The potential of under-utilized tetraploid (2N=4X=28) wheat genotypic diversity in recombination breeding

AABB

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This work is submitted as a dissertation in partial fulfillment for the award of the degree of

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In

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By

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PLAGIARISM CERTIFICATE

This is to certify that, **Maimoona Hussain**, registration number **02041311037** has completed Master of Philosophy in Plant Science from Department of Plant Sciences, Quaid-i-Azam University, and Islamabad. The title of thesis is ***"The potential of under-utilized tetraploid (2N=4X=28) wheat genotypic diversity in recombination breeding"***. This thesis has been checked on Turnitin for similarity index and found that it lies within the limit provided by HEC.

Supervisor: **Dr. Umar Masood Quraishi**

DECLARATION

The research work presented in this thesis was carried out by me in the Molecular plant breeding laboratory, Department of Plant Sciences, Quaid-I-Azam University Islamabad. The results, findings and conclusions were of my own investigation with discussion of my supervisor **Dr. Umar Masood Quraishi**. No part of this work has been presented for any other degree.

Maimoona Hussain

APPROVAL CERTIFICATE

This is to certify that the dissertation entitled ***"The Potential of Under-utilized Tetraploid (2N=4X=28) Wheat Genotypic Diversity in Recombination Breeding"*** submitted by Miss. **Maimoona Hussain** is accepted in its present form by the Department of Plant Sciences, Quaid-I-Azam University Islamabad, Pakistan, as to satisfy the partial fulfillment for the degree in **Plant Science** with specialization in **Molecular Plant Breeding**.

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DEDICATION

THIS EFFORT IS DEDICATED

TO

THE PERSONS WHO OWE MY BEING

THE BIGGEST BLESSINGS OF MY LIFE

(MY AMI ABU)

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LIST OF ABBREVIATIONS

LA	Leaf Area
CHL1	First reading of chlorophyll
CHL2	Second reading of chlorophyll
CHL3	Third reading of chlorophyll
DH	Days of Heading
DM	Days of physiological maturity
SPS	Spikelet per spike
SL	Spike length without awns
SLA	Spike length with awns
SPS	Spikelet per spike
G/S	Grain per spike
GY	Grain yield
TKW	Thousand kernel weight
ANOVA	Analysis of variance.
PCA	Principle component analysis
ETW	Extracted tetraploid wheat
MYA	Million years ago
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism

ANOSIM	Analysis of Homogeneous attributes
PCOORDA	principal coordinate analysis
CAPS	Cleaved Amplified Polymorphic Site
HTP	Height-throughput
SNPs	Single nucleotide polymorphism
SSR	Single sequence repeats

ABSTRACT

Wheat is an important staple food, providing 20% total calories to world population. World population is expected to increase to an alarming 9.6 billion in next 40 years. There is a need to increase the production of wheat in order to fulfil the demand of increasing population. There has not been a significant increase in grain yield, since Green revolution. Focus of all breeding programs has shifted to increasing both quality and the quantity of wheat. Domestication, continuous selections, green revolution and disease outbreaks have narrowed the gene pool increasing the need to explore the genetic diversity of different wild relatives of wheat. The under-utilized tetraploid wheat (2N=4X=28) genotypic diversity is one of such diverse source. In this study, the potential of under-utilised tetraploids (*Triticum carthilicum, Triticum dicoccum ,Triticum polonicum*) genetic diversity was explored to identify significant lines for the future breeding program. The data of different morphological parameters like LA, CHL, DH, DM, SL, SPS, G/S TKW, and presence or absence of awns are collected from these germplasms. Then this data was subjected to different statistical analysis. And descriptive statistics, ANOVA, correlation matrix and PCA of the data is obtained. Two components F1 and F2 contribute to 35.33% and 28.23% of the total variation respectively. And the total variation shown by these components is 63.55%. TKW as making obtuse angle with DH , DM, SPS, SLA, G/W so it has positive correlation with these factors and they will affect the yield as indicated by the small obtuse angles between their vectors (r=cos0=+1). So its value will directly affect yield. These analyses applied in the germplasms and ultimately it is utilised for the specific breeding purpose.

CHAPTER No. 1

INTRODUCTION

1.1 BACKGROUND:

Agriculture is and will remain the backbone of human civilization. Before agriculture, (Neolithic Revolution) hunter / gatherer lifestyle had supported some 4 million people worldwide (Cohen, 1995). Modern agriculture now feeds more than 6.77 billion people. This leap of this industrialized farming from old hunter era started from the domestication of primary cultures estimated 12,000 years (Salamini *et al.*, 2002) ago.

The man started exercising selection of species of agronomic interest. This selection is based primarily on the visual selection and has greatly improved the chances of various crops, especially cereals in the last two centuries. The Emergence of breeding companies at the end of the 19th century boasted advances in crop improvement. But the real propulsion was the rediscovery of Mendel's laws at the beginning of 20th century (Xu, 2005), which gave a genetic basis for the theory of selection and quantitative genetics. Mendel explained the inheritance of discrete traits, like vs. Purple white flower, smooth vs. Wrinkled seeds, for control through genetic factors or genes. However, most economically and / or agronomically important traits are not qualitative (discrete), but quantitative (continuous) in nature except, most of the traits of disease resistance. Visual selection also helped to improve these features too. Quantitative traits are usually influenced by several genes, with relatively weak individual effects and are influenced by the environment. Before 1990`s, the selection was performed without prior knowledge of "genes" involved in genetic variation. Even then, it leads to a steady increase in crop yield.

According to United Nation Report (2013), current world population of 7.2 billion will increase by almost a billion people in the next 12 years, and will reach by 8.1 billion in 2025, and 9.6 million in 2050. Most of the population increase will in the developing countries, which is expected to increase 5.9 billion in 2013 and 8.2 billion in 2050 (World Population Prospects: The 2012 Revision). These perturbing conditions have placed agricultural scientists and the researchers in the field of potency management to difficulty.

Now the need of the hour is to close a 69 % gap between the crops produced in 2006 and the crops of the world, need by 2050. (Crop breeding: renewing the global commitment; working paper June 2014).

1.2 EVOLUTION OF CEREALS:

Virtually all cereals belong to grass (Poaceae) family, which contains almost 10,000 species. Family Poaceae is the fourth most voluminous family of flowering plants and shows the prevalence of social, economic and ecological worldwide (Kellogg, 1998;Gaut, 2002). Chloridoideae family include millet, sorghum, millet; Panicoideae family includes maize and sorghum; Ehrhartoideae contains rice, Pooideae family consists of rye, oats, wheat and barley. Recent advances in sequencing has predicted that all cereal evolved from a common ancestor about 50 to 70 MYA (Salse *et al.*, 2008). Archaeological data shows that farming started or modern day agriculture started in parallel in three agro-climatic areas (about 5,000 - 10000 years from the Neolithic period). Three different centres in which the three main crops were domesticated and cultivated independently are Maize in Mexico, wheat in West Asia (Southern Region in the Fertile Crescent) rice both Southeast Asia and Africa (Harlan, 1975). Grasses and cereals are of great importance, which is growing even more in recent years due to their ascendant position in the economic, evolutionary history and diversity.

1.3 INTRODUCTION OF WHEAT:

Wheat is the staple food contributing about 30% of the world's edible dry matter and 60% of daily caloric intake in several developing countries (FAOSTAT, 2008). It is most consequential aliment crop of the world contributing 1/5th in human

caloric intake (Shiferaw *et al.*, 2013). It has more preponderant international trade volume as compared to other major cereal crops (Atchison *et al.*, 2010).

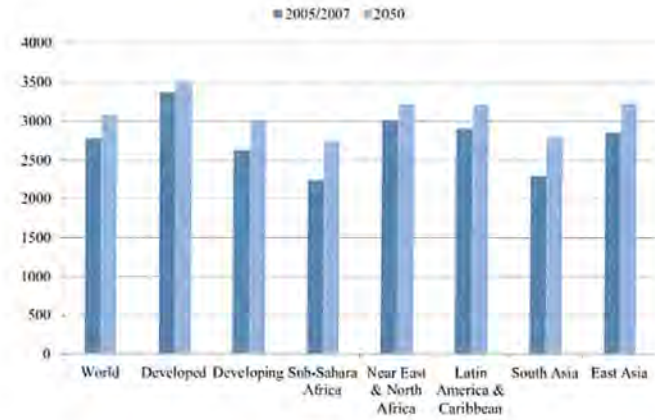

Figure 1.1 Graphical representation of per capita food consumption (kcal per person per day) (Alexandratos and Bruinsma, 2012).

It is expected that the wheat for human consumption will increase 2% per year in next decade and quality also has indirect effects on human health in different countries of the world. Currently, wheat is ranked 2nd after rice in terms of total production its alimental value is more preponderant than any other victuals source. Unlike other cereal crops, it is paramount and widely distributed throughout the world because of its agronomic adaptability i.e. from gelid near-arctic regions to stifling equatorial countries. Wheat cultivation is most thriving between latitudes 30°/60° N and 27° / 40° S (Nuttonson, 1955). The temperature for its growth has minimum values of 3° to 4°C and the maximum range is about 30° to 32°C. 25°C is its optimum growing temperature. It also ability to be grown in the area with wide precipitation ranges 250 to 1750 mm (Leonard and Martin, 1963).

Wheat was among the first domesticated grains by humans. In 5000 B.C. domestication began from Mediterranean region as the bread wheat has been cultivated in Nile valley and after that in other parts of the world. For example in Indus and Euphrates valley it was grown in 4000 B.C, in china it's cultivation started in 2000 B.C. and by 2000 B.C. It is cultivated in England.

1.3.1. NUTRITIONAL VALUE OF WHEAT:

Wheat not only provides complex carbohydrates, proteins, fats, but also is a vital source of vitamins, minerals and dietary fibers.

- **Carbohydrates:**

Major carbohydrates in wheat flour are starch and pentosans. They represent about 70-90% of the grain flour and provide the efficient source of energy for the human body (Eliason and Larsson, 1993).

- **Protein:**

Ecumenically, wheat is a rich source of protein content (10 to 18%) than either of maize or rice (9-7%) respectively. Therefore, it is leading source of protein in the human diet. It is facilely digestible by proximately 99% of the population except 1% of gluten sensitivity.

- **Other Minerals and Vitamins:**

Along with carbohydrates and proteins, it also contains affluent and diverse variety of mineral content like Iron, Zinc. It contains Vitamins including Vitamin A, folic acid, Vitamin B12, Vitamin B6, niacin, Vitamin E and D (Shetty *et al.*, 2006).

- **Dietary Fibbers:**

Wheat additionally provides dietary fibres when utilized as whole grains. There are sundry propitious effects of dietary fibres like the maintenance of the blood glucose and cholesterol level. It withal avail in insulin secretion, avert constipation and additionally ventricular disease (Shetty *et al.*, 2006;Weickert and Pfeiffer, 2008).

Due to all these nutritional benefits wheat is the first choice for human consumption.

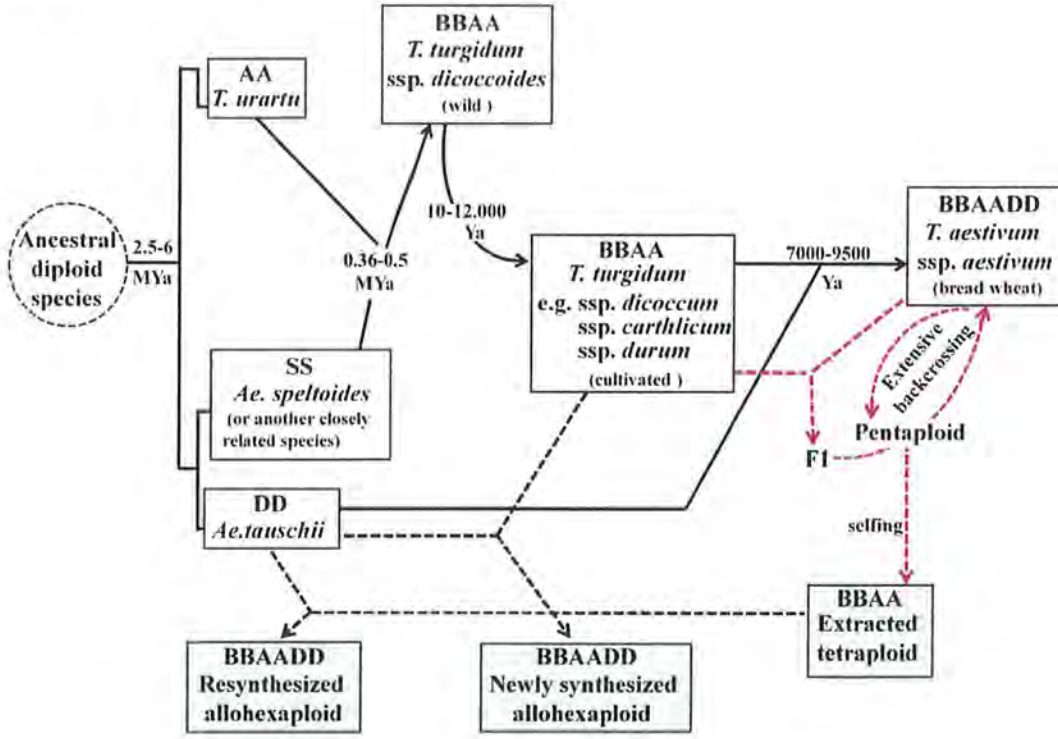

Figure 1.2 Evolution of Wheat (*Triticum*), Aegilops species and three synthetic polyploidy wheat lines (Zhang *et al.*, 2014)

1.3.4 WORLD WHEAT PRODUCTION:

Among all cereals, wheat is the most grown crop. Wheat is cultivated all over the world and the total engendered in 2010 was 651 million tons. As compared to other crops it's the most consumed and cultivated crop. Its production varies in different parts of the world according to its utilization.

In order to meet the needs of growing population wheat grain production need to be increased at an annual rate of 2 % without the increase of supplement land for cropping (Gill *et al.*, 2004).

