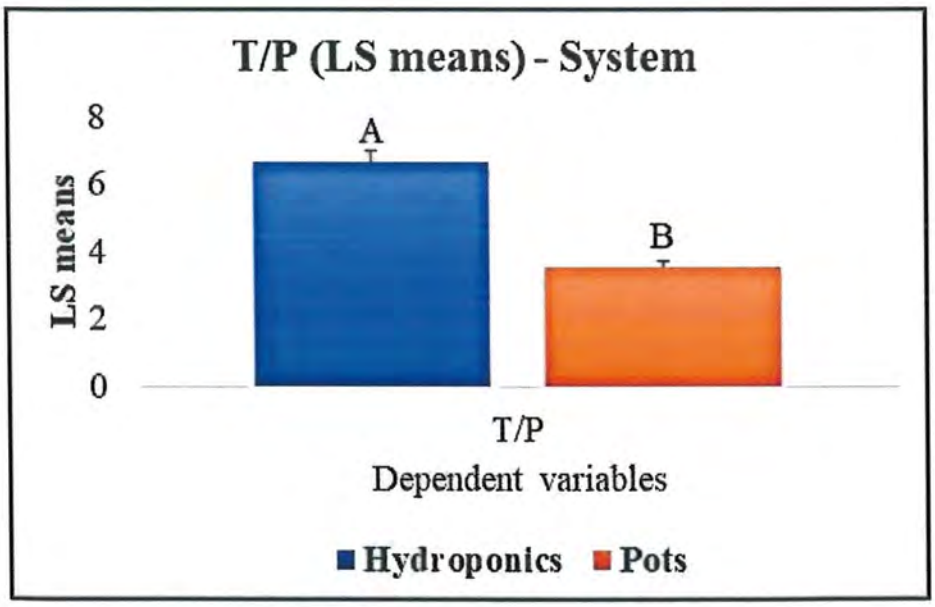

Figure 40. ANOVA for Tiller per Plant - System

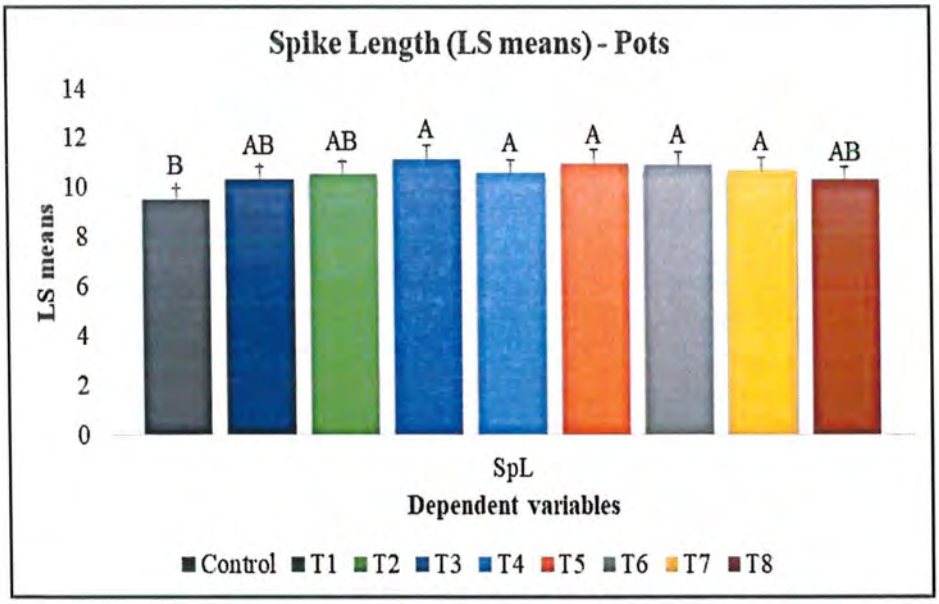

Figure 41. ANOVA for Spike Length - Pots

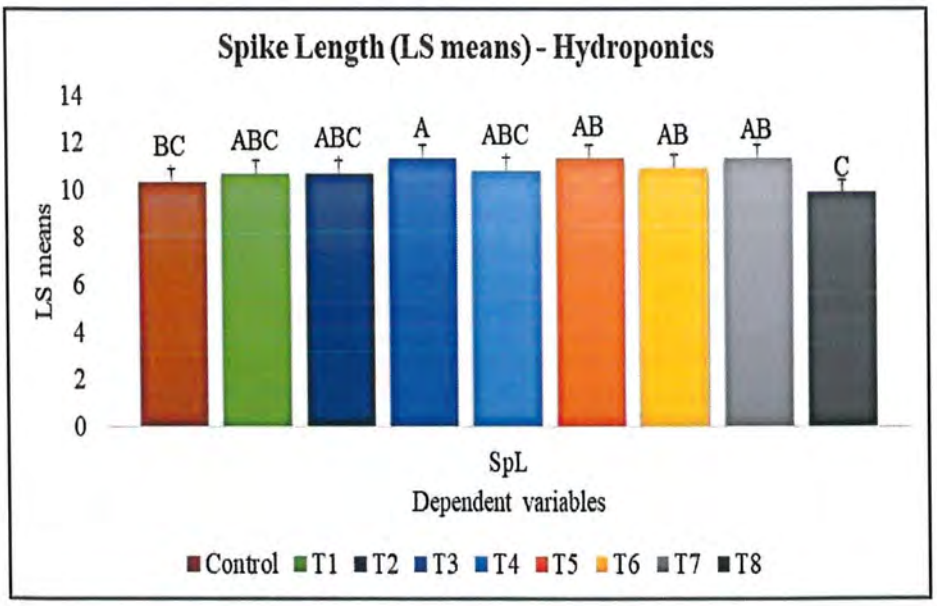

Figure 42. ANOVA for Spike Length - Hydroponics

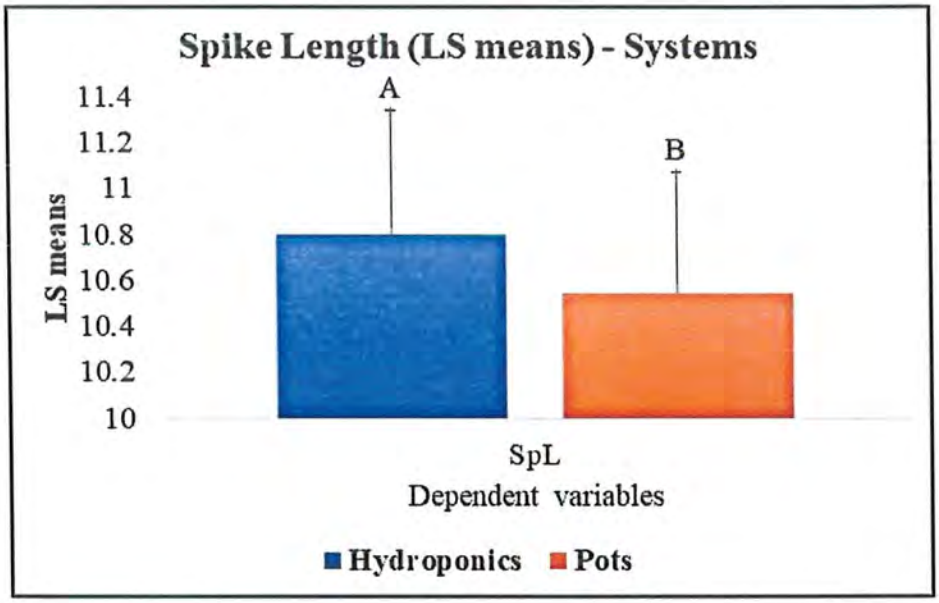

