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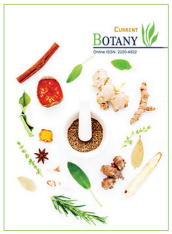
Review Article



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Stress breeding of neglected tetraploid primitive wheat (*Triticum dicoccum, Triticum carthlicum* and *Triticum polonicum*)

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ABSTRACT

Ancient wheats are progressively more considered as valuable resources for genes of interest especially in organic and health food markets which could be introduced into cultivated varieties. Better evaluation of primitive wheats that symbolize a valuable genetic resource may provide breeders with important sources for biotic and abiotic stress tolerance. Emmer wheat (*Triticum turgidum* subsp. *dicoccum*) is one of the most ancient of cultivated cereals, makes good bread, higher in fiber than common wheat, emmer is used is for making pasta and bread, with no need at all to use pesticides during growing season, grow in severe environments and minor lands, resistance to *Septroria* leaf blotch and resistance to Russian wheat aphid and Green bug. Persian wheat (*Triticum turgidum* ssp. *carthlicum*) described with many favorable characters, like being a good resistant species to stem rust and powdery mildew, plants have more tillers with good productivity, low temperature tolerant and pre-harvest budding and fairly resistant to fungus diseases. Polish wheat (*Triticum turgidum* subsp. *polonicum*) were used for bread making as many forms having grains with high protein content (27%), and it is a great source for high yielding wheat varieties characterized by plump grain, and could be used in genetic bio-fortification of durum wheat and common wheat. Lack of scientific researches and facilities to produce and marketing, in addition to concentrating on new varieties are considered as limitation factors of expanding these species. For better adaptation to climate change and for future food security, it is highly recommended to improve utilization and sustainable use of targeted species and cooperation between scientists and researcher on the national and international levels. This review is an attempt to highlight the value of targeted species with general information about classification, origin and distribution, importance domestication, characterization and conservation, traditional breeding and role of each of molecular biology, tissue culture and mutation in exploiting genetic variation in targeted species.

KEYWORDS: *T.dicoccum*, *T.carthlicum*, *T.polonicum*, Tetraploid, Primitive, Underutilized species

INTRODUCTION

In the future, global population is estimated to increase more and more to be around 9 billion in 2050, which means increasing in the demand for food. Food and other needs of the growing population enhance the demand for cereals. The demand of wheat is expected to increase in 2020 to seven hundred and sixty million tons [1], and in 2030 to eight hundred and thirteen million tons, and in 2050 will be higher than nine hundred million tons [2], according to these expectations and if we consider 2005 as a start point wheat production should increase in a rate of 1.6% up to 2020, while 1.2% up to 2030, and finally a rate of 0.9% during the period 2005-2050. It is predicted that wheat demand will raise more rapidly comparing to the demand of rice and will also be more convergent to the increase in worldwide inhabitants over the same period. Wheat is one of the most demanded cereals in the world with an average yield differs from lower than one to more than seven tones/ha according to the farming complexity and growing requirements, and agro-ecological situation. Wheat is broadly cultivated in the world, as China, India and Turkey are the most important wheat producers among developing countries. Approximately 45% of one hundred and twenty million hectares planted with wheat in developing countries are under rainfed conditions and may suffer from drought [3]. For that we need to exploit the genetic diversity in wheat and other main crops especially neglected species and adapted landraces to be used in breeding programs. Future breeding priorities will continue involve increasing yield and furthermore will vary particularly in developing countries according to changeable demands of market and environments [4].