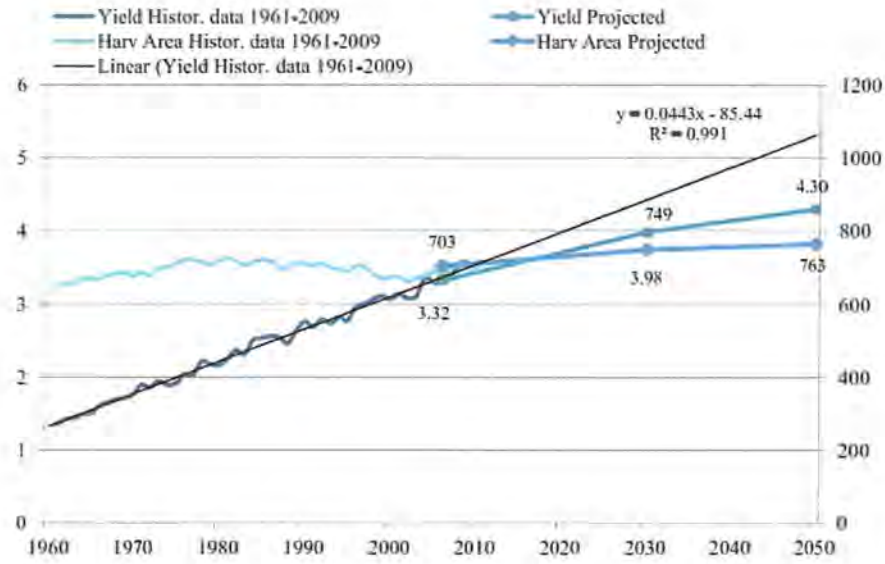

Figure 1.3 Graph depicting world cereal average yield and harvest area (Alexandratos and Bruinsma, 2012)

In order to fulfil the nutrition of the increasing population, sustainable wheat productivity is very important factor. Despite of the various advancement in agriculture, but the wheat production is still threatened by the harmful pathogens and pests (Moffat, 2001;Garelik, 2002) In 2008-09, wheat was grown in approximately 9.00 million hectares, with the total productivity of 24.00 million tons. This is 2.5 million tonnes successful production reflects about 11 percent increase. The increase in annual production is attributed to the increment of cultivated area from 8.2 to 9.0 million hectares and also nominal increase in yield of 0.05 tonnes per hectare (Ahmed *et al.*).

1.3.5 WHEAT PRODUCTION IN PAKISTAN:

Wheat is cultivated on 40% of the total arable land i.e. 8,463,000 hectares of Pakistan. It occupies 37% of the total cultivated area in our country that include 70% of Rabi (winter).The average yield of Pakistan is considerably low as compared to the worldwide production, the average yield of Pakistan is 2.6 tons / hectare. For Pakistan, a 4.5% increase / year were projected over five years, ending in 2011 to 2.9 tons per hectare, with the productivity of 26 million tons.

In a wheat production worldwide network, Pakistan ranks eighth, with the 9 million hectares, representing 40% of the total cultivated land in the country. In Pakistan the actions of the agriculture sector, 21% in total GDP with 3% share of GDP of the wheat crop (Economic Survey of Pakistan). Major wheat producing countries in Asia are Pakistan, China and India. China is the leading producer of wheat with an average production of 117 million metric tons country. In recent years, some factors such as limited water supply, rising input prices, extraordinary drought conditions etc. have seriously affected wheat production in Pakistan. Per capita consumption has increased in the country and there is a need to improve the performance to meet the demands of the growing population.

1.3.6 MORPHOLOGICAL DEVELOPMENT OF WHEAT:

The ripe wheat plant is completing all stages of growth and development. It has all structures such as leaves, growers and ears. The plant has root and shoot system. The shoot consists of repeating units phytomers calls, each containing PHYTOMER node, internode, leaf and a bud. While the root system contains two types of roots, that is the seminal roots and nodal roots.

- **Development of the wheat Plant:**

The growth and development of the wheat plant are complicated. A scale of growth provides a standard reference for describing the growth stages that provide better communication between farmers and researchers..

- **Scale for measuring growth:**

Worldwide, many scales have been developed to give numerical values to various stages of growth and development. Among them, the most used is Feekers and Zadoks scale. Growth stages are indicated either by its Feeks scale (germination, tillering, heading etc) or decimal code (Zadoks scale, 0-9). Zadoks proposed stages of growth decimal scale growth cereal crops. This scale is based on 10 early stages of growth (0-9) and each primary growth stage is divided into 10 second growth stage that specifies the number of parts of the plant in the main stem, thus broadening scale 00 to 99. Referring to a specific plant, each point of the plant described in two digits. The First digit indicates the growth stage and second indicates the part of the plant.

- **Example:**

Z13 means growth stage 1 with three leaves on the main stem like Z25 means the second stage 5 tillers. Due to various stages of growth go hand so that the plant can own more than 1 decimal code at a time like Z14, 22means four leaves on the main stem and two tillers.

(http://www.dpi.nsw.gov.au/data/assets/pdf_file/0006/449367/Procrop-wheat-growth-and-development.pdf).

1.3.7 WHEAT GRAIN DEVELOPMENT:

In general, development of the wheat kernel is divided into three phases comprising about 4 to 6 under weeks normal conditions. In the first phase called cell division phase, the increase in the number of cells in the endosperm (starch most storage protein and core) has been observed. Much weight does not accumulate during this phase. After 1 to 2 weeks after pollination, the second phase starts called grain filling phase (commonly referred to as the stage of development of the dough). During this phase kernel most of its dry weight accumulates. The transport of nutrients from the leaves, stem and spike to the developing seed takes place during this stage. Developing core physiological maturity at the end of grain filling stage though still contains about 30% water, that the core mass is hardened. This is the time when the final weight of the core is obtained. Finally, at the stage of drying (commonly known as maturing), the growth of the core decreases about three weeks in grain filling and reach their maximum weight. As the core approaches maturity, it ripens i.e. consistent moisture losses harden **"HARD KERNEL"**

1.3.8 WHEAT GRAIN YIELD:

Yield is the ultimate goal of a breeder, in some cases it is biological performance and still in some cases their grain yield. Yield can be defined as the return per year and is a complex trait, is affected by different physiological and environmental parameters as well, making her extremely complex genetic control. The grain yield per unit area is the main feature used by the wheat breeder for wheat breeding.

However to meet the awful need of wheat we need to have better perceptive about morphological and physiological aspect of wheat (Gupta *et al.*, 2006;Gupta *et al.*, 2008) (Shewry, 2009).Grain yield was explained physiologically by the following equation.

$$\mathbf{GY=BY \times HI}$$

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

The green revolution played an important role in yield improvement, but since last 40years or so, ground biomass yield has not changed. The main determinant of grain yield has state higher harvest rates in modern cultivars. It is also estimated that the harvest index for modern cultivars is near their threshold value (Slafer *et al.* 1996). Therefore, it can be deduced that both harvest index and biomass are the main factor for increasing the yield.

Historically, grain yield is correlated to grains in the square meter area, but the other major factor in grain yield is individual grain weight. By understanding these above two factors are quite difficult as they are negatively correlated (Slafer and Andrade, 1993)(Acreche and Salafer 2009).

1.4 MOLECULAR MARKERS:

A term DNA marker or solely of marker refers to specify polymorphism between individuals is defined as "DNA with known sequence and most probably the known location on chromosome and found to be associated with certain trait or phenotypes or genes". A genetic marker might be small DNA sequence like sequence surrounding the single bp (base pair) change called SNP (Single Nucleotide Polymorphism) c or it may be a long sequence like mini-satellites. There are different classes of molecular markers like low –throughput, hybridization-based markers or non PCR-based markers include RPLF (Restriction Fragment Length Polymorphism) (Botstein *et al.*, 1980) and VNTR (Variable Number of Tandem Repeats). VNTR are called minisatellites. Medium-throughput PCR-based molecular markers which includes SSR (Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism) (Vos *et al.*, 1995) CAPS (Cleaved Amplified Polymorphic Site) and RAPD (Random Amplified Polymorphic DNA) (Welsh and McClelland, 1990)

Height-throughput (HTP) sequence-based markers called SNPs (Wang *et al.*, 1998). SNP was first discovered in the human genome and proves to be universal as well as the most abundant form of genetic variation exhibition by individuals of the same species (Rafalski, 2002).

1.5 WILD WHEAT GENETIC DIVERSITY:

Triticum species are grouped in diploid (2n = 2x = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42). *Triticum aestivum,* common wheat bread contains 3 different genomes, but genetically linked (A, B and D) with a total genome size of 1.7x1010 bp, which is 500 times greater than that of the complex illustrating rice1 wheat genome nature. Prominently among other species of wheat grown in India instead.

A genetic study of the relationship RAPD primers was performed with 20 accessions of wheat from India (17 hexaploid, tetraploid and diploid 21). Out of 372 bands 323 (86.8%) were reported as a polymorphic by using RAPD primers25.The amplification products wide-ranging with an average of 14.8 ± 1.15 primer tapes from 5 primer (02-October), 30 (SPO-01).The result shows that the size of the PCR products varies from 162 bp to 2529 bp. Among the total number of bands, and the number of observed polymorphic bands a significant correlation i.e., $p<0.01$ were reported. For specific genotypes 30 unique bands were amplified from 16 primers, which should be useful for the design of future strategy of reproduction and also for identification of genotypes The similarity coefficient values ranged from 0.52 to 0.82 indicating a high genetic diversity among wheat genotypes, 20 wheat genotypes were grouped into two

- The Cluster had the diploid genotype *T. urartu,* tetraploid variety WH 896 and hexaploid variety PBW 175.

- The remaining 16 hexaploid and tetraploid varieties variety (Khapli) were placed in the other major group (Grewal *et al.*, 2007).

In plant breeding, the identification of genetic diversity is one of the most important tools now days. As compared to morphological and cytogenetic features, the molecular tool are most stable according to recent environmental condition,

molecular marker like Restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers have been used to evaluate the explore the genetic diversity of wheat. For the evaluation of genetic resources, the RAPD markers are most effectively used in wheat genotype.

The genetic diversity of 10 diploid and tetraploid wheat species was estimated using random amplified polymorphic DNA (RAPD) markers. Two species from diploid *T.boeticum* (wild), *T.monococcum*, and five from tetraploid wheat *T. dicoccoides var. arabicum* (wild), *T. dicoccum var. farru* , *T. dicoccum var. atratum* , *T. durum var. hordeiforme*, *T.durum var. leucurum* (Sharq), *T. turgidum var. alboyadurum* , *T. turgidum var. salomonis and T. persicum* were included for the analyses. To determine the genetic similarities Jacard's cluster analysis algorithm was used. The same genotypes were examined in field conditions for structural analyses, based on eight yield components. The dendrogram formed is then comparatively analysed and evaluated with the RAPD dendrogram. The differences were found between genetic and phenotypic similarity of the studied accessions. The final results indicate that the genetically similar genotypes are phenotypically similar (ALİYEV *et al.*, 2007).

Molecular analysis for a set of hexaploid (*Triticum aestvium*) and tetraploid (*Triticum durum*) wheat cultivars was investigated by applying 11 SSR primers set. The plant materials consisted of 45 genotypes 15 of which were *Triticum aestivum* and 30 of *T. durum* obtained from four different regions Egypt, Greece, Cyprus and Italy. For molecular analysis, the products of PCR were disunited on a 6% denaturing polyacrylamide gel electrophoresis and engendered a total of 3840 DNA fragments which were utilized for the molecular analysis. ANOSIM (Analysis of Homogeneous attributes), results showed that correlation was identically tantamount to 0.9048 ($P<0.0001$) designated that all the most kindred samples of genotypes are within the same population. The wheat varieties from the four distinct regions were clustered according to SSR data into two main clusters, durum wheat varieties and bread wheat varieties, the principal coordinate analysis (PCOORDA) validated the results of the dendrogram.

The two populations still had a moderate considerable level of genetic diversity and show little genetic differentiation among them. Understanding genetic variation

within and between populations is essential for the establishment of an efficacious breeding program concerning the intraspecific and interspecific hybridization.

In wheat breeding programs, molecular markers have been extensively used and they have many advantages as compared to morphological markers, including high polymorphism and environmental stresses on the physiological state of the plant. The molecular markers are used to assess genetic diversity and received much attention in the field of genetics. Many scientists have been reported that genetic diversity of wheat using several molecular markers such as RAPD, RFLP, AFLP, STS and ISSR. Simple sequence repeat (SSR) marker was used to investigate the genetic diversity due to its nature of multiple allelic, co-dominant inheritance, reproducibility, high abundance and wide attention of the genome in many crops (Abouzied *et al.*, 2013).

It is compulsory to characterize the genetic diversity of the plant resources for their efficacious utilization and protection. There are very prominent marker systems to analyse and define the plant genomes. Randomly Amplified Polymorphic DNA (RAPD) is one of the efficiently used techniques for distinguishing the genetic variation among the wheat species as well as the other plants. The aim of this research is to describe the genetic diversity of 11 wild emmer (*T. dicoccoides*) and 8 emmer (*T. dicoccon*) populations each of which is found in Turkey. For this purpose, wheat samples were analysed with 25 RAPD markers of which 20 were found to be informative. Of the total 178 amplification products, 85 were polymorphic. An average percentage of polymorphism was detected in 47.75%. An un-weighted pair-group method (UPGMA) is used to construct the cluster dendrograms with arithmetical averages. The UPGMA analysis shown the lowest homogeneous attribute was between emmer wheat recorded as TUR 02456 and wild emmer wheat recorded as TUR 03399, whereas, the genetic distance between two emmer wheat which are recorded as TUR 03562 and TUR 03564 was the highest. Consequently, RAPD very clearly evaluates the genetic diversity at inter and intraspecific levels and these species can be considered as valuable gene resources for future breeding and conservation programs. RAPD could limpidly assessed the genetic diversity at inter and intraspecific levels. In this aspect, RAPD-PCR can be considered as a reliable and congruous technique for genetic diversity studies and germplasm evaluations among

even close relative species. The obtained RAPD data are consistent with the data obtained from other marker systems (İzbırak, 2010).

The levels of genetic diversity of the population is a collection of 230 accessions of *Triticum turgidum* L. in which seven tetraploid subspecies were examined using 6 morphological protein with 9 SSP loci, 26 SSR markers and 970 Dart. The morphological traits and seed storage proteins variety was always lower in durum wheat as compared to the domesticated and wild emmer. Both sets of molecular markers distinguish the varieties of durum wheat from other tetraploid subspecies and two distinct subgroups within the subspecies of durum wheat by using Bayesian clustering (K = 2). The impact of genetic diversity was not detected for the molecular markers, where there was only a weak reduction. The SSR markers displayed a higher degree of resolution to Dart at 0.2K and identified a greater number of groups within each subspecies. On the basis of differentiation, marker DArT between typical subspecies of wheat loci showed that they could be linked to controlled genes of some agronomic traits. The selection gave 211 loci and 109 markers were determined and some are arranged at 2BL, 3BS and 4AL chromosome many quantitate trait loci (OTL) are involved in the domestication of tetraploid wheat, as tenacious glumes (Tg) and brittle rachis (Br) features. It shows that the structure of the population of tetraploid wheat collection partly gives the sign of the evolutionary history of *Triticum turgidum* L (Laidò *et al.*, 2013).