Figure 43. ANOVA for Spike Length - System

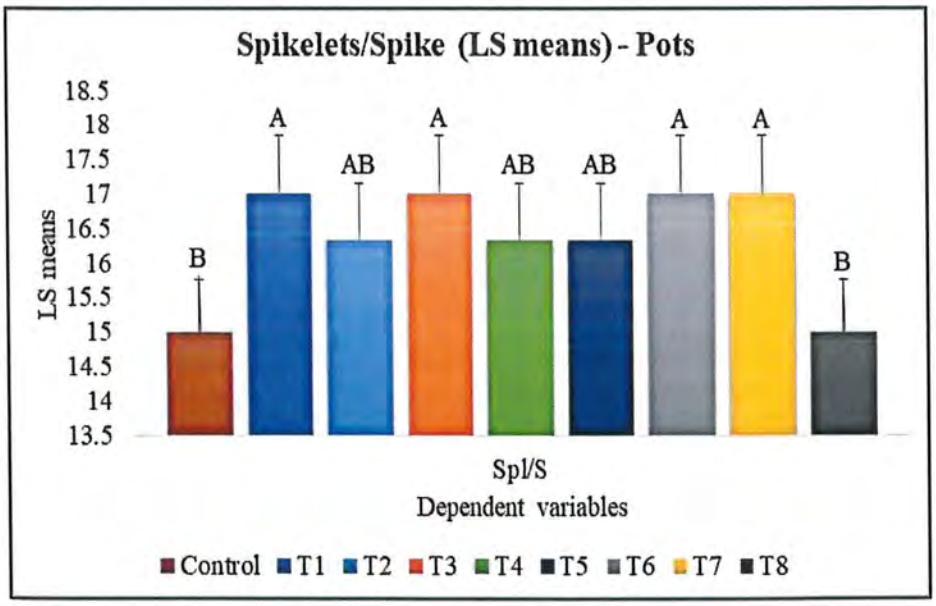

Figure 44. ANOVA for Spikelets/Spike - Pots

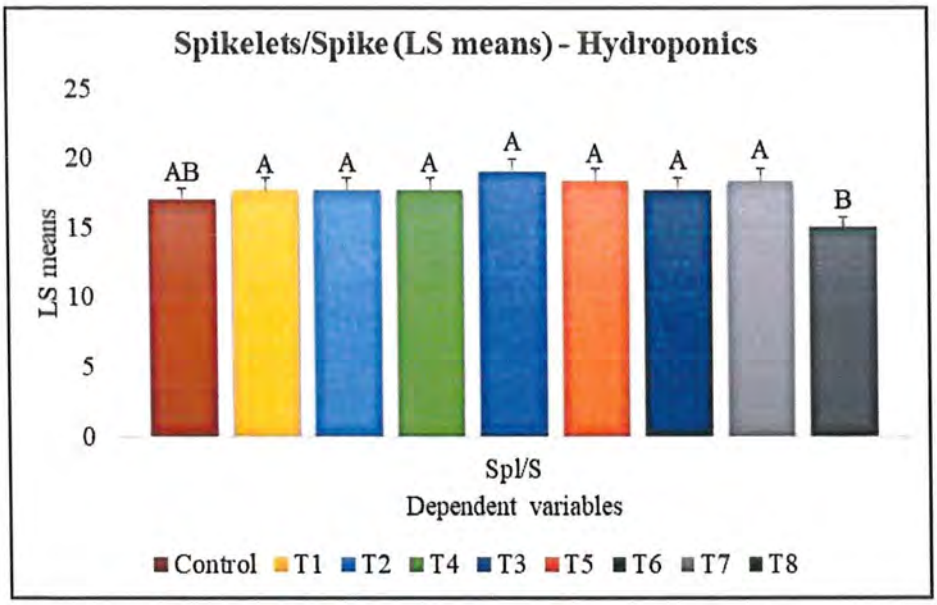

Figure 45. ANOVA for Spikelets/Spike - Hydroponics

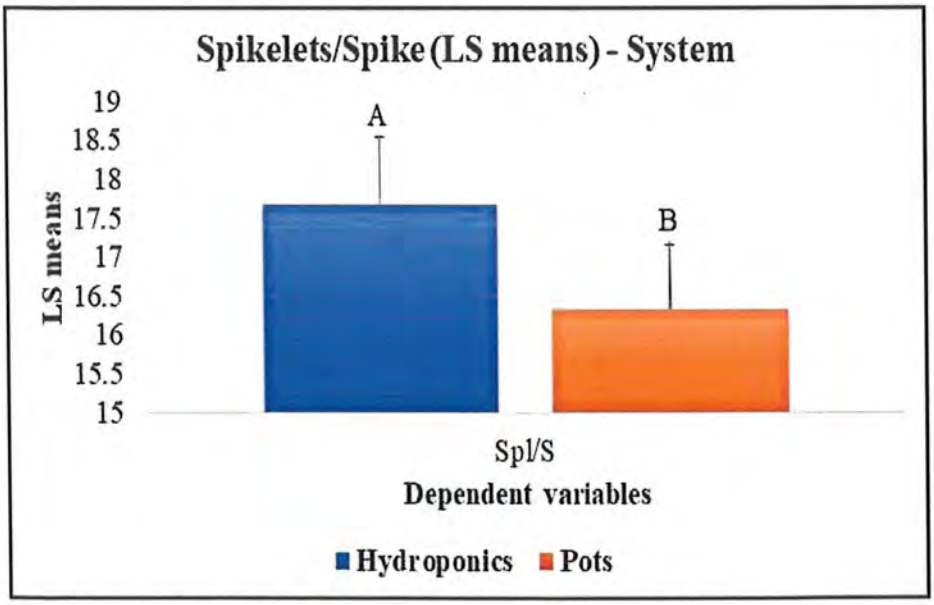