Classification of Wheat

Species of two genus *Aegilops* L and *Triticum* L. are used to have the name of wheat. *Triticum* genus consisting of both domestic and cultivated species while *Aegilops* contains only wild types of different species. Both of *Triticum* and *Aegilops* species are belonging to the Poaceae family, both are self-pollinated and annual species. It is predicted that wheat and maize have diverged about fifty to seventy million years ago [5]. Together with barley, wheat belongs to the tribe of Triticeae that took place in the Pooideae sub family [6]. *Aegilops* L. and *Triticum* L. include specie of diploid (2n=14), tetraploid (2n=28) and hexaploid (2n=42), in which un artificial crossing between species with chromosome sets (genomes) resulted in both of tetraploid and hexaploid [7]. *Triticum* L. genus comprises both wild and domesticated wheat species. Domestic types are unable at all to spread their seeds in contrast to the wild species, this means that the endurance of domesticated species cannot be continued unless farmers planting their seeds and provide all necessary inputs to keep them alive, they have hard rachis does not allow their seeds to spread, in contrast to wild species that have breakable rachis allows the seeds to spread [8] (Table 1). Another difference considered as classification key between what we call primitive wheat and modern wheat is that primitive wheat includes the first domesticated types of wheat which contribute in some way in evolution of modern types of *Triticum* including macaroni wheat (tetraploid *Triticum durum*) and common bread wheat (hexaploid *Triticum aestivum*), they are also described of free threshing or naked wheat in which the parts of rachis still connected together when threshing, whereas the other parts like glumes move away from each other, enabling the grain to release [9].

The grains of primitive wheat (as wild types) like *Triticum. monococcum* (AA), *Triticum. dicoccum* (AABB) and *Triticum. spelta* (AABBDD) (Table 2) when threshing have an enclosed cover, so they are called hulled wheat. After threshing the spike of hulled wheat, it divides to its spikelets containing hard glumes connected to a rachis and surrounding one seed or more. The hardened glumes and easily broken joints between the rachis internodes in spike are both the cause of having hulled trait [9]. There are diploid and tetraploid and hexaploid diverse levels in *Triticum* cultivated species such as *Triticum. monococcum* (2n=14) which is diploid, tetraploid (2n=28) like each of these species *Triticum durum*, *Triticum turanicum*, *Triticum carthlicum*, *Triticum polonicum*, *Triticum dicoccum* and *Triticum. timophevii*, and hexaploid species (2n=42) like *Triticum. aestivum*, *Triticum. compactum*, *Triticum. spelta.* [10].

Table 1: Classification table for *Triticum* L [10]

Local Name	Scientific name	Genome	Status
Wild einkorn	*T. urartu*	AA	Wild
Wild einkorn	*T. boeoticum*		
Einkorn	*T. monococcum*		Domesticated
-	*T. sinskajae*		
-	*T. aethiopicum*	AABB	Domesticated
Persian wheat	*T. carthlicum*		
Wild emmer	*T. dicoccoides*		Wild
Emmer	*T. dicoccum*		Domesticated
Macaroni wheat	*T. durum*		
-	*T. ispahanicum*		
Polish wheat	*T. polonicum*		
Khorasan wheat	*T. turanicum*		
Rivet wheat	*T. turgidum*		
-	*T. araraticum*	AAGG	Wild
-	*T. timopheevii*		Domesticated
Bread wheat	*T. aestivum*	AABBDD	
Compact wheat	*T. compactum*		
-	*T. macha*		
Spelt	*T. spelta*		
-	*T.sphaerococcum*		
-	*T. vavilovii*		
-	*T. zhukovskyi*	AAAAGG	

Origin and Distribution

The first known area where domestication started is the Fertile Crescent from northern Syria through southeast Turkey, northern Iraq and western Iran, this area is the place of plants originator as the wild species of wheat (wild emmer and wild einkorn) and barley were found [8]. According to [13] the beginning of cultivation started in the Near East until seven thousand 7000 BC, then after that expanded to northern Africa and Europe, and moreover freely establishment of cultivation started in south eastern Asia and central America. We can say that cultivation was familiar all over the world during a few millennia. According to historical evidences, the growth and expand of agriculture look as if it had occurred in a quite small period. The Fertile Crescent mountains in southwestern Asia which is located between Tigris and Euphrates from the east to the Mediterranean coast through the desert of Syria is considered as the primary center of diversity and origin of the *Triticum* genus [14]. It is believed that the south-west of the Fertile Crescent is the center of diversity of the tetraploid wild and primitive wheat (Figure 1).