Collection of wild wheat species of genus *Triticum*, which supports, study and stored in the IPGR-Sadovo which is represented by 45 plant species. This huge collection contains 783 accessions. The regions of origin determine the diversity of this maintained germplasm. The large parts of accessions are from Russia, Bulgaria and Germany. And others are from Spain, USA, India, etc. These are the accessions that are identified as original germplasm their identity must be controlled while their storage and reproduction. The available gene pool is the basis for successful plant breeding. It can be improved by maintaining original germplasm for research (Desheva *et al.*, 2014).

T.Polonicum and other *Triticum* accessions were subjected to AFLP analysis to explain the origin of *T. petropavlovskyi.* Akond, Watanabe and co-workers produced a total of 91 putative loci by four primer combinations. Among them, there

were 56 polymorphic loci, which make 61.53% of the total number of putative loci. They found that the genetic diversity among 11 accessions of *T. petropavlovskyi* was tight due to the lower number of polymorphic loci among different species of wheat. 44 polymorphic loci were seen in *T. aestivum* and *T. compactum* while the highest polymorphism was found in *T. polonicum.*

T. petropavlovskyi was noticed that most closely related to T. Chinese accessions between *T. polonicum polonicum* other countries on the basis of the UPGMA clustering and grouping PCO and genetic similarity estimates of AFLP. Two accessions of *T. aestivum* were grouped with *T. petropavlovskyi,* in UPGMA clustering. Regarding peak structure, i.e., the presence of edge, and glume awn pubescence presence both were similar to *T. petropavlovskyi.* Six loci in *T. Chinese polonicum* were absent in almost all accessions of *T.petropavlovskyi.* The result of the study reduced the likelihood of an independent allopolyploidization event in the origin of *T. petropavlovskyi* .It also indicate a greater degree of gene flow between *T. aestivum* and *T. polonicum* and leading to *T.petropavlovskyi* .Chances are that the P gene of *T. petropavlovskyi* hexaploid wheat was introduced from *T. aestivum T. polonicum* (Akond *et al.*, 2007).

The extent and patterns of genetic diversity in landraces germplasm collected from the major wheat growing regions Ethiopian tetraploid wheat were evaluated using the traits of phenol qualitative agro morphological, cereal proteins and molecular markers. The degree of genetic erosion of local varieties of tetraploid wheat germplasm Ethiopia was also evaluated. Field evaluation of agro-morphological features and laboratory examination of cereal proteins and molecular markers (ISSR) showed the presence of a wide genetic variation among accessions grown in various Ethiopian regions. Based on the agro-morphological characters, all accessions included in the study were grouped into 15 groups, with nine solo remaining accessions. The first 5, 4 and 3 principal components analysis were involved in explaining most of the variation in the region of origin, species and altitude, respectively classes. The biomass yield per plot, the nature of edge, day of departure,

lower glume shoulder width, kernel (seed) colour and footing the bill in the emergence were always important in explaining variation in all studied accessions by region of origin, species and altitude class. Of the physical and qualitative traits

evaluated in all accessions of the source region, the highest Shannon-Weaver diversity index (H ') was mainly due to plant height, an important agronomic character in durum wheat in except local varieties of Arsi and Bale, which was due to edge colour.

Characterization Ethiopian germplasm for tetraploid wheat glutenin subunit composition resulted in the identification of new alleles at locus Glu-1. About 39% of durum wheat studied subunits containing Glu-A1x, which are rare in other durum wheat. Although there is a monomorphism, in accessions of number of gliadin and glutenin subunits, it was found that the B genome to be more polymorphic than one genome. The DNA polymorphism analysis with primers produced ISSR polymorphic bands 128 and allowed separation of 60 tetraploid accessions wheat genotypes. Nei's genetic distance for all accessions ranged 0, 0090-, 8574 and that the source region from 0.045 to 0.138. The molecular characterization of four using ISSR *Triticum* species revealed that these species were clearly separated with *T. durum* being the most diverse, followed by *T. turgidum*, and *T. aethiopicum T. dicoccom* in that order. *Triticum durum* was more closely related to *T. turgidum* than the other species. The wide diversity of Ethiopian tetraploid wheat germplasm shown here can be used for the genetic improvement of crops through selection and hybridization. However, this wide genetic diversity is threatened by genetic erosion. For conservation reasons and practical application is necessary to embark on the most comprehensive and systematic collection of germplasm from all over Ethiopia, with ex-situ conservation and proper in situ conservation in the place of origin of local varieties. This would also help prevent genetic erosion derived from local varieties being replaced by improved varieties of hexaploid and / or tetraploid wheat (Hailu, 2011) .

The aim of the study was to determine the degree of quantitative variation morphological features and quality for the genetic resources of hexaploid and tetraploid wheat accessions from Ethiopia. A total of 126 accessions: *Triticum aestivum (L.)* (53) and *T. turgidum* (L.) (50, hard and paniccum subspecies), and 23 accessions of spelled (*T. dicoccum*) were obtained from the Institute of Conservation biodiversity, Ethiopia (IBC / e), and investigated by parameters including days to heading, spike density, edge length, thousand grain weight, score of accommodation, score powdery mildew, yellow pigment (ppm) and protein (%). Adhesions were originally collected from different parts of Ethiopia and Eritrea and preserved in the

IBC / E. The results indicated that considerable variation within species for all traits was observed. Principal component analysis showed that the first three factors accounted for 70.91% and 71.23% of the total variation in *T. aestivum* and *T. turgidum*, respectively. Cluster analysis showed that all accessions were grouped into four three subgroups *T.aestivum* and *T. turgidum*, respectively. For both species groups did not follow any regional or altitudinal pattern. The *T.aestivum* with high grain weight and are mainly grouped accessions from northern Ethiopia. The study showed that the accessions of Ethiopian wheat are rich in diversity. However, the degree of change varies with the species of wheat and features. It is suggested that the accessions studied are useful for breeding work on improving agronomic characteristics and specific quality sources (Geleta and Grausgruber).

1.6 OBJECTIVES:

The main objectives of the study are design by keeping the whole above scenario in mind.

- To assess the diversity for morphological traits in wheat germ plasm.
- To explore the genetic diversity of the under-utilized Tetraploid wheat.
- To utilize the genetic diversity of Tetraploid wheat in recombination breeding.

CHAPTER No. 2

MATERIALS AND METHODS

Research work carried out at Quaid-i-Azam University Islamabad and National Agriculture Research Centre (NARC) Islamabad Pakistan with the collaboration of Wheat Wild Crosses Lab. Plant material provided by Dr. Kazi was planted in pots at NARC on 27th December 2013. According to environmental condition normal agronomics and cultural practices was applied.

2.1 GERMPLASM USED:

The species of *Triticum* are grouped into diploids (2n=2x=14), tetraploids (2n=4x=28) and hexaploids (2n=6x=42). Wild wheat is the prototype, or the "early ancestor" of the wheat that is commonly known and used today. This "ancestor" of wild wheat was discovered in 1906 by the early Zionist agronomist Aharon Aharonson in Rosh Pina (near Safed). He believed this discovery would be able to improve cultivated wheat, and today, 100 years later, his vision is being fulfilled. The wild types of wheat include different genotypes like *T.dicoccoides, T.carthilicum, T.polonicum, T.dicoccum, T.speltoides, Ae.tauschii, T.urartu, T.sphaerococcum.* The spikes of this germplasm are as shown below in the figure.

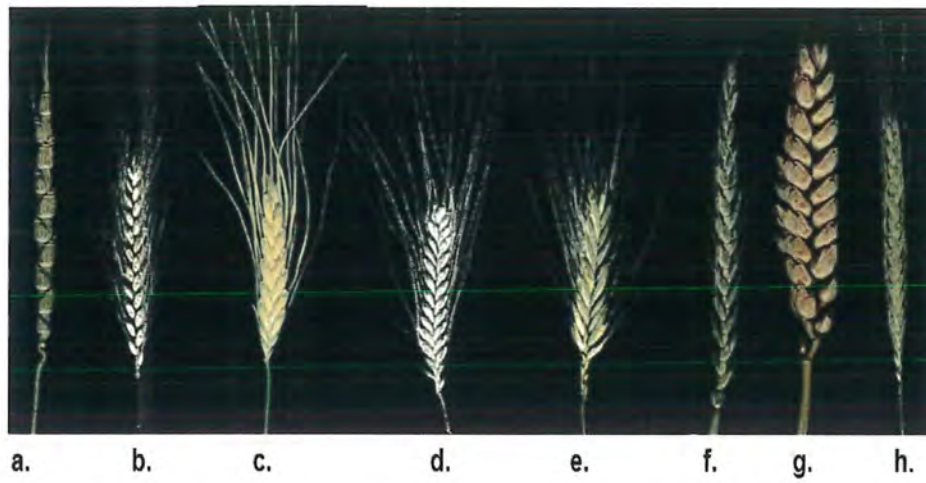

Fig 2.1 : Spike of wild relatives of bread wheat. A: ***A. Tauschii*** **, B:** ***T. carthilicum,*** **C:** ***T. dicocoids,*** **D:** ***T. dicocum*****, E:** ***T. polonicum*****, F:** ***T.spelta,*** **G:** ***T. shaerococcum,*** **H:** ***T. urartu***

(1) ***T.carthilicum:***

Triticum carthlicum Nevski (1934) is an accepted name of *T.carthilicum.* This name is the accepted name of a species in the genus *Triticum* (family *Poaceae*).It is also called as **"Persian wheat".**

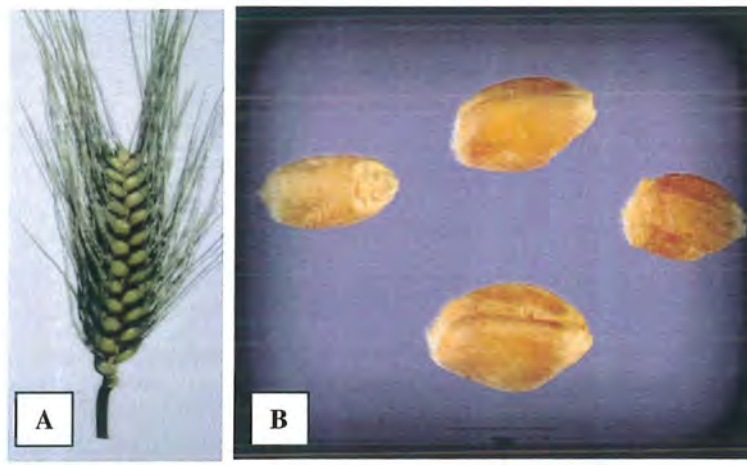

Figure 2.1(1) ***A: T.carthilicum (A) Spike (B) Grain***

(2) *T.polonicum:*

It is an ordinary variant of wheat. It's a tetraploid wheat found in small areas of the Mediterranean region, Ethiopia, Russia and in other regions of Asia. Carl Linnaeus describes it for the first time in 1762. *Triticum polonicum*, also called as the **"Polish wheat".**

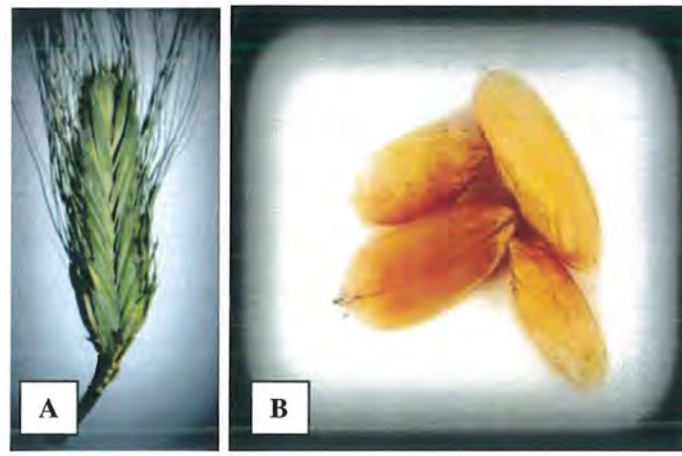

Figure 2.1*(2) *B: T.polonicum (A) Spike (B) Grain

(3) *T. dicoccum:*

It is the domesticated species of Emmer wheat. It is also called as **"Hulled wheat".** *T.dicoccum* is tetraploid awned wheat.

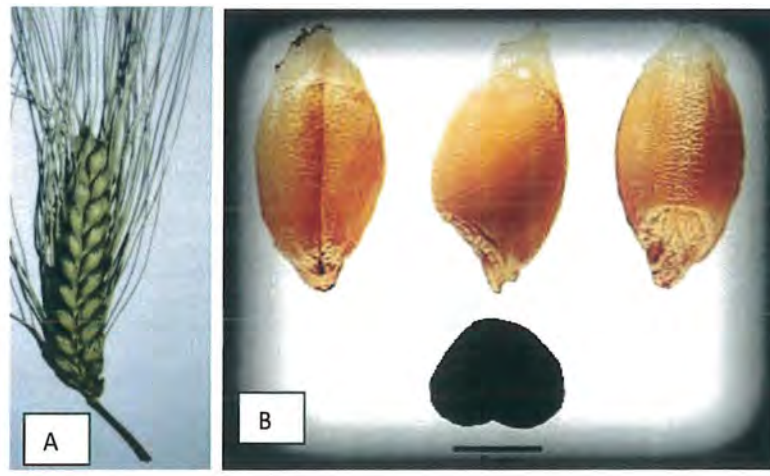

Figure 2.1 (3): *T.dicoccum (A) Spike (B) Grain*

2.1.1 PEDIGREE OF DIVERSITY PANEL USED:

A core collection of 120 lines was used in this study comprising of three types of germ plasm. That is durum (pasta) wheat (a tetraploid). The name of the three types of germ plasm *is Triticum Carthilicum, Triticum dicoccum , Triticum polonicum.* Forty entries of each of these three germplasm were used in the study. The material was selected for genotypic performance (days to Maturity) in last year trials. This collection contains lines having gene pool yet not characterized. So, this material was expected to provide us the information for the use in the breeding programs. The pedigree of the selected genotypes is described in table 1 along with their accession numbers.

2.2 PHENOTYPIC EVALUATION:

Phenotypic data was evaluated by measuring different yield related traits. These traits are as follows.