Figure 46. ANOVA for Spikelets/Spike - System

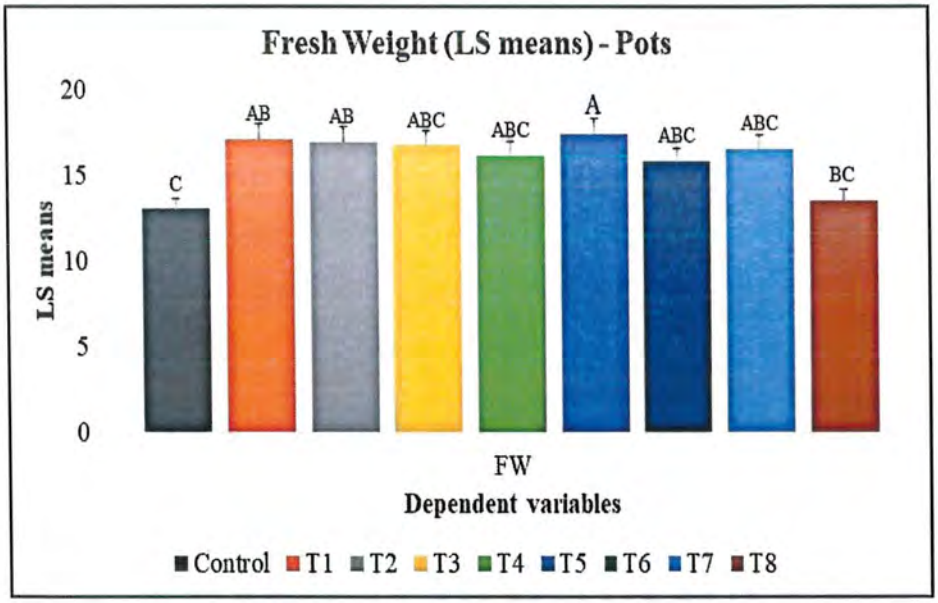

Figure 47. ANOVA for Fresh Weight - Pots

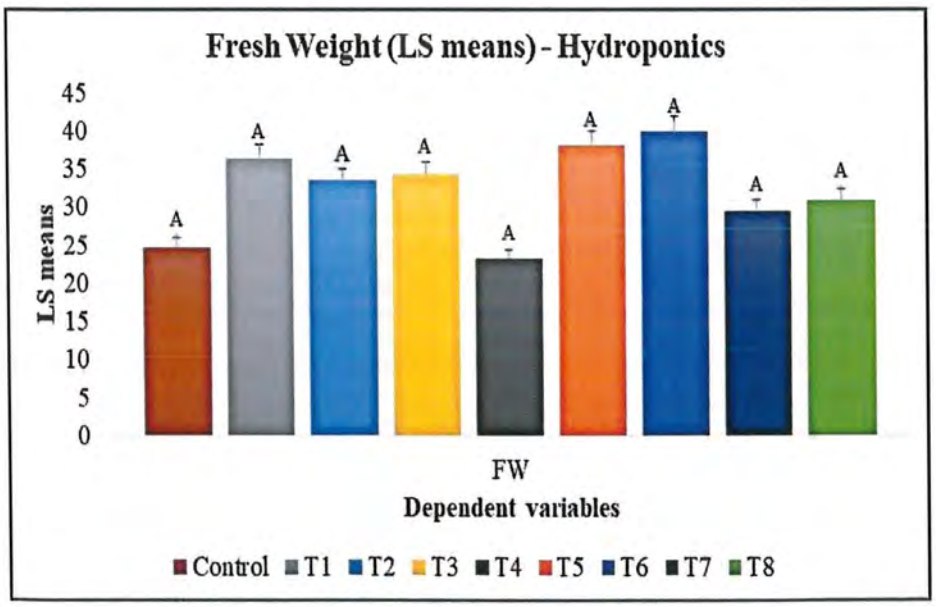

Figure 48. Figure 47. ANOVA for Fresh Weight - Hydroponics

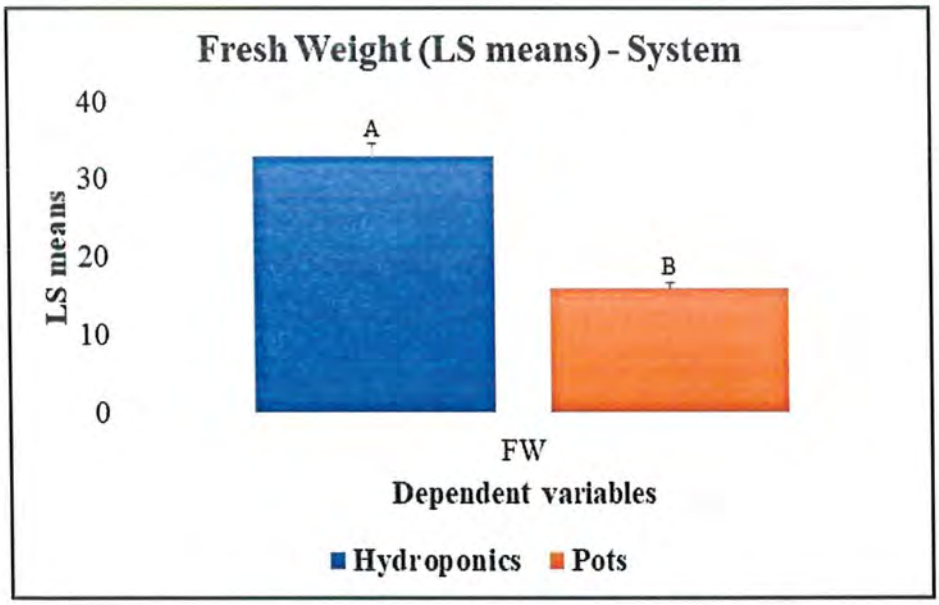