Economic and Health Importance

Interest in ancient wheat and better quality varieties (concerning nutrition) increased in some countries especially in Germany, emmer wheat can be harvested under moderate environmentally conditions such as poor soils and water deficiency [15], so it is recommended with other ancient wheat types for organic production, where no synthetic fertilizers or pesticides are used to support the plant. An additional

Table 2: Most important primitive wheat species [11, 12]

Common name	Species	Ploidy
Einkorn	*T.monococcum*L.	Diploid (2n=2x=14)
Emmer	*T. dicoccum*Schrank (Schuebl.) *T.palaeocolchicum*Menabde *T.ispahanicum*Heslot *T. timopheevi*(Zhuk.) Zhuk	Tetraploid (2n=4x=28)
Polish	*T. polonicumL.*	
Persian	*T. carthlicumNevski*	
Spelt	*T. spelta*L. *T. zhukovskyi* Menabde.et. *T.aestivum. vavilovii*(Thum.) Jakubz	Hexaploid (2n=6x=42)
Compact	*T. aestivum. compactum*Host	
Macha	*T. aestivum. macha*Dekapr. et Menabde	

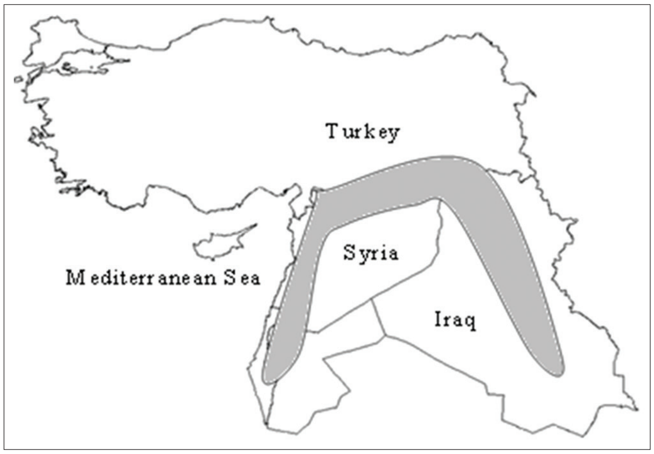

Figure 1: Map origin of the *Triticum* genus (Map created by M.M. Saleh)

motivation is the production of a healthy food, mainly of complete grain food products. Researchers announced that one way of decreasing the high blood pressure is to consume that complete grain food of wheat [16]. Products of the whole grain usually refer to ancient wheat, as it plays an important role in health comparing to food made by white flour. Breeders are continually trying to exploit the genetic diversity to find genotypes rich in necessary minerals for the nutrition of humans because the most of the new varieties of wheat (in contrast to ancient wheat) are notably poorer in mineral content of some elements such as phosphorous, magnesium, zinc, copper, selenium, manganese and iron. A good example of that is the Polish wheat which is planted in small areas of Europe, Asia and Africa which must have exceptional consideration in this point of view. Nutrient value of polish wheat in competition with durum wheat and bread wheat was examined, Results showed that the grain of *Triticum polonicum* had the considerably best content of iron (39.1 mg/kg), phosphorus (4.55 g/kg), zinc (49.5 mg/kg), sulphur (1.82 g/kg), magnesium (1.42 g/kg), and boron (0.56 mg/kg), in addition to a lower content of aluminum (1.04 mg/kg). The nutrient report of the majority of studied genotypes of polish wheat varied totally from that of bread wheat and durum wheat. The principal component analysis maintained the distinguish of 7 polish wheat genotypes described as healthy for human nutrition for having the maximum content of zinc, iron and copper, in addition to the minimum concentration of undesirable elements in food such as barium, aluminum and strontium. For that reason, breeders specified *Triticum polonicum* as a proper genetic resource to enhance nutrition value of modern varieties of wheat, specify that polish wheat could be used in genetic bio-fortification of durum wheat and bread wheat [17]. Nowadays, some people in Italy and Switzerland consume emmer (*Triticum dicoccum*) bread which is baked by themselves or by buying it from bakeries where they can found it, they prefer it more than other types of bread because of the high content of fibers in emmer, and also they consume emmer wheat as pasta [18]. Historical investigations also indicate that people in Turkey and Egypt eat emmer bread. As emmer does not need a lot of input, with no need at all to use pesticides, it is considering as proper for planting in difficult environmental circumstances and in minor areas of cultivation [19].