2.2.1 FLAG LEAF AREA (cm^2):

Flag leaf was measured using the AM350 compact portable leaf area meter. It is suitable for measuring non-destructive measurement of leaf area and associated parameters. It contains a high-resolution scanner with the board for integrated data analysis. Leaf area was measured by placing the leaves on scanner board or separate independent surface. On an average basis, three readings were taken. Leaf area can also be calculated with the help of simple ruler in centimetre. For this purpose length and width of the flag, leaf area is measured. And then leaf area is calculated by using the following formula (Maqbool *et al.*, 2010).

Flag Leaf Area (cm^2) = Leaf Length (cm) × Leaf width (cm) × (0.74)

2.2.2 CHLOROPHYLL CONTENT:

Chlorophyll content of the plant was recorded at different stages of the plant. It was calculated with the help of chlorophyll meter. Chlorophyll content was measured 3 times, and the gap between these three readings should be equal. We take

the three reading with the gap of a week between each reading. Three values were measured from three different leaves so that average can be calculated.

2.2.3 DAYS TO HEADING:

When the field plants reached 50% flowering days to heading were calculated starting from germination data (80% plants are germinated) using day's calculator online. (http://www.timeanddate.com/date/duration.html).

2.2.4 DAYS TO PHYSIOLOGICAL MATURITY:

It was calculated from the date of sowing to the stage of physiological maturity. When 50% of field plants start showing senescence and leaf colour turns brown, this stays when the plant is physiologically mature. Days to physiological maturity were calculating using online day's calculator that starts from the germination date (80% plants are germinated).

2.2.5 SPIKE LENGTH WITH AND WITHOUT AWNS (cm):

Total of 5 spikes per entry were collected from the field and the spike length was calculated using meter rod in centimetres, the spike length was taken both with and without awns and then average was taken.

2.2.6 NUMBER OF SPIKELET PER SPIKE:

Smaller units of the wheat spike are called spikelet, which remains attached to the rachis. Each spikelet contains 3-5 floral bearing seeds. From the collected sample of a spike as described above 5 spikes per entry were used to count the spikelet per spike and then average was taken.

2.2.7 GRAINS PER SPIKE:

Three spikes from the single plant were harvested and threshed. Grains collected from these spikes were weighted using weighing balance. Then grains per spike are calculated by dividing the total number of grains of three spikes by 3. And if the grain yield per plant is to be calculated then the total spike from the single plant

are harvested and weighted. Grains collected from these spike, weighed and total weight is called grain yield per plant.

2.2.8 PRESENCE AND ABSENCE OF AWNS:

Presence and absence of the awns in the spikes of the plants of three different germ plasm was observed.

2.2.9 1000 GRAIN WEIGHT (G):

This is an essential physiological parameter and it has a direct effect on the yield.5 to 10 plants were selected from each entry to harvest the spike.1000 grains were counted using 500 grains counter try and weighed using a weighing balance in grams. The formula use for this is as follows.

$$\text{Thousand Kernel weight (g)} = \frac{\text{Weight of seeds}}{\text{Total no of seeds}} \times 1000$$

All observations were taken from plantation in the screen house in NARC, Islamabad.

2.3 DATA COLLECTION AND ANALYSIS:

Data were collected on both qualitative and quantitative phenotypic characters from the pots present in the screen house of NARC, Islamabad (Wheat Wide Crosses). Data is collected for all 120 entries.40 entries for each germplasm. This data consists of all the agronomic traits as mentioned above in detail. All data collected were subjected to the following statistical analyses.

- **Descriptive statistics** is used to describe the basic features of the data in a study. Provide simple summaries about the sample and measures. Descriptive statistics is used to present quantitative descriptions in a manageable way. In this study, descriptive statistics was used to describe the data in terms of mean, standard deviation, standard error of the mean, efficient co-variation and describe the maximum and minimum data values.

- **Analysis of variance** (ANOVA) is a collection of statistical models used in order to analyse the differences between group means and their associated procedures (such as "variation" among and between groups), developed by R. A. Fisher.

- **Correlation matrix** is used to describe the correlation among the different traits. That can be positive or negative or may be zero. They may be perfectly correlated or they may increase or decrease according to one another according to the value present in the correlation matrix.

  Principle component analysis (PCA) when the analysis is designed to account for all of the variances including that found in the correlation coefficients and error variance; this is called a principal components analysis. It contains the following important things.

A. Scree plot:

A scree plot displays the eigenvalues associated with a component or factor in descending order versus the number of the component or factor. You can use scree plots in principal components analysis and factor analysis to visually assess which components or factors explain most of the variability in the data.

B. Eigenvalues:

Eigenvalues are commonly reported in factor analysis. They are calculated and used in deciding how many factors to extract in the overall factor analysis.

C. Eigenvectors:

Eigenvectors are a special set of vectors associated with a linear system of equations (i.e., a matrix equation) that are sometimes also known as characteristic vectors, proper vectors, or latent vectors.

D. PCA biplots:

These are the graphs which use to interpret the variability and the correlation between the different traits or factors.

CHAPTER No 3

RESULTS

3.1 MORPHOLOGICAL ANALYSIS:

This study includes following three types of germplasm. *T.carthilicum, T.polonicum, T.dicoccum.* The spikes of these genotypes are shown in the figure below.

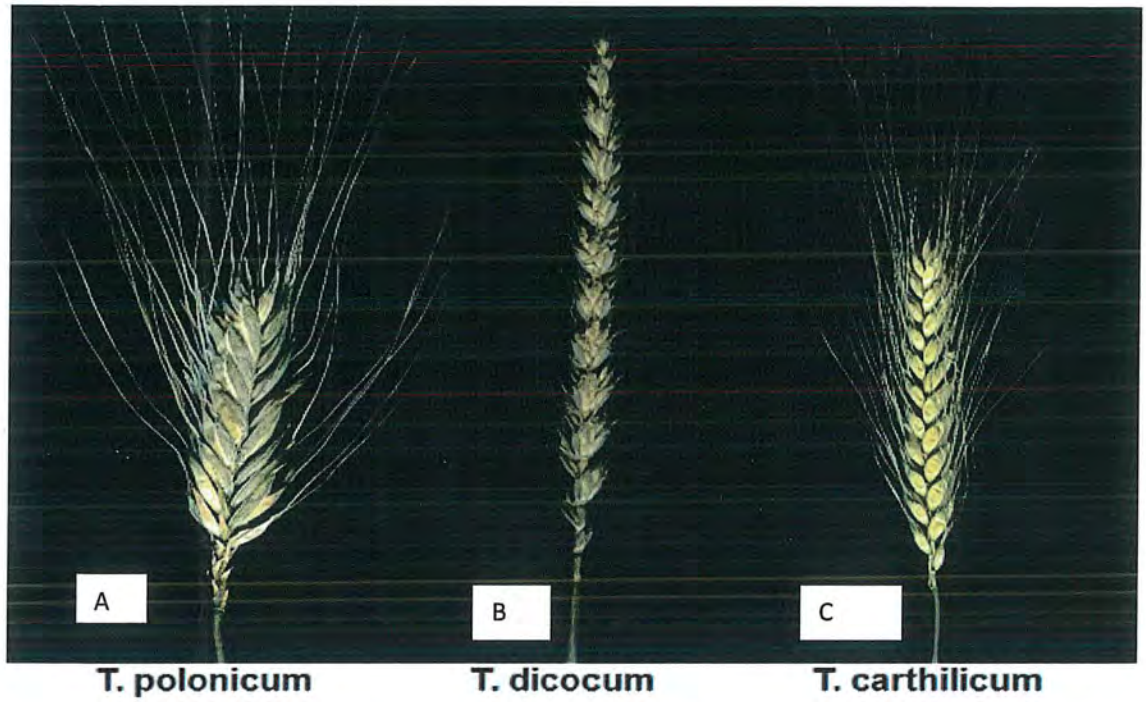

Figure 3.1 Morphology of wild relatives of wheat

In the above figure 3.1 the difference in the wheat morphology is shown. The first spike shown is *T.polonicum* spike is wider in appearance as compared to the other two, dark green in color when at senescence stage.It has brittle rachis.Awns are long and tough. The spike of *T.dicocum* spike is normal in length and consists of the non-brittle rachis.It also consists of awns.And the third one *T.carthilicum* is long and it also consists of brittle rachis it also consists of awns.

3.2 DESCRIPTIVE STATISTICS:

In this study, descriptive statistics were used to describe the data in terms of mean, standard deviation, standard error of the mean, the coefficient of variation and describe the maximum and minimum data values. Here is the picture that showed the descriptive statistics of this study.

The descriptive statistic of all yield related agronomic traits of wild relatives of bread wheat (*T.carthilicum, T.polonicum, and T.dicoccum*) was averaged from field trial given in Table 3.1. The leaf area ranged from 5.7600 to 59.500 with average of 26.283, SD 26.283, SE of mean 1.313, and C.V 54.724. The first reading of chlorophyll from 17.733 to 55.533 with an average of 39.511, SD 9.0009, SE of mean 0.8217, and CV 22.781.The second reading of chlorophyll from 11.133 to 60.667with average of 32.481, S.D 14.435, SE of mean 1.3177, and C.V 44.441.The third reading of chlorophyll from 1.2000 to 46.333 with the average of 11.827, S.D 12.608, SE of mean 1.1509, and CV 1.6.63.

The grain per spike from 0.000 to 56.000 with the average of 27.995, S.D 12.353, SE of mean 1.1276, and C.V 44.124.The spike length without awns from 0.000 and 18.667 with the average of 8.8778, S.D 3.3456, S.E of mean 0.3054, and C.V 37.685.The spike length with awns from 0.000 to 27.667 with the average of 16.955, S.D 4.613, SE of mean 0.4302 and C.V 27.21. The days of heading from 0.000 to 123.00, with the average of 111.22, S.D 18.564, SE of mean 1.6947, and C.V 16.692.The days of maturity from 0.000 to145.00 with the average of 137.36, S.D 22.77, S.E of mean 2.0786 and C.V 16.577.The spikelets per spike from 0.000 to 27.000 with the average of 19.478, S.D 5.099, S.E of mean 0.4655, and C.V 26.178.Thousand kernel weight range from 0.000 to 56.000, with the average 27.995, SD 12.353, SE of mean 11.877 and C.V 41.951.

3.3 ANALYSIS OF VARIANCE (ANOVA):

The results of the two-way analysis of variance for all yield related agronomic traits *T.carthilicum, T.polonicum, and T.dicoccum* is shown in the table 3. Acorrding to this the difference between the LA of different germplasms was found highly significant at the P value of 0.000.The difference between the LA of replicates was

found significant at the P value of 0.148827 and the difference between the LA of germplasm and the replicates was found non-significant at the P value of 0.748348.

According to the two-way analysis of variance, the difference between the CHL1 of different germplasms was found highly significant with the P value of 0.000.The difference between the CHL1 of replicates was found non-significant with the P value of 0.903988. The difference between the CHL1 of germplasm and the replicates was found non-significant with the P value of 0.918487. The difference between the CHL2 of the different germplasms was found highly significant with the P value of 0.000.The different between the CHL2 of replicates was found significant with the P value of 0.536695. And the difference between the CHL2 of germplasm and the replicates was found non-significant with the P value of 0.938892.The difference between the CHL3 of different germ plasms was found highly significant with the P value of 0.000.The difference between the CHL3 of the replicates was found non-significant with the P value of 0.878953. The difference between the CHL3 of germplasm and the replicates was found non-significant with the P value of 0.986457.

According to the two-way analysis of variance, the difference between the SL (without awns) of different germplasms was found highly significant with the P value of 0.000.The difference between the SL (without awns) of the replicates was found non-significant with the P value of 0.611919. The difference between the SL (without awns) of germplasm and the replicates was found non-significant with the P value of 0.917084.The difference between the SL A(with awns) of the different germplasms was found highly significant with the P value of 0.000. The difference between the SL A (with awns) of the replicates was found non- significant with the P value of 0.644771.The different between the SL A (with awns) of replicates and the germ plasm was found non- significant with the P value of 0.950030.

According to the two-way analysis of variance, the difference between the SP/S of different germplasms was found highly significant with P value of 0.000. The difference between the SP/S of the replicates was found non-significant with the P value of 0.636095.The difference between the SL/A of the germplasm and replicates was found significant with the P value of 0.395753.

The difference between the Grains per spike (G) of the different germplasms was found highly significant with the P value of 0.000. The difference between the Grains per spike (G) of the replicates was found non-significant with the P value of 0.990310. The difference between the Grains per spike (G) of the germplasm and the replicates was found non-significant with the P value of 0.999961.

3.4 CORRELATION MATRIX:

According to the Correlation matrix Table 4 the LA has a positive correlation with CHL1, CHL2, CHL3, SL, and SLA with the values 0.567, 0.597, 0.458, 0.254 and 0.216 respectively. And LA correlates negatively with DH, DM and SPS with the values of -0.179, -0.201 and -0.669 respectively. LA has little or some correlation with G/S and TKW with the values of 0.099 and 0.098 respectively. CHL1 correlates positively with CHL2, CHL3 DH, DM, SL, and SLA with the values of 0.702, 0.459, 0.255, 0.216, 0.516 and 0.417 respectively. CHL1 has a negative correlation with SPS with the value of -0.268.CHL1 also has little or some correlation with G/S and TKW with the values 0.001 and 0.013 respectively.CHL2 correlates positively with CHL3, DH, DM, SL and SLA with the values of 0.524, 0.212, 0.179, 0.512 and 0.379 respectively. CHL2 has negative correlation with SPS with the value of -0.316. CHL2 has a little correlation with G/S and TKW with the value of 0.061 and 0.058 respectively. CHL3 has a positive correlation with SL with the value 0.256, and it has negative correlation with SPS, G/S, TKW with the values of -0.329, -0.031, and -0.049 respectively. CHL3 has a little correlation with DH, DM and SLA with the value of 0.051, 0.043 and 0.061 respectively.

DH has a positive correlation with DM, SL, SLA, SPS, G/S and TKW with the values of 0.993, 0.517, 0.634, 0.544, 0.302 and 0.317 respectively.DM is positively correlated with SL, SLA, SPS, G/S and TKW, having values 0.486, 0.615, 0.561, 0.327 and 0.343 respectively. SL is positively correlated SLA, with the values of 0.814. SL have negatively correlated with SPS, G/S, and TKW with the value of -0.017, -0.050 and -0.035 respectively. SLA is positively correlated with G/S and TKW having values 0.210 and 0.240 respectively. SLA has a little correlation with SPS with the value of 0.140. SPS is positively correlated with G/S and TKW 0.205

and 0.200 respectively. G/S is positively correlated with TKW, with the value of 0.980.