Figure 49. Figure 47. ANOVA for Fresh Weight - System

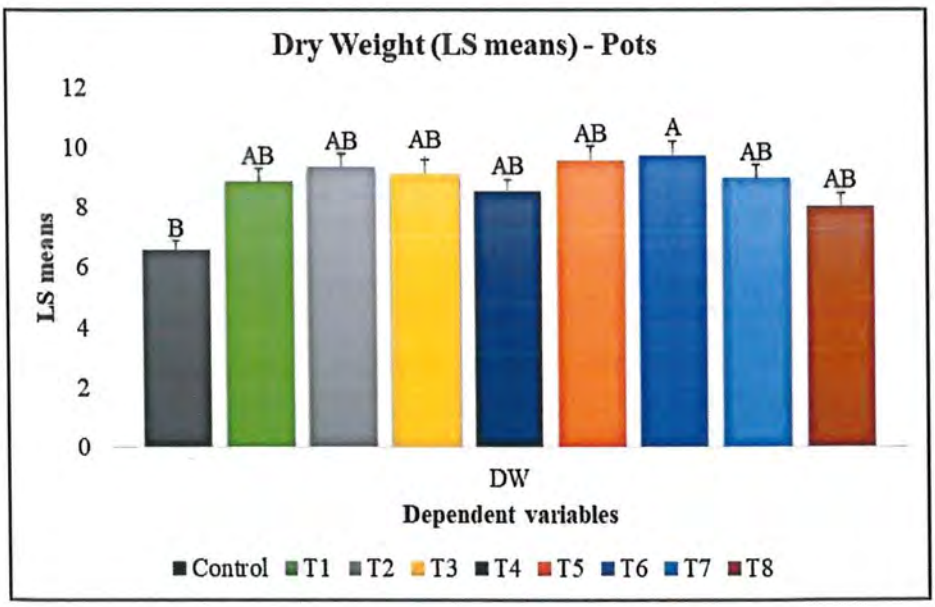

Figure 50. Figure 47. ANOVA for Dry Weight - Pots

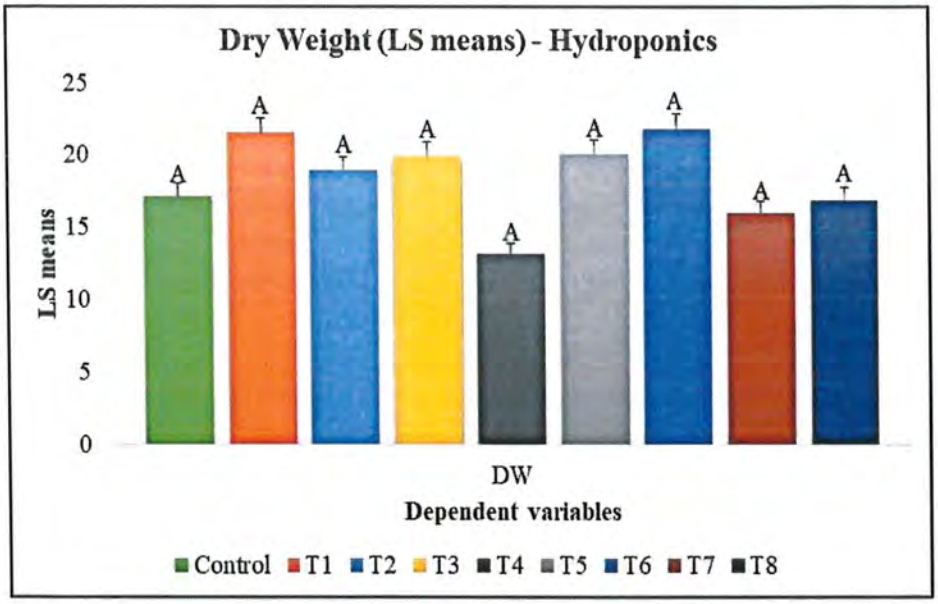

Figure 51. Figure 47. ANOVA for Dry Weight - Hydroponics

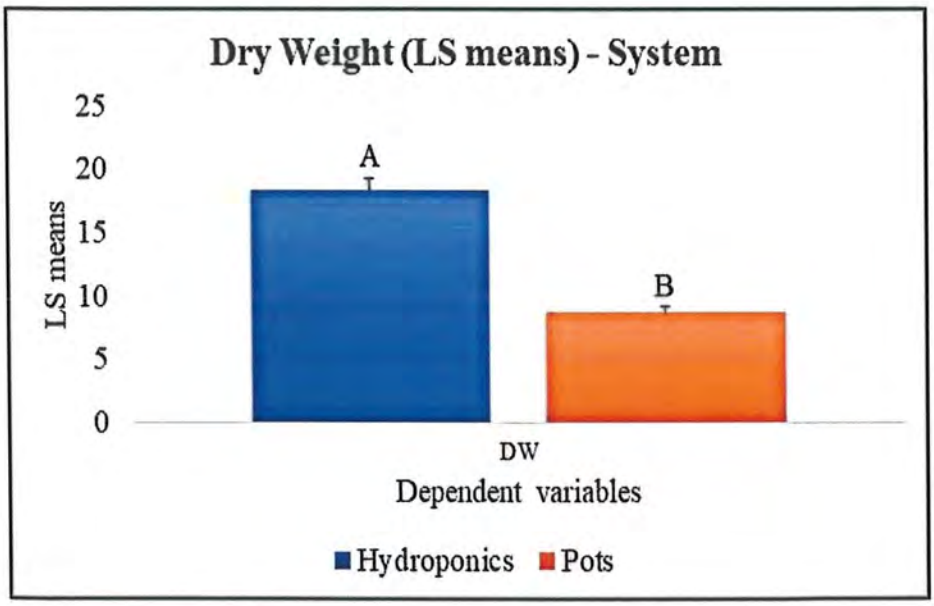