Domestication, Selection and Early Improvements

The genetic changes of wild species that guiding to the organized manage of its selection and reproduction could be determined as a domestication of species. One of the first domesticated species is emmer. Species extended as domesticated crops all over the Fertile Crescent followed their spread to the closest areas like southern the Caspian Sea or in the direction of the Nile valley, then worldwide, in particular central and eastern Asia and Europe [8]. At the same time, crops were shared between inhabitant growers and also with migrant farmers carrying their crops. Emmer wheat evolved from wild emmer and was domesticated in the Fertile Crescent around 9,500 to 9,000 years back [11]. Two populations of wild emmer are found in northern and southern of the Fertile Crescent. Based on genetic analysis [20], chloroplast DNA microsatellite variations [21], it is suspected that northern population of wild emmer (south Turkey, Iran and Iraq and parts of the Fertile Crescent) is a real progenitor of cultivated emmer and place of their domestication correspond to the focal point where agriculture started, Karacadag region in Southeast Turkey [22]. Brittle rachis was the main trait that was altered through domestication, thus cultivated emmer hasnon-brittlerachis that helps to keep spikelet's intact on spike until manually harvested [22,23]. Cultivated emmer is easy to thresh compared to wild emmer but still cannot be freely threshed, as it is hulled too. Cultivation of emmer wheat expanded broadly across and further than the Near East. It expanded toward the east to India and westward through Anatolia to Europe and the region of Mediterranean coast, and it was one of the most important crops in these areas for about six thousand years [24]. Durum wheat (*T. turgidum subsp. durum*) may have derived from domesticated emmer wheat in the eastern Mediterranean region [25]. Persian wheat could be a result of segregation from a hybrid cross between domesticated emmer wheat and *T. aestivum* [26].

CULTIVATION AND TRADITIONAL BREEDING

Current Cultivation Practices

In harsh environmental conditions, Emmer has been cultivated by farmers in the Italian mountains (Apennines) for centuries as a result of being highly adaptable, but emmer became to be neglected along with the growing of durum wheat, which caused a reduction in emmer's cultivated area in the 1970s to only a few thousand square meters [27]. In Italy yield of emmer was about 3.5 t/ha [28] and the cultivated area about 2500 ha [29]. The production of emmer has become increasingly widespread in Italy [11], while in the region of Black sea in Turkey there is just a tiny area in provinces of Kastamonu and Sinop which is cultivated of emmer. Emmer is planted in north of Ethiopia (Tigray area) with other species of wheat or further crops of cereals in the ratio of 1:2–1:1. Emmer is planted from time to time in the company of other wheat and barley in ratio of one emmer and two wheats and two barleys in order to enhance the final products value [30]. It is mostly produced for house hold consumption and infrequently for regional market [30]. In Turkey villages, farming of soil is proper in the duration of the uncultivated year, in which soil is very compressed and needs to be prepared well using high traction power. The farmer's common strategy to facilitate the agronomic operations in the field is delaying cultivation of the soil until the first rain. The lack of proper equipment is another reason for late sowing. Soil is plowed using animal traction only once before seeding. Depending on the distance of fields from the villages and the soil conditions, If plowing cannot be completed in the autumn, it will be completed in next spring. In autumn villagers usually plant the neighboring fields firstly. Seeds are distributed after plowing the soil previously, then covered by soil with an ancient tool made of wood. Normally in case of producing cereals the applied quantity of fertilizer needed is low and rarely herbicide is used.

Current Agricultural Challenges

As in every genus, neglected species has been pushed out of production as in every genus, neglected species has been pushed out of production by higher yielding and more profitable crops. As a result of some influencing factors like genetic, agronomic, financial and cultural ones, most of the underutilized species (often ignored by scientist and policy-makers), are in danger of becoming extinct [31,32]. Challenges in producing and marketing emmer for example in Turkey are: Inadequate studies(because of finances shortage and lack of resources) on nutritional properties, and the absence of confirmed official results, genetic studies on the diversity of emmer are not enough, current technology renders processing difficult and time consuming, shortage of suitable handling equipment to develop emmer quality and the need to control quality, low official support for emmer production, weak market transparency as farmers of emmer are far from seller, improper infrastructure, Farmers' migration to the cities and changes in life style are leading to the disappearance of emmer producers, very low local consumption and lack of good packaging and storage.