3.4.1 CORRELATION MAP:

The correlation map (fig 3.4) uses a red-blue (hot-cold) scale to show the correlations. The blue colour corresponds to a correlation close to -1 and red corresponds to a correlation near 1. Green corresponds to a correlation close to 0.

3.5 PRINCIPAL COMPONENT ANALYSES (PCA BIPLOTS):

3.5.1 SCREE PLOT:

A scree plot for data analyzed in table 6 is presented in the figure below.

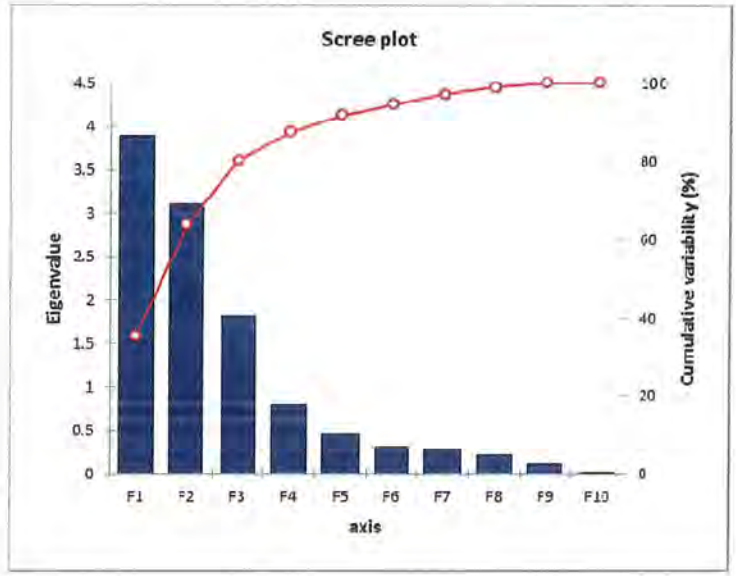

Figure 3.2: Eigenvalues plotted against factor numbers

The PCA rationalizes the data by converting number of related traits into a smaller number of the variables as factors F1, F2, F3...F11. In the above figure 3.2 (a), the scree plot shows most variability in the first six or seven factors (F1 to F7), as the scree plot is showing the curve. And the remaining factors (F7 to F11) explain a very small proportion of the variability or no variability and are likely unimportant. It seems straight line after F7 to F11. According to the table 6, F1 accounted for about 35.420 of the variation, F2 for 25.252, F3 for 16.234, F4 for 7.277, F5 for 4.229, F6

for 2.850, F7 for 2.561, F8 for 1.931, F9 for 1.023, F10 for 0.170 and F11 for 0.052 .(Table 6)

3.5.2 FACTOR ANALYSIS:

The factor analysis divides the eleven traits into eleven groups or factors. The factor which made the largest contribution accounted for 35.328 of the total variation and was composed of the some components of grain yield including LA, CHL, SL, SPS, and TKW (Table 6). Increasing spikelet number and grain number would the most effective for increasing the yield. Similarly F2, F3, F4, F5, F6, F7, F8, F9, F10, accounted for 28.226, 16.525, 7.253, 4.231, 2.871, 2.560, 1.925, 1.027, and 0.052 of total variation respectively. Grain yield of the these germplasms may be regarded as being composed of leaf area, chlorophyll content, spike length spikelet per spike, grains per spike and the 1,000 seed weight.

To better understand the relationships among the measured traits of these germplasms *(T.carthilicum, T.polonicum, T.dicoccum)* the relationships are graphically displayed in a plot of factor-1 and factor-2 in figure 3.3

3.5.3 PCA BIPLOT:

Principal component analysis was drawn to check the relationship between variables based on BIPLOT of different components. The horizontal axis relates to the first components and the vertical axis relates to the second component.

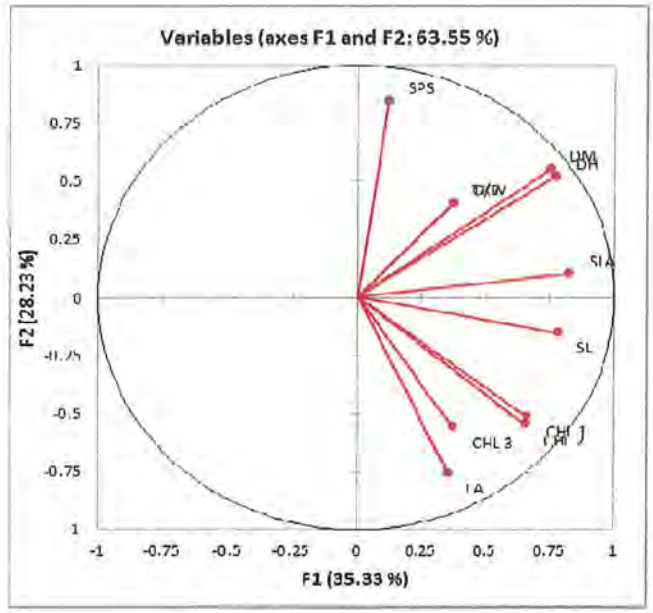

Figure: 3.3 PCA biplot of relationship between all variables and factor (F1 and F2)

Principal component analysis was drawn to check the relationship between variables based on BIPLOT of different components. The horizontal axis relates to the first components and the vertical axis relates to the second component. In this, as shown in the figure, two components F1 and F2 contribute to 35.33% and 28.23% of the total variation respectively. And the total variation shown by these components is 63.55%. In the figure, the correlation between the component can be calculated by having a look on the angle between the variables.TKW, G/S, SPS are the important traits. They are with other will affect the yield. In the above figure as TKW as making obtuse angle with DH , DM, SPS, SLA.so it has positive correlation with these factors and they will affect the yield as indicated by the small obtuse angles between their vectors (r=cos0=+1). TKW has a very strong positive correlation with G/S with almost 0 angle (r=cos0=+1). So its value will directly affect yield.LA as making obtuse angle with CHL1, CHL2, CHL3, SL and SLA showing the strong positive relation. LA has little correlation with G/S and TKW as angle near to 90°.And LA has negative correlation with DH, DM and SPS as angle greater than 90° and approximately 180° with each other (r=cos180= –1). Similarly CHL1, CHL2 has strong positive correction with other and with SL, and SLA. But they have negative correlation with SPS as having an angle greater than 90°.

They have a little correlation with G/S and TKW.As the figure is showing CHL3 away, so CHL3 will have a positive correlataion with DH, DM and SL.as making an obtuse angle i.e. (r=cos0=+1).

And it has negative relationship with SPS, G/S, and TKW as angle greater than 90^{o} and approximately 180^{o} with each other (r=cos180= –1). Both DH, DM as in the middle of all making an obtuse angle and hence having positive correlation with all other traits.

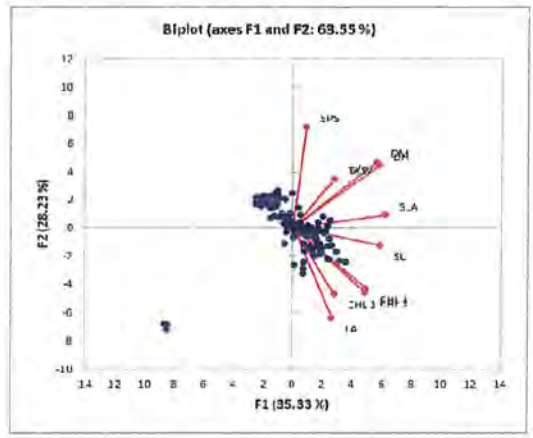

Figure: 3.4 PCA biplot of relationship between all variables and factor (F1 and F2)

CHAPTER No 4

DISCUSSION

Breeders are making efforts to meet the growing food demand, with the major focus on identifying germplasm with desired traits or amend the less congruous lines with important characteristics. Wild uncultivated amphiploid are one of the unexplored important genetic resources in the primary gene pool, but the challenge is to incorporate this genetic material into subsisting aliment crops. To identify the potential of under-utilized tetraploid wheat genotypic diversity, *T.carthilicum, T.dicoccum, and T.polonicum* were used in this study. The material was selected for genotypic performance (days to Maturity) in last year trails. This collection contains lines having gene pool yet not characterized. This knowledge of the extent of genetic variability for specific traits will play pivotal role breeding programs.

The values of the date of heading and maturity showed that among the three wild relatives in the study, *T.carthilicum* had the early maturity as compared to the other two *T.dicoccum, and T.polonicum* varieties. This variation can be used in breeding program for drought escape trait. *T. dicocum,* on the other hand, showed the late maturity, which can be used in development and characterization of stay-green trait for yield enhancement. The chlorophyll values at booting stage of the three germ plasm showed that *T. poloniocum* lines have higher chlorophyll contents as compared to the other, which ultimately converts to higher photosynthetic assimilates resulting in higher yield.

Recent studies (Teklu and Hammer, 2009)(Geleta and Grausgruber 2013) regarding yield related traits of tetra and hexaploid wheat in Ethiopia found heading days variation ranged from 121 to 135, which coincide with our findings. TKW of tetraploid wheat is reported to be between 26.9 to 49.5 (Pecetti and Damania, 1996).TKW values for the studied genotypes ranged from 14-56. Genotype with high TKW can be used in future breeding programs for higher production of wheat germ plasm.

Correlation matrix showed that LA with CHL1 and CHL2, CHL1 and CHL1, G/S and TKW, SL and SLA, LA and SL have the strong positive correlation. In this

study, a positive significant correlation is found between DH and TKW, G/S & SPA .Similarly, (Siahbidi *et al.*, 2012) Factor analysis of agro-morphological characters in durum wheat lines found the same positive correlation between the DH and SPS, G/S & TKW (Chaturvedi and Gupta, 1995;Aruna and Raghavaiah, 1997) (Sharma and Rao, 1989) and Sharma, Subhani and Khaliq, Sharma studies revealed similar results between the G/S and the other morphological characters like SPS, TKW and DH and DM. (Sharma and Rao, 1989) (Khaliq, 1994;Singh and Sharma, 1994) In this study, the principle component analysis was used to check the relationship between the variables based on the bi plots between the components. The total variation found by PCA analysis was 63.55%. F1 contribution in total variation was 35.33% and F2 contributed 28.23% .TKW as making obtuse angle with DH , DM, SPS, SLA, so it has positive correlation with these factors and they will effect the yield as indicated by the small obtuse angles between their vectors (r=cos0=+1).TKW has very strong positive correlation with G/S with almost 0 angle (r=cos0=+1).So its value will directly affect yield.

Teklu and Hammer, (2008) also computed PCA on the diversity index for 13 traits. In this the first and second component accounted for 29.8% and 20.5% of the total variability respectively that 82%.They also found the correlation among the different traits in Ethiopian tetraploid wheat. Siahbidi *et al,* (2012) (Hailu *et al.*, 2005) (Janmohammadi *et al.*, 2014) also employed this strategy of PCA for analysis the correlation among the different agronomic traits. In this study, the factor which made the largest contribution accounted for 35.328% of the total variation and was composed of the some components of grain yield including LA, CHL, SL, SPS, and TKW. Similarly F2, F3, F4, F5, F6, F7, F8, F9, F10, accounted for 28.226, 16.525, 7.253, 4.231, 2.871, 2.560, 1.925, 1.027, and 0.052 of total variation respectively(Beheshtizadeh *et al.*, 2013) employed the same method of PCA for bread wheat (*Triticum aestivum L.*) genotypes in which 4 components account for 76% of total variance. The four principal components, which accounted for about 38%, 15%, 12% and 11% of the total variation respectively, composed of the traits spike weight, number of seed/spike, spike yield, spike yield , tillering, plant height, 100seed weight, no of tillers and seed yields. Ahmad *et al,* (2014) also employ this strategy while study the multivariate analysis on bread wheat. The first component show 39.17% on the x-axis and the second component on the y-axis show 21.89% variability together it

makes 61.06% variability. That is nearly similar to our value. This show large variability among the different traits and this germplasm possesses large genetic diversity that facilitate in breeding process.

CONCLUSION:

Wheat improvement is a prerequisite for global food security issues. Wheat wild relatives are the good source for widening the genetic diversity of new germplasm that is affected by continuous selection. These tetraploids can be used in the development of high yielding synthetic hexaploid. Enhanced wheat production will be achieved by enforcing crop improvement protocols based upon the utilization of genetic diversity, crucial for the durability of stress resistances and tolerances and for ensuring sustainability. Major emphasis is devoted to consideration of the exploitation of 'alien' genetic diversity, encompassing interspecific and intergeneric hybridization categories. Characterization of these lines helps us to identify new genes for further breeding programs.