Figure 52. Figure 47. ANOVA for Dry Weight - System

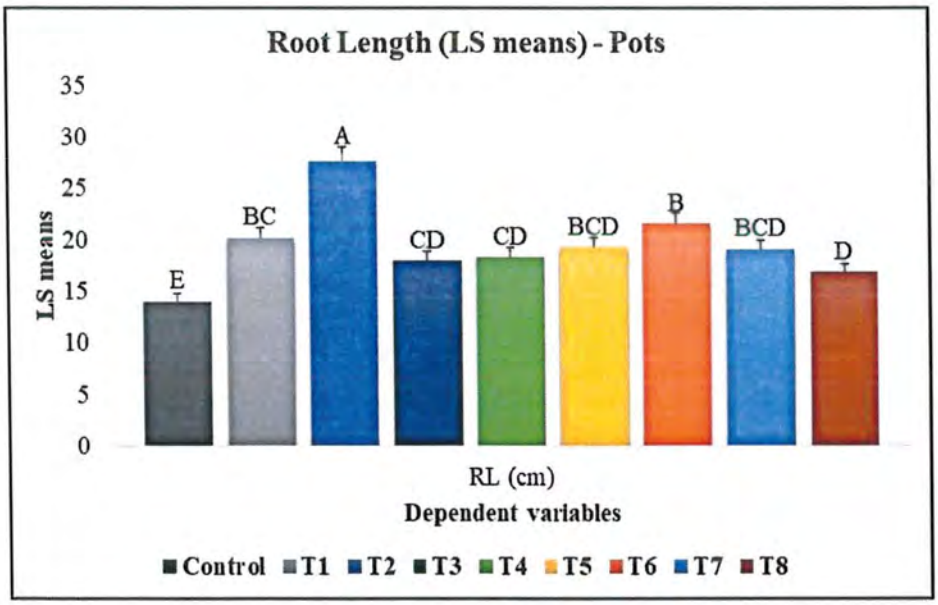

Figure 53. Figure 47. ANOVA for Root Length - Pots

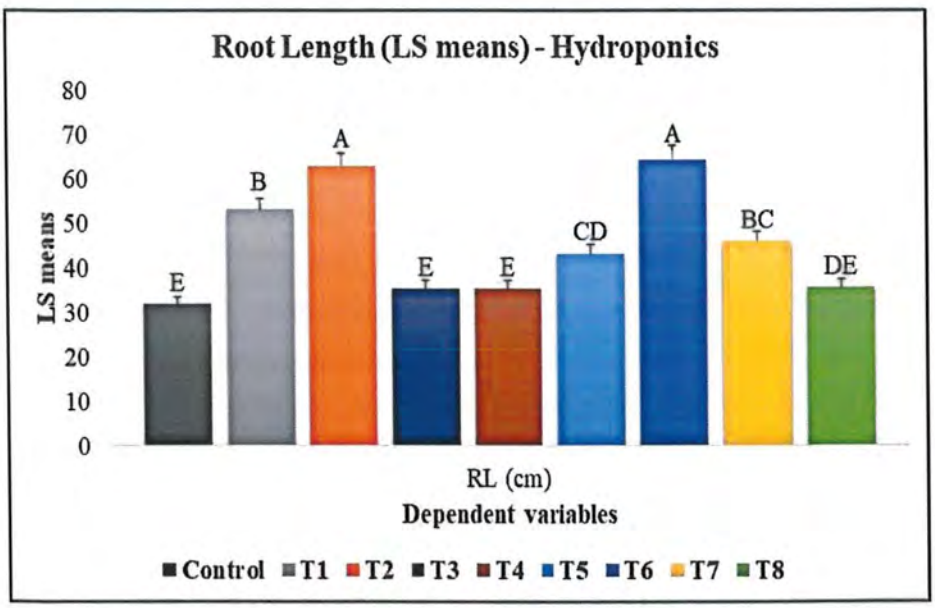

Figure 54. ANOVA for Root Length - Hydroponics

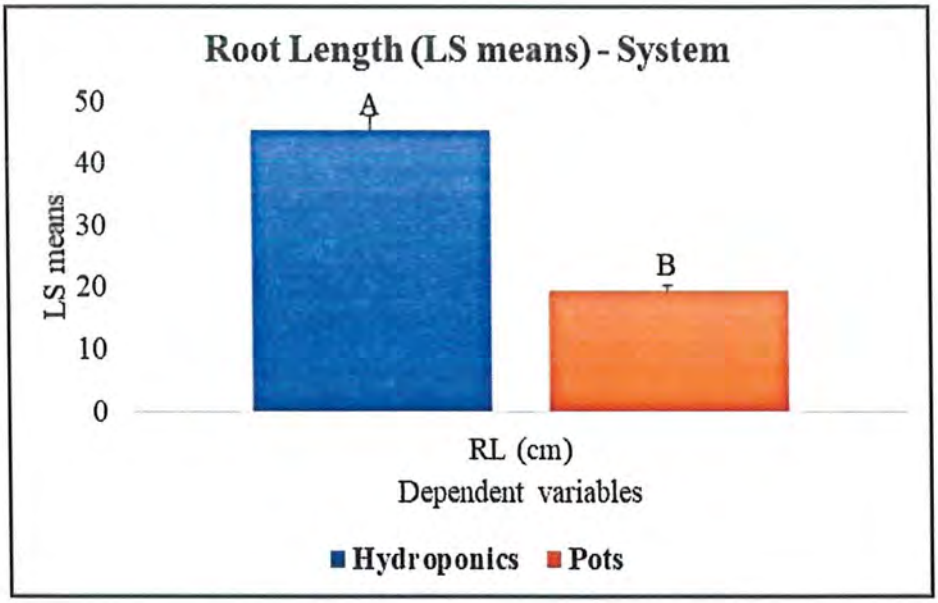

Figure 55. ANOVA for Root Length - System