Improvement of Strategies

Water deficiency and drought stress factors are considered as the most important limiting factors in global wheat growing [33]. Emmer wheat is a typical wheat cultivar which can play the role of a donor of drought tolerance in the wheat breeding process. Researcher now aims to discover genetic variation in primitive tetraploid wheat like emmer and Persian and polish wheat to be used in breeding program especially for biotic and abiotic stress and for other purposes like improving food quality and increasing yield. Scientists in genetic resources are collecting neglected and ancient wheat and conserve these valuable materials for future food security.

Traditional Breeding Methodologies and Limitations

Unfortunately, modern varieties of wheat have a narrow genetic basis in which affect negatively their resistance for different unexpected stresses and endangered the future enhancement of wheat. For that it is very important and crucial to discover and evaluate more genetic types of wheat which having desirable genes. The introgression of primitive emmer into bread wheat resulted in appearance of *Triticum spelta* in Europe according to the genetic investigations of some researchers [34,35]. [36], during the searching for an attractive gene source to increase the resistance to Fusarium head blight in wheat working on polish wheat, they studied 9 spring lines of *Triticum polonicum* L. and compared them with *T. turanicum* called Kamut wheat besides two common wheat cultivars, studied traits were (plant height, spike length, density of spike, weight of grains per spike, weight of single grain, the content of each of protein, ash, fat, crude fiber and minerals in grain). It was clear from results that the polish wheat genotypes had a moderately weak reaction to infection with Fusarium culmorum through the reduction in the weight of grain in spike and also weight of single grain by thirteen percent and more than six percent respectively. Polish wheat grain was distinguished by a protein and ash content considerably higher compared to the common cultivars (by 19.8 and 23.7 %, respectively) and significant concentrations of fat and nutritional fiber were higher comparing to Kamut wheat (by 30.2 and 17.4 %, respectively). Grain of studied polish wheat was significantly (in contrast to common cultivars) more rich in zinc, iron, copper, molybdenum, potassium, sulfur, and magnesium, with lowest amount of strontium and aluminum. Levels of Fusarium toxin in grain for all examined genotypes were significantly increased because of the vaccination, and the average concentrations of the mentioned elements were lower in polish wheat grains than in bread wheat. For all of that *Triticum polonicum* might represent a precious genetic resource for breeders to improve high quality wheat varieties rich of nutrients with acceptable resistance Fusarium head blight FHB.

Role of Biotechnology

In the 1990s, the increase in interest in natural and organic products has led to a "rediscovery" of hulled wheat, which has health characteristics associated with high starch-resistant content [37]. Moreover, it has an ability to grow at low temperatures, in soils with limited fertility, and utilizes low input techniques. It is also a source of genes for breeding wheat [38]. The interest toward *T. dicoccum* was to evaluate the available germplasm [39], to select strains among old landraces and to develop new genotypes between interspecific crosses [40]. For these reasons it has become of interest to know the amount of diversity existing both between and within emmer populations. Many difficulties in recovery of transgenic plants and gene delivery are still found in wheat transformation [41]. By using biolistic method, [42] obtained the first transgenic herbicide resistant wheat, then attempts frequency had improved to about 16.7% [43]. According to [44] and [45], wheat grain quality was improved by modification the starch and protein profiles through the application of transgenic methods, which will complement the conventional plant breeding [46,47]. Some particles like gold are coated with target genes and shot into wheat cells through the biolistic particle or micro-projectile bombardment which is a widely used gene transfer technique in wheat. [48] and [49] mentioned that transient expression of ß-glucuronidase gene subsequent bombardment of cell suspensions was achieved by biolistic particle (as a gene delivery) technique. Another technique in is Agrobacterium-Mediated wheat transformation which fewer successful cases have been attained compared to the biolistic technique. Scientists improved the efficiency and repeatability of wheat transformation after consideration of the factors that affect Agrobacterium-mediated transformation, and extended wheat genotypes. [50] referred to the successful attempts for wheat transformation mediated by Agrobacterium tumefaciens using emmer wheat *Triticum. Dicoccum* named *(DDK1001, DDK1009)* [51].