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APPENDICES

Table 1: Germ plasm And Accession Numbers

SR NO.	ENTRY	GERMPLASM	SR NO.	ENTRY	GERMPLASM
1	26-171	T.carthillicum	31	26-207	T.carthillicum
2	26-172	T.carthillicum	32	26-211	T.carthillicum
3	26-173	T.carthillicum	33	26-212	T.carthillicum
4	26-175	T.carthillicum	34	26-213	T.carthillicum
5	26-176	T.carthillicum	35	26-214	T.carthillicum
6	26-177	T.carthillicum	36	26-215	T.carthillicum
7	26-178	T.carthillicum	37	26-216	T.carthillicum
8	26-179	T.carthillicum	38	26-218	T.carthillicum
9	26-180	T.carthillicum	39	26-219	T.carthillicum
10	26-181	T.carthillicum	40	26-220	T.carthillicum
11	26-182	T.carthillicum	41	26-321	T.Polonicum
12	26-184	T.carthillicum	42	26-322	T.Polonicum
13	26-187	T.carthillicum	43	26-323	T.Polonicum
14	26-188	T.carthillicum	44	26-324	T.Polonicum
15	26-189	T.carthillicum	45	26-325	T.Polonicum
16	26-190	T.carthillicum	46	26-326	T.Polonicum
17	26-191	T.carthillicum	47	26-327	T.Polonicum
18	26-192	T.carthillicum	48	26-328	T.Polonicum
19	26-193	T.carthillicum	49	26-329	T.Polonicum
20	26-195	T.carthillicum	50	26-330	T.Polonicum
21	26-197	T.carthillicum	51	26-331	T.Polonicum
22	26-198	T.carthillicum	52	26-332	T.Polonicum
23	26-199	T.carthillicum	53	26-333	T.Polonicum
24	26-200	T.carthillicum	54	26-334	T.Polonicum
25	26-201	T.carthillicum	55	26-335	T.Polonicum
26	26-202	T.carthillicum	56	26-336	T.Polonicum
27	26-203	T.carthillicum	57	26-337	T.Polonicum
28	26-204	T.carthillicum	58	26-338	T.Polonicum
29	26-205	T.carthillicum	59	26-339	T.Polonicum
30	26-206	T.carthillicum	60	26-340	T.Polonicum

SR NO.	ENTRY	GERMPLASM	SR NO.	ENTRY	GERMPLASM
61	26-341	T.Polonicum	91	28-44	T.dicoccum
62	26-343	T.Polonicum	92	28-45	T.dicoccum
63	26-344	T.Polonicum	93	28-46	T.dicoccum
64	26-345	T.Polonicum	94	28-47	T.dicoccum
65	26-346	T.Polonicum	95	28-48	T.dicoccum
66	26-347	T.Polonicum	96	28-49	T.dicoccum
67	26-348	T.Polonicum	97	28-50	T.dicoccum
68	26-349	T.Polonicum	98	28-51	T.dicoccum
69	26-350	T.Polonicum	99	28-52	T.dicoccum
70	26-351	T.Polonicum	100	28-53	T.dicoccum
71	26-352	T.Polonicum	101	28-54	T.dicoccum
72	26-353	T.Polonicum	102	28-55	T.dicoccum
73	26-354	T.Polonicum	103	28-56	T.dicoccum
74	26-355	T.Polonicum	104	28-57	T.dicoccum
75	26-356	T.Polonicum	105	28-58	T.dicoccum
76	26-357	T.Polonicum	106	28-59	T.dicoccum
77	26-358	T.Polonicum	107	28-60	T.dicoccum
78	26-359	T.Polonicum	108	28-61	T.dicoccum
79	26-360	T.Polonicum	109	28-62	T.dicoccum
80	26-361	T.Polonicum	110	28-63	T.dicoccum
81	28-33	T.dicoccum	111	28-64	T.dicoccum
82	28-34	T.dicoccum	112	28-65	T.dicoccum
83	28-35	T.dicoccum	113	28-66	T.dicoccum
84	28-36	T.dicoccum	114	28-67	T.dicoccum
85	28-37	T.dicoccum	115	28-68	T.dicoccum
86	28-38	T.dicoccum	116	28-69	T.dicoccum
87	28-39	T.dicoccum	117	28-70	T.dicoccum
88	28-41	T.dicoccum	118	28-71	T.dicoccum
89	28-42	T.dicoccum	119	28-72	T.dicoccum
90	28-43	T.dicoccum	120	28-73	T.dicoccum

Table 2: Descriptive Statistical Analysis of the data regarding yield parameters of Tetraploid wheat Genotypes (*T.carthilicum, T.polonicum, and T.dicoccum)*

Traits	N	Range	Mean	SD	SE Mean	C.V.
LA	120	5.7600-59.500	26.283	26.283	1.313	54.724
CHL1	120	17.733-55.533	39.511	9.0009	0.8217	22.781
CHL2	120	11.133-60.667	32.481	14.435	1.3177	44.441
CHL3	120	1.2000-46.333	11.827	12.608	1.1509	106.6
DH	120	0.0000-123.00	111.22	18.564	1.6947	16.692
DM	120	0.000-145.00	137.36	22.77	2.0786	16.577
SL	120	0.0000-18.667	8.8778	3.3456	0.3054	37.685
SLA	115	0.000-27.667	16.955	4.6135	0.4302	27.21
SPS	120	0.0000-27.000	19.478	5.099	0.4655	26.178
G/S	120	0.000-56.000	27.995	12.353	1.1276	44.124
TKW	120	0.000-56.000	27.995	12.353	11.877	41.951

Table 3: Correlation Metrix of the data regarding yield parameters of Tetraploid wheat Genotypes *(T.carthilicum, T.polonicum, and T.dicoccum).*

Variables	LA	CHL 1	CHL 2	CHL 3	DH	DM	SL	SLA	SPS	G/S	TKW
LA	1	**0.567**	**0.597**	**0.485**	-0.179	**-0.201**	**0.254**	**0.216**	**-0.669**	0.099	0.098
CHL 1	**0.567**	1	**0.702**	**0.459**	**0.255**	**0.216**	**0.516**	**0.417**	**-0.268**	0.001	0.013
CHL 2	**0.597**	**0.702**	1	**0.524**	**0.212**	0.179	**0.512**	**0.379**	**-0.316**	0.061	0.058
CHL 3	**0.485**	**0.459**	**0.524**	1	0.051	0.043	**0.256**	0.061	**-0.329**	-0.031	-0.049
DH	-0.179	**0.255**	**0.212**	0.051	1	**0.993**	**0.517**	**0.634**	**0.544**	**0.302**	**0.317**
DM	**-0.201**	**0.216**	0.179	0.043	**0.993**	1	**0.486**	**0.615**	**0.561**	**0.327**	**0.343**
SL	**0.254**	**0.516**	**0.512**	**0.256**	**0.517**	**0.486**	1	**0.810**	-0.017	-0.050	-0.035
SLA	**0.216**	**0.417**	**0.379**	0.061	**0.634**	**0.615**	**0.810**	1	0.140	**0.210**	**0.240**
SPS	**-0.669**	**-0.268**	**-0.316**	**-0.329**	**0.544**	**0.561**	-0.017	0.140	1	**0.205**	**0.200**
G/S	0.099	0.001	0.061	-0.031	**0.302**	**0.327**	-0.050	**0.210**	**0.205**	1	**0.980**
TKW	0.098	0.013	0.058	-0.049	**0.317**	**0.343**	-0.035	**0.240**	**0.200**	**0.980**	1

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

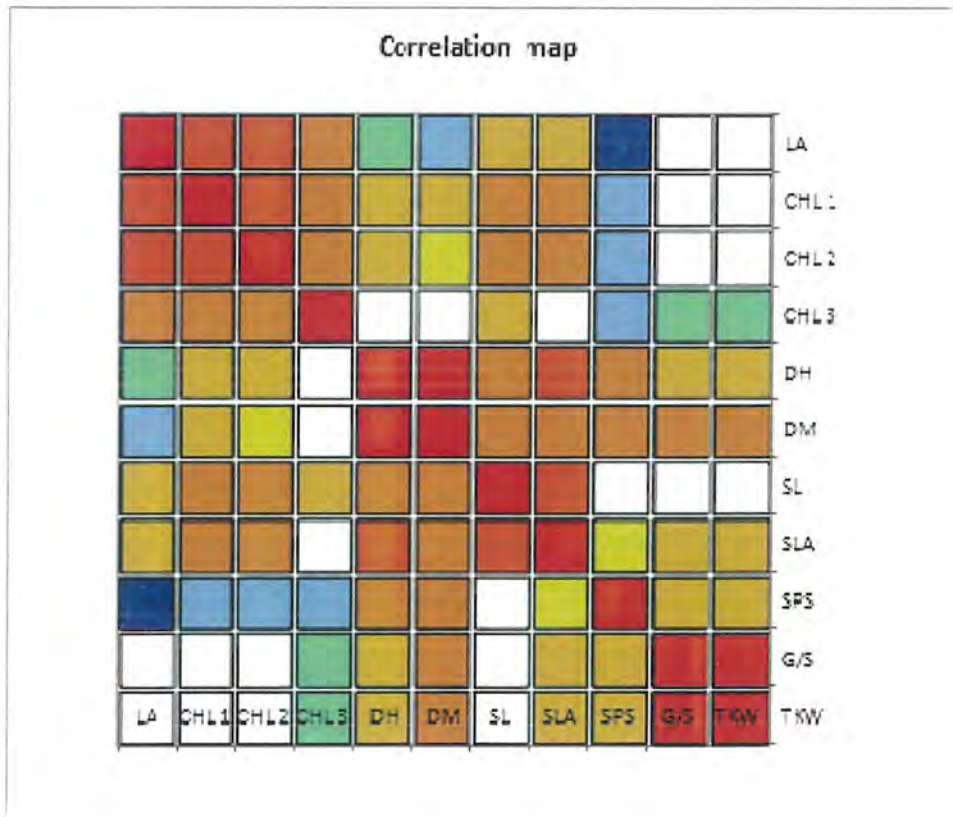

Figure 3.5: Correlation Map

Table 4: Eigenvectors and factor analysis of the data regarding yield parameters of Tetraploid wheat Genotypes *(T.carthilicum, T.polonicum, and T.dicoccum).*

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
LA	0.181	-0.431	0.262	-0.169	-0.034	-0.355	0.061	0.671	-0.320	-0.003
CHL 1	0.336	-0.293	-0.032	0.091	0.609	-0.208	-0.560	-0.248	0.047	-0.021
CHL 2	0.333	-0.312	0.041	0.141	0.365	0.537	0.573	0.044	0.135	-0.012
CHL 3	0.190	-0.318	0.063	0.690	-0.547	0.092	-0.231	0.007	0.149	0.016
DH	0.392	0.294	-0.156	0.185	0.014	-0.353	0.255	-0.047	-0.069	0.710
DM	0.382	0.312	-0.138	0.199	-0.018	-0.362	0.261	-0.041	-0.047	-0.703
SL	0.397	-0.087	-0.285	-0.316	-0.298	0.333	-0.140	-0.249	-0.610	-0.017
SLA	0.419	0.056	-0.140	-0.472	-0.235	0.056	-0.162	0.211	0.670	0.007
SPS	0.062	0.479	-0.154	0.257	0.222	0.383	-0.336	0.588	-0.151	-0.002
G/S	0.189	0.229	0.615	-0.051	-0.030	0.094	-0.066	-0.122	-0.040	0.008
TKW	0.189	0.229	0.615	-0.051	-0.030	0.094	-0.066	-0.122	-0.040	0.008

Table 5: Factor loading values (-1 to 1) for different variables of the data regarding yield parameters of Tetraploid wheat Genotypes *(T.carthilicum, T.polonicum, and T.dicoccum).*

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
LA	0.356	-0.760	0.354	-0.151	-0.023	-0.199	0.032	0.309	-0.108	0.000
CHL 1	0.661	-0.516	-0.043	0.081	0.416	-0.117	-0.297	-0.114	0.016	-0.002
CHL 2	0.656	-0.551	0.056	0.126	0.249	0.302	0.304	0.020	0.045	-0.001
CHL 3	0.374	-0.560	0.084	0.616	-0.373	0.052	-0.123	0.003	0.050	0.001
DH	0.773	0.519	-0.210	0.165	0.010	-0.199	0.135	-0.022	-0.023	0.054
DM	0.753	0.550	-0.186	0.178	-0.013	-0.204	0.138	-0.019	-0.016	-0.053
SL	0.782	-0.153	-0.384	-0.282	-0.203	0.187	-0.074	-0.115	-0.205	-0.001
SLA	0.826	0.099	-0.189	-0.421	-0.161	0.031	-0.086	0.097	0.225	0.001
SPS	0.122	0.844	-0.207	0.229	0.151	0.215	-0.178	0.271	-0.051	0.000
G/S	0.373	0.404	0.829	-0.045	-0.020	0.053	-0.035	-0.056	-0.014	0.001
TKW	0.373	0.404	0.829	-0.045	-0.020	0.053	-0.035	-0.056	-0.014	0.001
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Eigenvalue	3.886	3.105	1.818	0.798	0.465	0.316	0.282	0.212	0.113	0.006
Variability	35.328	28.226	16.525	7.253	4.231	2.871	2.560	1.925	1.027	0.052
Cumulativ	35.328	63.554	80.079	87.332	91.564	94.435	96.995	98.921	99.948	100.000

Table 6: Correlation between the variable and factors of the data regarding yield parameters of Tetraploid wheat Genotypes *(T.carthilicum, T.polonicum, and T.dicoccum)*

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
LA	0.356	-0.760	0.354	-0.151	-0.023	-0.199	0.032	0.309	-0.108	0.000
CHL 1	0.661	-0.516	-0.043	0.081	0.416	-0.117	-0.297	-0.114	0.016	-0.002
CHL 2	0.656	-0.551	0.056	0.126	0.249	0.302	0.304	0.020	0.045	-0.001
CHL 3	0.374	-0.560	0.084	0.616	-0.373	0.052	-0.123	0.003	0.050	0.001
DH	0.773	0.519	-0.210	0.165	0.010	-0.199	0.135	-0.022	-0.023	0.054
DM	0.753	0.550	-0.186	0.178	-0.013	-0.204	0.138	-0.019	-0.016	-0.053
SL	0.782	-0.153	-0.384	-0.282	-0.203	0.187	-0.074	-0.115	-0.205	-0.001
SLA	0.826	0.099	-0.189	-0.421	-0.161	0.031	-0.086	0.097	0.225	0.001
SPS	0.122	0.844	-0.207	0.229	0.151	0.215	-0.178	0.271	-0.051	0.000
G/S	0.373	0.404	0.829	-0.045	-0.020	0.053	-0.035	-0.056	-0.014	0.001
TKW	0.373	0.404	0.829	-0.045	-0.020	0.053	-0.035	-0.056	-0.014	0.001

Table 7: Contribution of the variables of the data regarding yield parameters of Tetraploid wheat Genotypes *(T.carthilicum, T.polonicum, and T.dicoccum)*

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
LA	3.153	18.743	7.024	2.619	0.138	11.786	0.549	46.244	9.714	0.030	0.001
CHL 1	11.094	8.719	0.107	0.794	37.119	5.234	30.786	5.880	0.190	0.028	0.050
CHL 2	10.843	10.009	0.124	2.032	13.424	30.437	31.179	0.102	1.822	0.016	0.012
CHL 3	3.456	10.350	0.249	47.372	29.917	0.824	5.622	0.000	2.172	0.012	0.028
DH	15.492	8.458	2.487	3.450	0.016	12.127	6.978	0.175	0.433	0.254	50.130
DM	14.715	9.537	1.929	4.017	0.042	12.646	7.280	0.134	0.208	0.157	49.336
SL	15.639	0.808	8.315	10.427	8.642	10.285	2.135	6.009	37.631	0.089	0.020
SLA	17.615	0.293	1.802	22.276	5.528	0.331	2.666	4.302	44.972	0.214	0.001
SPS	0.418	22.811	2.530	6.598	4.984	14.474	11.838	34.106	2.156	0.084	0.000
G/S	3.638	5.059	38.193	0.068	0.117	1.303	0.432	1.244	0.700	49.109	0.136
TKW	3.937	5.214	37.241	0.346	0.072	0.554	0.535	1.805	0.003	50.007	0.286