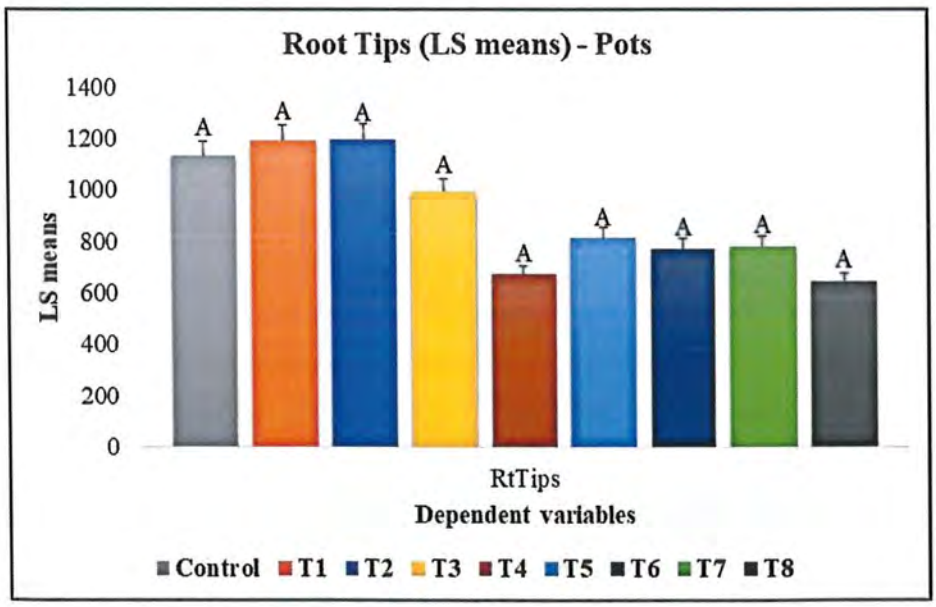

Figure 56. ANOVA for Root Tips - Pots

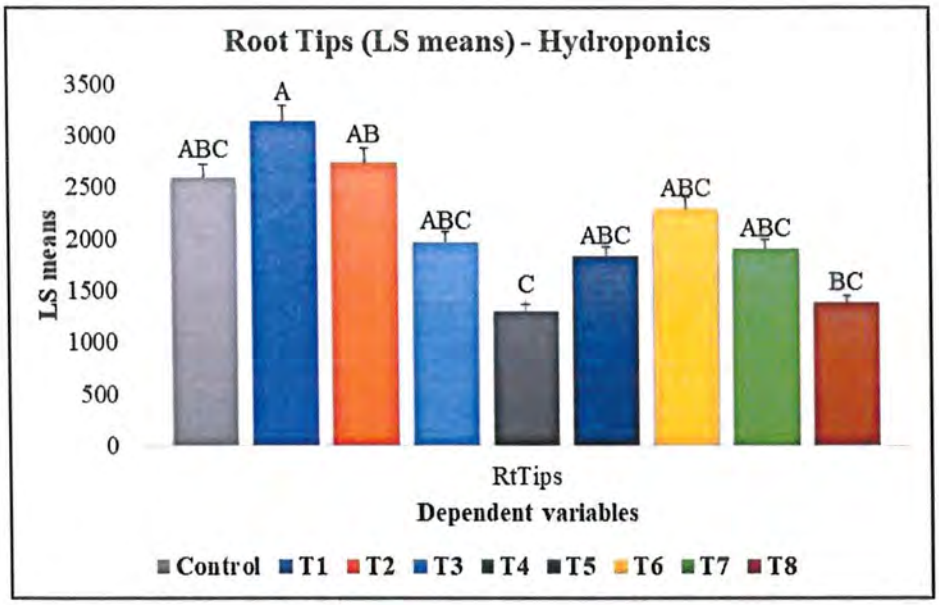

Figure 57. ANOVA for Root Tips - Hydroponics

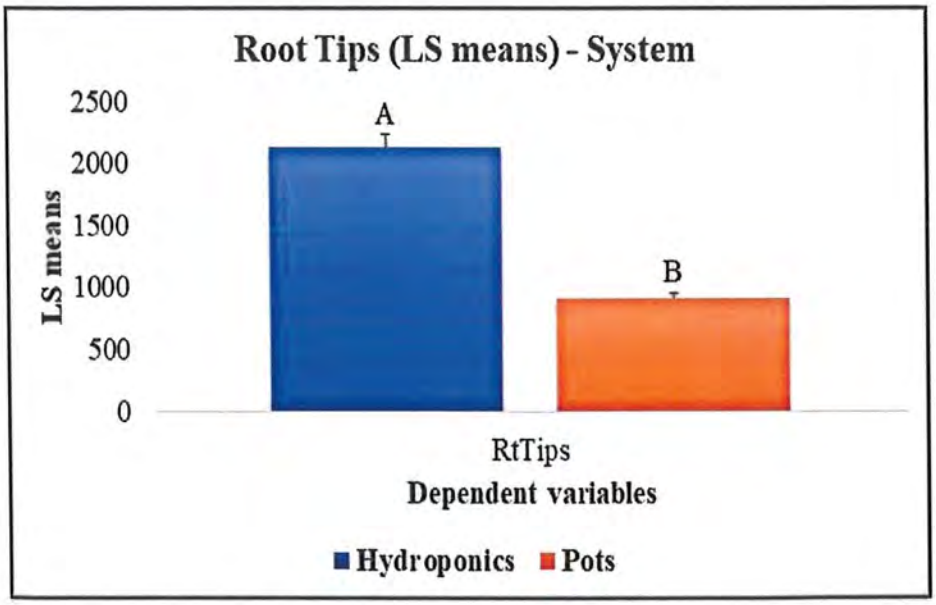

Figure 58. ANOVA for Root Tips - System

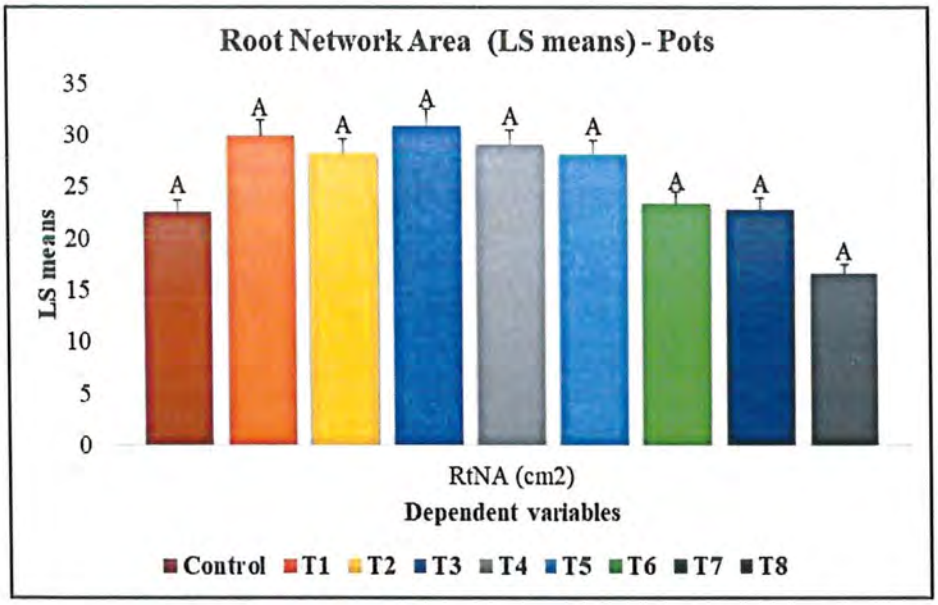