GENETIC DIVERSITY AND CONSERVATION

Breeders develop new varieties depending on many germplasm raw materials for improvement including different genotypes such as wild and weedy relatives, wild species, primitive cultivars, natural hybrids, ancient cultivars, elite lines, breeding lines, mutants, interspecific and intergeneric hybrids improved systematically [52].

Genetic Diversity

Genetic diversity is gradually lost during the crop domestication and further in the breeding programs which are focused towards few traits, hindering long-term crop improvement [53]. The genetic diversity of species and related crops can significantly contribute to the improvement of crop characteristics [54] with the purpose of emphasize the resistance to diseases or develop traits of varieties [55]. Most of studies, showed that these free-threshing tetraploids evolved from the natural stands of cultivated emmer [56,57]. Their origin was the result of post-domestication diversification [22]. This diversification happened either due to the pressure of local agro-ecological conditions or driven by natural hybridization. Neglected primitive species of wheat contribute in broaden wheat germplasm diversity, as the durum wheat *Triticum turgidum* subsp. *durum* is suspected to have evolved from domesticated emmer *Triticum turgidum* subsp. *dicoccum* in the eastern Mediterranean region due to the adaptation to the local ecological conditions, and similar theory is applicable to the other tetraploid species namely polish wheat *T. turgidum* L. subsp. *polonicum*, these species might have also emerged due to agro-ecological pressures too. Another possibility of species diversification is inter-ploidy introgression like persian wheat *T. carthlicum* which is believed to be a segregation from a cross between domesticated emmer and *T. aestivum* [26].

Cultivars Characterization

Emmer wheat (*T. turgidum* subsp. *dicoccum*): Tetraploid wheat, consider as one of the first ancient cultivated cereals. With two types of habit winter and spring, usually have a pubescent leave, very dense and flattened spikes. Spikelet are usually including two flowers and normally are awned. Kernels are red or white stay after threshing together in the glumes. They are small and sharp at both ends (Figure 2). In 1996 *Triticum dicoccum* occupied only one percent of the total world area cultivated. Emmer is cultivated in some countries and regions such as Ethiopia, Iran, eastern Turkey, central Europe, Italy, Spain, Transcaucasia, former Yugoslavia, the Volga Basin [58], Emmer was former grown in the United States for feed on a limited acreage but then has substantially disappeared from cultivation. In Ethiopia emmer still an essential crop while it is an unimportant crop in each of India, Italy and Turkey [59] Bread Wheat evolved from the crossing between cultivated emmer (*Triticum dicoccum*, AABB) and goat grass (*Aegilops. tauschii*, DD). cultivated emmer has also contributed a number of important genes into the wheat gene pool like resistance to *Septrorianodorum* leaf blotch [60,61], resistance to Russian wheat aphid [62] and resistance to Green bug [63].

Figure 2: *Triticum dicoccum* Schuebl (Photo by Y.J. Jabbour)

Persian wheat is the common name of *Triticum turgidum* ssp. *carthlicum* L. tetraploid wheat, early-maturing with spring habit, has not been exploited that much for wheat improvement. There are only a few reports for novel disease sources, such as *fusarium* head blight resistance sources [64]. Persian wheat is essentially cultivated in each of Transcaucasia, Russian Federation Dagest, Georgia and northeastern Turkey and eastern Mediterranean. Species like *T. turgidum* subsp. *carthlicum* are being underexplored and studied, a better evaluation of their germplasm may provide researchers with important sources for abiotic and biotic stress tolerance, it is fairly resistant to fungus. The stem is tough yellow colored to light red, stretchy spikes, tending to bend over, in spite of existing of many flowers in each spikelet only 3 of them typically grow to kernels (Figure 3). Kernels are free-threshing, hard, and normally red. It is an important crop for human consumption. It has several valuable characters, like good resistance to stem rust and powdery mildew dust brand, more tillers and fecundity, good fertility, low temperature tolerance and pre-harvest budding. For a lot of useful characters and simplicity of the transfer of genes to common wheat, *T. carthlicum* has been recommended as one of the best favorable donors to improve bread wheat [65,66].