Table 8: Factor Scores of data regarding yield parameters of Tetraploid wheat Genotypes *(T.carthilicum, T.polonicum, and T.dicoccum)*

Observatio	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Obs1	0.062	0.197	-0.377	-0.678	-0.374	-0.380	-0.040	-0.437	-0.015	-0.008
Obs2	-0.283	0.052	-0.653	-0.275	0.305	-0.658	-0.120	-0.009	-0.119	0.049
Obs3	0.066	-0.230	-0.987	0.240	1.249	0.688	0.273	-0.179	0.127	0.059
Obs4	-0.147	0.956	-1.735	-0.641	-0.148	-0.178	-0.781	0.379	-0.140	0.194
Obs5	0.560	-0.461	-1.319	-0.259	0.956	-0.138	0.384	-0.760	0.148	-0.161
Obs6	-0.593	0.775	-0.525	-1.128	-0.745	-0.640	-0.178	-0.482	-0.130	-0.040
Obs7	-0.917	0.666	-1.046	-0.402	-0.397	-0.178	0.284	0.073	-0.380	-0.049
Obs8	-0.246	0.317	-0.147	0.157	0.470	-0.257	0.027	-0.333	0.631	-0.041
Obs9	-0.716	0.988	-1.515	-0.096	-0.255	0.262	0.674	0.542	-0.345	0.016
Obs10	0.903	-0.394	-1.002	1.549	0.051	0.561	-0.011	0.484	-0.150	-0.218
Obs11	0.130	-0.591	-0.705	-0.057	0.898	-0.383	1.026	-1.015	0.490	0.030
Obs12	1.584	-0.561	-1.185	-1.458	-0.110	0.393	0.463	-1.018	0.150	-0.028
Obs13	-0.291	0.579	-1.040	-0.090	0.396	0.099	0.229	0.145	-0.213	-0.047
Obs14	-0.025	0.373	-1.157	-0.385	0.327	-0.038	-0.492	0.139	-0.005	-0.075
Obs15	0.503	0.302	-1.234	0.462	0.303	0.145	-0.638	0.242	0.049	-0.037
Obs16	1.011	0.100	-0.320	0.091	1.468	0.766	-0.363	0.247	-0.121	0.005
Obs17	0.604	0.043	-0.622	-1.009	0.033	0.216	0.393	0.209	-0.410	0.004
Obs18	-0.393	-0.403	-1.507	-0.185	0.300	0.274	0.550	-0.292	0.046	0.004
Obs19	1.190	-0.054	-0.988	-1.004	-0.376	-0.152	-0.058	-0.383	-0.323	-0.025
Obs20	1.447	-0.794	1.005	-0.009	-0.247	0.582	0.173	-1.255	0.457	0.006
Obs21	-0.447	0.786	2.879	-0.105	0.186	-0.479	0.087	-1.579	-0.499	0.060
Obs22	0.402	-0.127	-1.477	-1.291	-0.856	-0.508	-0.460	-0.455	-0.121	-0.057
Obs23	0.492	0.870	-1.668	-0.536	-0.749	0.223	-1.333	0.001	-0.148	0.020
Obs24	-0.129	0.414	-0.599	-0.697	0.101	-0.516	-0.129	-0.146	-0.129	-0.150
Obs25	1.595	-0.808	-1.270	0.862	0.237	1.056	-0.663	0.632	0.610	0.023
Obs26	-0.211	0.686	-1.166	-0.620	0.210	0.070	-0.871	0.125	-0.331	0.041
Obs27	0.981	-0.669	-0.695	-0.637	0.941	0.685	0.135	-0.540	0.112	0.286
Obs28	0.037	-0.182	-1.110	-0.496	0.336	-0.172	0.585	-0.426	0.055	-0.020
Obs29	-0.538	-1.175	-2.925	-0.513	0.265	-0.374	0.882	-0.056	0.386	-0.062
Obs30	1.747	0.322	-1.515	-0.467	0.865	1.578	0.398	0.766	0.047	0.041
Obs31	1.738	-0.046	-0.746	-1.024	0.342	1.091	0.354	-0.396	-0.155	-0.106
Obs32	2.467	-0.817	-1.983	0.406	-0.329	1.073	-0.574	0.261	0.198	-0.036
Obs33	0.220	0.022	-1.381	-0.394	-0.476	-0.381	0.091	-0.210	-0.297	0.083
Obs34	-0.397	0.501	-1.721	-0.022	0.463	-0.342	-0.775	0.151	-0.197	-0.135
Obs35	0.636	-0.459	-1.021	-0.105	0.671	-0.049	0.517	-0.244	-0.079	-0.012
Obs36	1.907	-0.248	-1.292	-1.147	0.096	0.946	0.537	-0.354	-0.046	-0.018
Obs37	0.075	-0.791	-3.349	-1.208	0.089	-0.912	-1.035	0.168	0.278	-0.044
Obs38	1.141	-0.204	-1.550	-1.171	-0.092	-0.012	-1.143	0.346	-0.588	0.003
Obs39	1.305	-0.242	-0.060	1.125	-0.540	0.491	-0.880	-0.285	-0.687	0.076
Obs40	0.625	-0.059	-1.106	-0.767	0.437	0.412	-0.095	0.447	-0.190	-0.062

Obs41	1.234	-0.141	2.185	-1.170	0.507	-0.753	-0.353	0.603	0.182	-0.057
Obs42	0.446	0.315	2.439	-1.099	0.134	-1.199	0.069	0.322	0.061	-0.017
Obs43	2.146	-0.465	3.305	0.304	-0.436	0.495	0.219	0.444	0.774	-0.007
Obs44	1.451	-0.217	3.321	-0.930	0.663	-0.750	0.019	0.228	-0.163	-0.076
Obs45	2.250	-0.291	1.416	-1.340	-3.234	1.284	0.509	0.164	0.132	-0.010
Obs46	1.861	-1.184	-0.822	0.281	-0.724	-0.290	-0.386	0.499	0.818	0.000
Obs47	1.411	-0.683	1.063	-0.050	-0.153	-0.820	-1.743	-0.031	0.196	-0.030
Obs48	1.383	-0.214	0.740	-0.608	-0.310	-0.232	-1.376	-1.052	0.131	0.032
Obs49	1.593	-0.997	0.652	0.306	-0.032	0.009	-0.684	0.165	0.109	0.004
Obs50	1.911	-1.586	0.449	1.155	-0.191	0.298	-0.840	0.252	-0.529	0.031
Obs51	3.584	-2.495	-0.189	-1.713	-1.682	0.043	0.121	-0.513	-0.127	0.017
Obs52	1.428	-2.056	1.194	0.425	-0.723	-1.222	0.782	0.271	-0.221	0.083
Obs53	1.924	-1.885	0.596	0.319	0.499	0.200	-0.470	-0.011	-0.646	0.021
Obs54	2.036	-1.502	0.738	0.237	0.002	-0.044	0.305	0.258	-0.828	-0.003
Obs55	-8.344	-6.902	1.234	-1.261	1.184	0.868	0.051	-0.143	-0.056	-0.028
Obs56	0.802	-0.578	-0.798	-0.356	0.734	-0.302	0.203	0.591	0.617	0.016
Obs57	3.173	-2.354	2.590	1.100	-0.011	-0.219	-0.050	0.265	0.348	0.059
Obs58	1.643	-0.943	2.216	-0.903	1.449	-0.355	0.422	-0.191	0.149	-0.016
Obs59	2.403	-1.339	0.072	-1.977	0.153	0.312	0.210	0.222	-0.309	-0.044
Obs60	2.371	0.084	2.381	-0.690	-0.001	-0.036	-0.302	0.976	0.039	0.033
Obs61	1.347	-0.195	-0.115	-1.474	0.089	0.290	-0.050	-0.210	0.069	-0.015
Obs62	1.768	-1.937	1.188	1.276	-0.785	-0.578	0.218	0.239	0.083	-0.435
Obs63	0.728	-2.506	-2.937	-0.573	-0.284	-0.257	0.649	-0.033	0.168	0.089
Obs64	0.724	-1.189	0.674	0.403	-1.021	-0.570	1.065	0.243	-0.267	0.150
Obs65	0.802	-1.699	1.030	-0.328	-0.942	-1.205	0.285	0.429	0.117	0.065
Obs66	2.422	-2.294	0.248	0.157	-1.264	0.661	0.454	-0.746	0.692	0.048
Obs67	2.920	-1.779	3.329	0.501	0.280	-0.052	-0.157	0.356	0.004	0.061
Obs68	-8.390	-7.248	1.437	-0.085	-0.646	0.637	-0.494	0.227	-0.001	0.026
Obs69	1.151	-1.330	0.262	-0.569	1.369	-0.465	0.036	0.298	0.159	0.097
Obs70	0.708	-2.958	-2.521	1.872	0.493	-0.344	0.640	-0.146	0.350	0.002
Obs71	2.515	0.471	2.199	-0.595	0.925	0.697	0.591	0.230	-0.002	0.006
Obs72	-8.588	-6.896	1.312	-0.572	-0.198	0.422	-0.668	-0.005	-0.070	0.014
Obs73	0.934	-0.137	-0.044	-0.012	0.715	-0.632	-0.004	0.177	-0.992	0.021
Obs74	1.302	-1.633	0.690	0.993	1.396	-0.619	0.714	-0.203	-0.587	-0.066
Obs75	2.629	-1.271	-0.293	1.345	-1.284	0.644	-0.619	0.069	-0.096	0.046
Obs76	1.509	-0.667	2.742	-0.474	0.425	-0.432	0.318	0.533	-0.096	-0.008
Obs77	0.692	-3.313	-2.228	2.507	-0.284	-0.839	0.481	0.384	0.018	0.039
Obs78	0.128	-2.667	-2.192	1.473	-0.168	-1.023	1.174	0.948	-0.468	0.007
Obs79	1.643	-1.408	0.152	1.306	-1.045	-0.314	-0.257	-1.182	0.359	-0.048
Obs80	2.081	0.737	1.714	-0.104	1.378	0.803	0.493	0.712	-0.198	0.068
Obs81	0.005	2.391	2.019	0.895	0.480	0.992	0.820	-0.857	-0.351	-0.017
Obs82	-0.857	1.984	0.070	0.296	0.439	0.669	0.136	-0.037	0.198	-0.060
Obs83	-1.943	1.886	-0.587	-0.138	-0.631	-0.278	0.296	0.385	0.161	-0.009
Obs84	-1.865	2.152	0.150	0.173	-0.582	0.032	0.442	0.462	-0.088	0.001
Obs85	-1.170	2.355	-0.081	0.354	-0.047	-0.228	0.048	0.357	0.239	0.146
Obs86	-0.126	0.428	-0.280	1.743	-0.417	1.326	-0.197	-0.316	-0.693	0.012
Obs87	-1.624	1.916	0.153	0.158	-0.138	-0.149	0.248	-0.149	0.087	-0.007
Obs88	-1.204	2.039	0.483	0.129	-0.816	-0.150	-0.243	0.246	0.156	0.012
Obs89	-1.439	1.446	-0.041	0.089	0.292	0.232	-0.054	-0.632	0.011	-0.070
Obs90	-1.473	2.193	1.006	-0.148	-0.222	0.091	-0.403	-0.096	0.042	-0.042

Obs91	-1.150	1.673	0.108	-0.346	-0.338	-0.173	0.089	0.473	0.251	-0.017
Obs92	0.422	1.351	0.715	-0.384	0.618	-0.645	-0.612	-0.599	0.270	0.081
Obs93	-1.469	0.632	-3.212	0.058	0.021	-0.817	0.458	0.373	0.302	0.134
Obs94	-1.956	2.020	-0.226	0.324	-0.167	0.010	0.636	0.459	0.203	-0.001
Obs95	-1.281	1.843	0.023	0.080	0.252	-0.057	-0.361	0.217	0.106	0.032
Obs96	-1.999	1.870	0.325	0.246	-0.015	0.132	0.012	0.230	0.046	-0.056
Obs97	-2.216	1.975	0.491	0.016	-0.380	-0.115	0.290	0.358	0.186	-0.001
Obs98	-1.986	2.195	0.970	0.049	-0.212	0.289	-0.260	0.496	0.107	-0.031
Obs99	-2.366	1.747	0.805	0.292	0.037	0.044	0.314	-0.196	0.098	0.038
Obs100	-1.859	2.172	0.654	-0.084	-0.472	0.546	0.151	0.626	0.037	0.013
Obs101	-2.394	1.996	0.541	0.437	-0.383	0.246	0.729	0.044	-0.439	-0.068
Obs102	-1.764	1.696	0.382	-0.032	-0.292	-0.456	0.278	-0.202	0.104	0.022
Obs103	-2.072	1.530	0.832	-0.375	-0.742	-0.174	0.695	-0.398	0.254	-0.006
Obs104	-1.016	1.510	0.436	-0.268	0.000	-0.252	-0.077	-0.194	0.267	0.075
Obs105	-2.379	1.615	-0.018	-0.195	-0.724	-0.078	0.365	0.123	0.253	-0.057
Obs106	-1.614	1.548	0.635	-0.168	-0.112	0.043	-0.208	0.217	-0.027	-0.007
Obs107	-0.914	2.251	0.094	0.057	0.169	-0.104	-0.509	0.061	0.051	0.001
Obs108	-0.482	1.935	0.385	0.630	0.319	0.294	-0.234	0.022	0.370	0.010
Obs109	-0.997	2.553	0.642	0.307	0.023	-0.006	-0.117	-0.235	-0.278	0.066
Obs110	0.564	0.127	0.153	2.430	0.001	0.638	-0.246	0.015	0.503	0.086
Obs111	-2.062	1.475	0.484	-0.231	-0.533	-0.165	0.227	0.385	-0.117	0.005
Obs112	-0.554	0.249	0.382	1.425	0.658	0.837	-0.405	0.057	0.474	-0.035
Obs113	-1.487	1.354	0.397	0.248	0.625	-0.001	-0.498	-0.044	0.096	-0.020
Obs114	-2.374	2.098	0.544	-0.207	-1.000	0.173	0.635	-0.036	0.205	0.037
Obs115	-1.373	1.329	-0.011	0.461	1.085	-0.858	-0.733	-0.594	0.330	0.028
Obs116	-1.296	1.490	-0.269	0.233	0.116	-0.269	-0.081	0.498	0.141	-0.022
Obs117	-1.057	1.731	0.210	0.211	0.655	-0.123	-0.506	-0.112	0.213	-0.039
Obs118	-1.429	2.094	-0.243	-0.019	-0.648	0.573	0.309	-0.198	-1.018	0.009
Obs119	-0.332	0.046	-0.023	3.312	-0.015	-0.063	-0.113	-0.509	0.025	-0.055
Obs120	-1.238	0.820	0.286	2.847	-0.178	-0.982	-0.315	-0.839	-0.659	0.072