Figure 59. ANOVA for Root Network Area- Pots

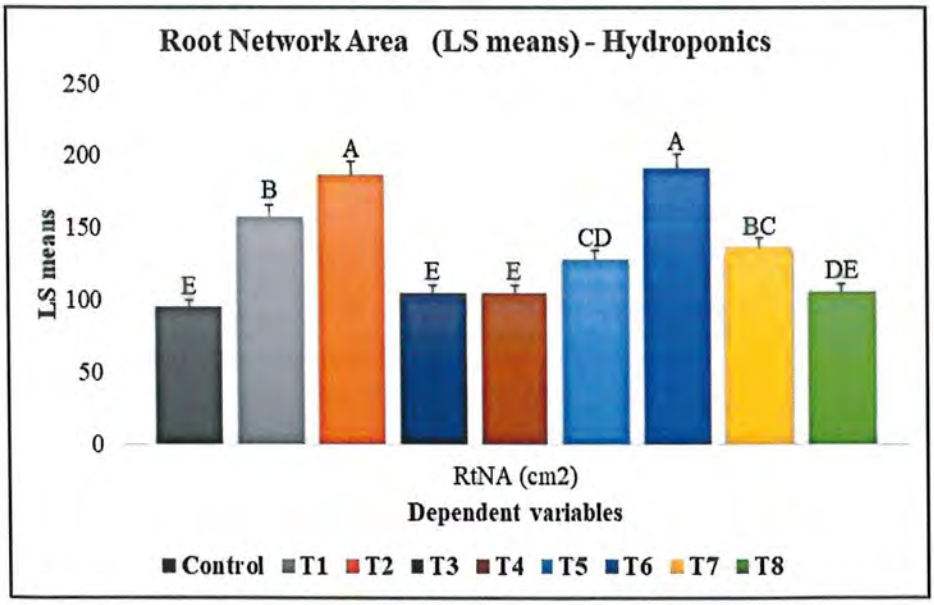

Figure 60. ANOVA for Root Network Area - Hydroponics

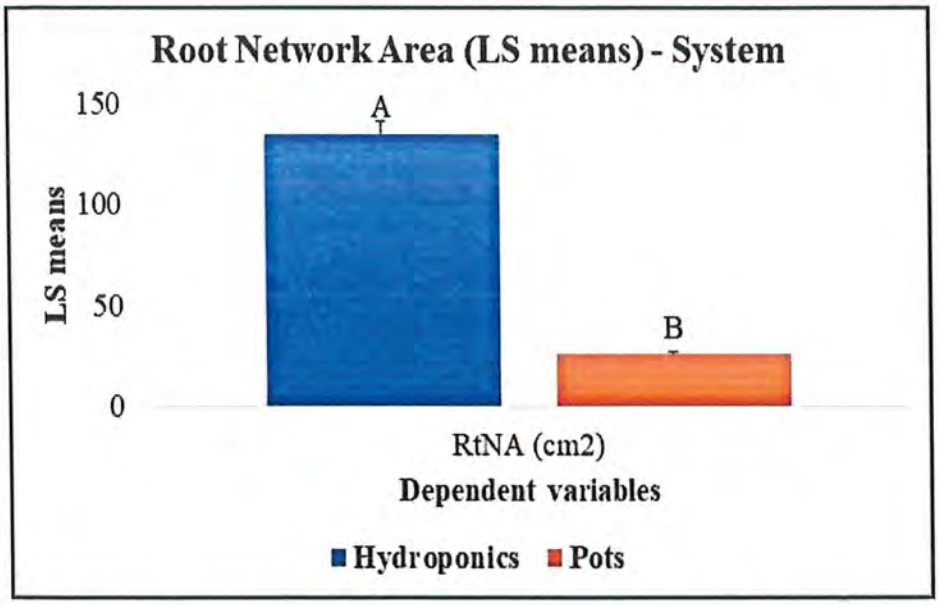

Figure 61. ANOVA for Root Network Area - System

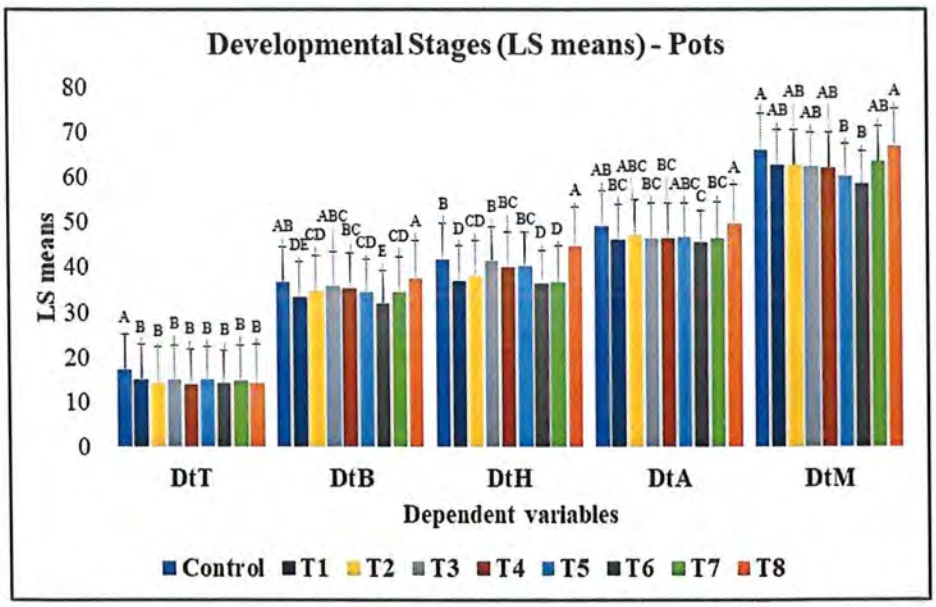

Figure 62. ANOVA for Developmental Stages - Pots

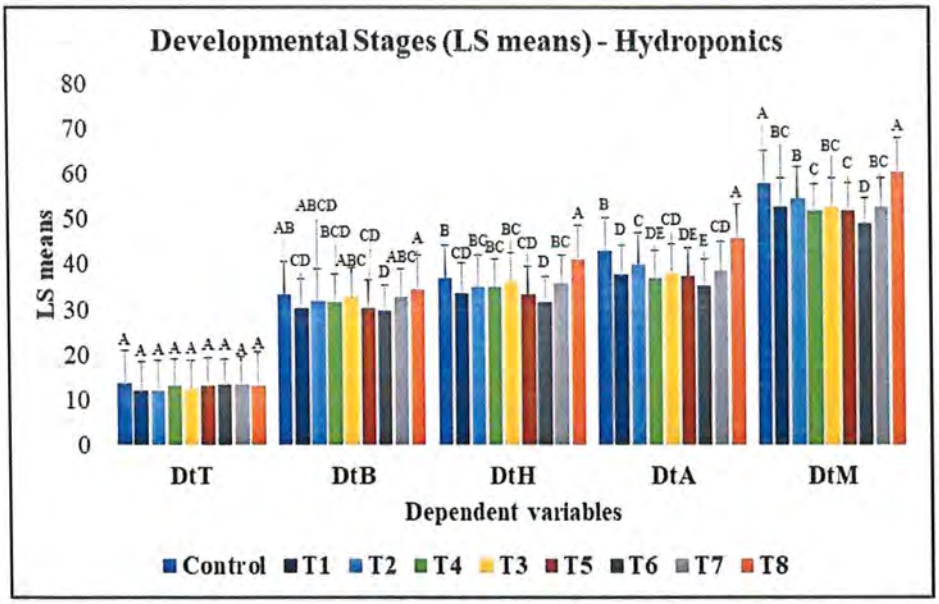

Figure 63. ANOVA for Developmental Stages - Hydroponics

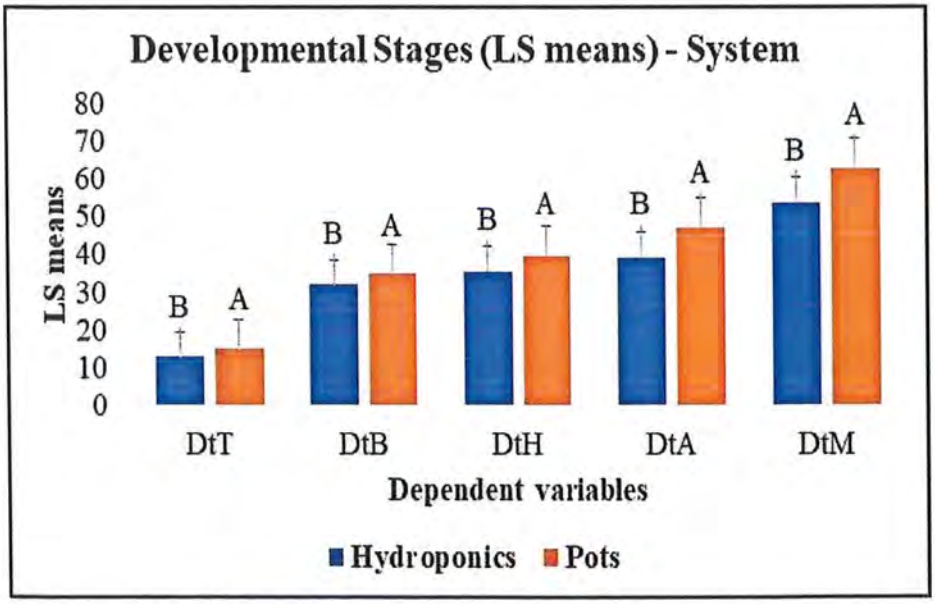

Figure 64. ANOVA for Developmental Stages - System

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