Table 9: Contribution of Observation (%):

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Obs1	0.001	0.010	0.065	0.480	0.251	0.382	0.005	0.753	0.002	0.009
Obs2	0.017	0.001	0.195	0.079	0.166	1.142	0.043	0.000	0.104	0.344
Obs3	0.001	0.014	0.447	0.060	2.794	1.248	0.221	0.127	0.119	0.499
Obs4	0.005	0.245	1.379	0.429	0.039	0.084	1.805	0.566	0.145	5.433
Obs5	0.067	0.057	0.798	0.070	1.637	0.050	0.436	2.272	0.162	3.752
Obs6	0.075	0.161	0.126	1.329	0.993	1.080	0.094	0.915	0.125	0.231
Obs7	0.180	0.119	0.502	0.169	0.282	0.083	0.238	0.021	1.064	0.341
Obs8	0.013	0.027	0.010	0.026	0.395	0.174	0.002	0.437	2.936	0.245
Obs9	0.110	0.262	1.052	0.010	0.117	0.182	1.343	1.155	0.880	0.037
Obs10	0.175	0.042	0.461	2.506	0.005	0.830	0.000	0.922	0.165	6.867
Obs11	0.004	0.094	0.228	0.003	1.445	0.387	3.112	4.055	1.770	0.130
Obs12	0.538	0.084	0.644	2.220	0.022	0.408	0.634	4.081	0.166	0.110
Obs13	0.018	0.090	0.496	0.008	0.281	0.026	0.155	0.082	0.334	0.325
Obs14	0.000	0.037	0.613	0.155	0.191	0.004	0.717	0.076	0.000	0.815
Obs15	0.054	0.024	0.698	0.223	0.164	0.056	1.205	0.230	0.018	0.197
Obs16	0.219	0.003	0.047	0.009	3.856	1.549	0.390	0.240	0.109	0.003
Obs17	0.078	0.000	0.177	1.064	0.002	0.123	0.456	0.172	1.239	0.002
Obs18	0.033	0.044	1.041	0.036	0.162	0.198	0.895	0.335	0.015	0.002
Obs19	0.303	0.001	0.448	1.053	0.253	0.061	0.010	0.578	0.768	0.091
Obs20	0.449	0.169	0.463	0.000	0.110	0.895	0.089	6.196	1.542	0.005
Obs21	0.043	0.166	3.800	0.011	0.062	0.606	0.022	9.812	1.840	0.524
Obs22	0.035	0.004	1.000	1.740	1.313	0.680	0.625	0.816	0.108	0.477
Obs23	0.052	0.203	1.275	0.300	1.004	0.131	5.258	0.000	0.162	0.058
Obs24	0.004	0.046	0.164	0.507	0.018	0.703	0.050	0.084	0.122	3.240
Obs25	0.546	0.175	0.739	0.775	0.101	2.943	1.302	1.572	2.747	0.076
Obs26	0.010	0.126	0.623	0.402	0.079	0.013	2.245	0.061	0.808	0.242
Obs27	0.206	0.120	0.221	0.424	1.584	1.239	0.054	1.145	0.092	11.819
Obs28	0.000	0.009	0.564	0.257	0.202	0.078	1.012	0.713	0.022	0.058
Obs29	0.062	0.371	3.922	0.274	0.126	0.370	2.302	0.012	1.098	0.559
Obs30	0.655	0.028	1.052	0.227	1.340	6.573	0.468	2.310	0.016	0.242
Obs31	0.648	0.001	0.255	1.095	0.209	3.138	0.370	0.618	0.178	1.622
Obs32	1.305	0.179	1.803	0.172	0.194	3.039	0.975	0.268	0.290	0.185
Obs33	0.010	0.000	0.874	0.162	0.405	0.383	0.024	0.174	0.649	1.002
Obs34	0.034	0.067	1.358	0.001	0.384	0.309	1.775	0.089	0.285	2.634
Obs35	0.087	0.057	0.477	0.011	0.806	0.006	0.792	0.234	0.046	0.020
Obs36	0.780	0.016	0.765	1.373	0.016	2.363	0.852	0.494	0.016	0.047
Obs37	0.001	0.168	5.141	1.525	0.014	2.196	3.167	0.112	0.571	0.282
Obs38	0.279	0.011	1.101	1.432	0.015	0.000	3.866	0.472	2.550	0.001
Obs39	0.365	0.016	0.002	1.323	0.523	0.635	2.291	0.321	3.482	0.827
Obs40	0.084	0.001	0.561	0.614	0.342	0.449	0.027	0.785	0.268	0.561
Obs41	0.327	0.005	2.190	1.430	0.461	1.494	0.368	1.431	0.243	0.469
Obs42	0.043	0.027	2.726	1.262	0.032	3.792	0.014	0.408	0.027	0.043
Obs43	0.988	0.058	5.008	0.096	0.340	0.645	0.142	0.775	4.418	0.007
Obs44	0.451	0.013	5.056	0.904	0.786	1.484	0.001	0.204	0.196	0.828
Obs45	1.086	0.023	0.919	1.877	18.721	4.351	0.766	0.106	0.128	0.014

Obs46	0.743	0.376	0.310	0.082	0.938	0.222	0.440	0.981	4.934	0.000
Obs47	0.427	0.125	0.518	0.003	0.042	1.774	8.987	0.004	0.284	0.129
Obs48	0.410	0.012	0.251	0.386	0.172	0.142	5.601	4.353	0.127	0.145
Obs49	0.544	0.267	0.195	0.098	0.002	0.000	1.383	0.107	0.088	0.003
Obs50	0.783	0.675	0.092	1.394	0.065	0.235	2.086	0.251	2.067	0.138
Obs51	2.755	1.671	0.016	3.066	5.067	0.005	0.043	1.034	0.119	0.044
Obs52	0.438	1.135	0.653	0.188	0.935	3.940	1.808	0.288	0.361	0.989
Obs53	0.794	0.953	0.163	0.107	0.446	0.105	0.655	0.000	3.082	0.063
Obs54	0.889	0.606	0.249	0.059	0.000	0.005	0.276	0.262	5.061	0.002
Obs55	14.930	12.786	0.698	1.660	2.508	1.986	0.008	0.081	0.023	0.117
Obs56	0.138	0.090	0.292	0.133	0.965	0.241	0.121	1.374	2.809	0.038
Obs57	2.158	1.487	3.076	1.263	0.000	0.127	0.007	0.277	0.893	0.500
Obs58	0.579	0.239	2.250	0.853	3.758	0.333	0.527	0.144	0.163	0.036
Obs59	1.238	0.481	0.002	4.081	0.042	0.257	0.130	0.194	0.705	0.285
Obs60	1.206	0.002	2.598	0.498	0.000	0.003	0.271	3.747	0.011	0.155
Obs61	0.389	0.010	0.006	2.269	0.014	0.222	0.008	0.174	0.036	0.035
Obs62	0.670	1.007	0.647	1.700	1.102	0.882	0.140	0.225	0.051	27.374
Obs63	0.114	1.686	3.955	0.343	0.144	0.175	1.247	0.004	0.207	1.149
Obs64	0.112	0.379	0.208	0.170	1.867	0.857	3.355	0.233	0.524	3.271
Obs65	0.138	0.775	0.487	0.113	1.587	3.828	0.241	0.725	0.100	0.619
Obs66	1.258	1.412	0.028	0.026	2.862	1.152	0.610	2.188	3.529	0.327
Obs67	1.828	0.850	5.079	0.262	0.140	0.007	0.073	0.499	0.000	0.542
Obs68	15.095	14.098	0.947	0.007	0.748	1.070	0.721	0.203	0.000	0.099
Obs69	0.284	0.475	0.031	0.338	3.356	0.571	0.004	0.349	0.187	1.347
Obs70	0.107	2.348	2.913	3.661	0.435	0.313	1.213	0.084	0.905	0.000
Obs71	1.356	0.060	2.217	0.370	1.532	1.283	1.033	0.207	0.000	0.005
Obs72	15.814	12.765	0.789	0.342	0.070	0.470	1.319	0.000	0.036	0.027
Obs73	0.187	0.005	0.001	0.000	0.915	1.053	0.000	0.124	7.263	0.062
Obs74	0.363	0.716	0.218	1.030	3.488	1.012	1.507	0.162	2.544	0.639
Obs75	1.482	0.434	0.039	1.889	2.951	1.095	1.133	0.018	0.068	0.307
Obs76	0.488	0.119	3.446	0.234	0.323	0.492	0.299	1.116	0.067	0.009
Obs77	0.103	2.947	2.276	6.563	0.145	1.855	0.685	0.581	0.002	0.225
Obs78	0.003	1.909	2.203	2.267	0.051	2.763	4.076	3.537	1.617	0.008
Obs79	0.579	0.532	0.011	1.781	1.954	0.261	0.195	5.500	0.952	0.332
Obs80	0.928	0.146	1.346	0.011	3.398	1.700	0.720	1.992	0.288	0.672
Obs81	0.000	1.534	1.869	0.837	0.413	2.595	1.992	2.889	0.907	0.040
Obs82	0.157	1.057	0.002	0.092	0.345	1.180	0.055	0.006	0.289	0.514
Obs83	0.810	0.954	0.158	0.020	0.714	0.203	0.260	0.582	0.190	0.011
Obs84	0.746	1.243	0.010	0.031	0.606	0.003	0.578	0.841	0.057	0.000
Obs85	0.294	1.489	0.003	0.131	0.004	0.137	0.007	0.500	0.421	3.100
Obs86	0.003	0.049	0.036	3.173	0.312	4.639	0.115	0.393	3.541	0.020
Obs87	0.565	0.985	0.011	0.026	0.034	0.059	0.182	0.087	0.056	0.007
Obs88	0.311	1.115	0.107	0.017	1.193	0.060	0.174	0.239	0.181	0.020
Obs89	0.444	0.561	0.001	0.008	0.152	0.141	0.009	1.572	0.001	0.709
Obs90	0.465	1.291	0.464	0.023	0.089	0.022	0.480	0.036	0.013	0.256
Obs91	0.284	0.752	0.005	0.125	0.205	0.079	0.023	0.879	0.465	0.041
Obs92	0.038	0.490	0.234	0.154	0.684	1.096	1.110	1.412	0.539	0.955
Obs93	0.463	0.107	4.728	0.004	0.001	1.763	0.621	0.548	0.671	2.601
Obs94	0.821	1.095	0.023	0.110	0.050	0.000	1.195	0.828	0.304	0.000
Obs95	0.352	0.912	0.000	0.007	0.114	0.009	0.386	0.186	0.083	0.149

Obs96	0.857	0.938	0.048	0.063	0.000	0.046	0.000	0.208	0.016	0.452
Obs97	1.053	1.047	0.110	0.000	0.259	0.035	0.249	0.504	0.254	0.000
Obs98	0.846	1.294	0.431	0.003	0.081	0.220	0.200	0.970	0.085	0.139
Obs99	1.200	0.820	0.297	0.089	0.002	0.005	0.291	0.151	0.070	0.206
Obs100	0.741	1.267	0.196	0.007	0.399	0.785	0.068	1.541	0.010	0.026
Obs101	1.229	1.069	0.134	0.200	0.262	0.160	1.572	0.008	1.420	0.672
Obs102	0.667	0.772	0.067	0.001	0.153	0.550	0.229	0.161	0.079	0.070
Obs103	0.921	0.629	0.317	0.147	0.986	0.080	1.428	0.623	0.476	0.006
Obs104	0.221	0.612	0.087	0.075	0.000	0.167	0.017	0.148	0.527	0.810
Obs105	1.214	0.700	0.000	0.040	0.938	0.016	0.395	0.060	0.474	0.476
Obs106	0.559	0.643	0.185	0.030	0.023	0.005	0.128	0.184	0.005	0.007
Obs107	0.179	1.360	0.004	0.003	0.051	0.029	0.766	0.015	0.019	0.000
Obs108	0.050	1.005	0.068	0.414	0.182	0.228	0.163	0.002	1.007	0.015
Obs109	0.213	1.750	0.189	0.099	0.001	0.000	0.040	0.216	0.570	0.623
Obs110	0.068	0.004	0.011	6.169	0.000	1.076	0.179	0.001	1.863	1.062
Obs111	0.911	0.584	0.107	0.056	0.509	0.072	0.152	0.583	0.101	0.004
Obs112	0.066	0.017	0.067	2.121	0.775	1.850	0.486	0.013	1.659	0.180
Obs113	0.474	0.492	0.072	0.064	0.700	0.000	0.733	0.008	0.068	0.055
Obs114	1.208	1.181	0.135	0.045	1.790	0.079	1.193	0.005	0.309	0.196
Obs115	0.404	0.474	0.000	0.222	2.107	1.942	1.591	1.390	0.803	0.109
Obs116	0.360	0.596	0.033	0.057	0.024	0.190	0.019	0.976	0.148	0.072
Obs117	0.240	0.804	0.020	0.046	0.769	0.040	0.759	0.049	0.334	0.222
Obs118	0.438	1.176	0.027	0.000	0.751	0.867	0.282	0.154	7.642	0.011
Obs119	0.024	0.001	0.000	11.456	0.000	0.011	0.038	1.019	0.005	0.443
Obs120	0.328	0.180	0.038	8.465	0.057	2.543	0.294	2.767	3.206	0.751

Table 10: Summary Statistics:

Variable	Observatiowith missinithout miss			Minimum	Maximum	Mean	td. deviatic
LA	120	0	120	5.760	59.500	26.283	14.383
CHL 1	120	0	120	17.733	55.533	39.511	9.001
CHL 2	120	0	120	11.133	60.667	32.481	14.435
CHL 3	120	0	120	1.200	46.333	11.827	12.608
DH	120	0	120	0.000	123.000	111.217	18.564
DM	120	0	120	0.000	154.000	137.358	22.770
SL	120	0	120	0.000	18.667	8.878	3.346
SLA	120	0	120	0.000	27.667	16.955	4.516
SPS	120	0	120	0.000	27.000	19.478	5.099
G/S	120	0	120	0.000	56.000	27.995	12.353
TKW	120	0	120	0.000	56.000	27.995	12.353

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