Polish wheat (*Triticum turgidum* subsp. *polonicum*): Tetraploid spring wheat, stem is tall, described of large open or dense spikes, spike shape is rectangular or square in cross-section and awned, too long, thin, and tough grains. They thresh free of glumes, it is characterized by large glumes size up to 4.5 cm, and thousand kernel weight may reach up to 80 gm (Figure 4). This species was cultivated in the Mediterranean region south of Europe as it needs a warm weather. Grains in several types contains high content of protein reach to twenty-seven percent, polish wheat was used as bread [67]. Polish wheat is not that popular as a crop although it is grown sporadically in warm climates of southern Spain. Italy, Ukraine and warmer parts of Asia, Algeria, and Ethiopia [68]. Hybrids developed by crossing polish wheat with *Aegilops* species record yielded 80 tones/h but had high fertilizer needs. It can be said that polish wheat is a great source for high yielding wheat varieties characterized by plump grain [69].

Genetic Resources Ex Situ Conservation

Ex Situ is one way of conservation of plant genetic resources which means preservation of these resources mainly in gene banks and botanical gardens, or research centers concerning biodiversity of agriculture [70]. The information on the status of *Ex Situ* conservation of *T. dicoccum* has been gathered by experts from European and Middle Eastern countries. Today *T. dicoccum* remain as relic crop in Spain, Italy, Turkey, the Balkans and India. Genetic resources unit in the International Center for Agricultural Research in the Dry Areas (ICARDA) has a larger number of accessions belonging to *T. dicoccum* and has been distributed to collaborators and breeders in different countries. In Italy many accessions of *T. dicoccum* are being conserved at the germplasm institute (National Research Council) in Bari. Collecting activities have guided to decision of the existence of emmer wheat in various parts south of Italy where was measured as about vanished crop. The scientific statistics with local information on neglected wheat have raised up public understanding of the situation of conservation regarding neglected wheat, so in Italy a lot of growers are planting them, and in close association with the gene bank of Bari crop growing is frequently supported, which manages and controls the situation of genetic degradation of these important species. In Greece *T. dicoccum* cultivation has been reported last century, it was found also during the period 1981-1988 through the collecting missions hold by the Greek gene bank. There is strong evidence that *T. dicoccum* is still maintained under cultivation in small island near southern coast of Crete. Such material is conserved at the Greek genebank. In Turkey collection and conservation activities of the Turkish plant genetic resources became well organized since 1963, accessions of *T. dicoccum* and other neglected species are also maintained by the genebank. Researchers are still collecting species where these crops are still being grown in different regions of Turkey. In Spain, the major conservation center for *T. dicoccum* and hulled wheat is the Centro de Recursos Fitogeneticos (CRF) of the Spanish Plant Genetic Resources Centre in Madrid. A number of Spain collected genotypes is further more preserved for investigation determinations at the Institute of John Innes of Norwich, England. Some accessions of *T. dicoccum* originated in Spain are being preserved at the Spanish Plant Genetic Resources Centre in Madrid.

Figure 3: *Triticum carthlicum* Nevski (Photo by Y.J. Jabbour)

Figure 4: *Triticum polonicum* L (Photo by Y.J. Jabbour)

Genetic Resources *In Situ* Conservation

In situ conservation is a type of crop genetic resource conservation that means the maintenance of genetic resources on farm or in the natural habitats [71,72]. In situ conservation can be defined similarly to CBD definition as the preservation of natural habitats and protection of ecosystems and the enhancement and recovery of living species populations in their natural environments, and also in the environment where cultivated or domesticated species have improved their unique traits. Two types of *in situ* conservation can be distinguished. First type of *in situ* conservation indicates the continually presence of different genotypes in their natural habitats, taking an account all spots where daily practices of farmers to enhance the genetic diversity on their farm. This type is a notable experience, but today in particular observable in areas where cultivators enhance local and different crop cultivars as landraces, although new, largely adapted or superior varieties in yield are available [73]. Second type of *in situ* conservation indicates precise programs and projects to sustain and emphasize crop diversity, supported by national governments, international programs, and private organizations [73]. The first step for active conservation is natural *in situ* conservation units for the reason that their environmental and demographic conditions permit for self-motivated gene protection completely managed by the natural disturbance of the environment [74]. In Georgia some neglected species of wheat are found naturally like *T. dicoccum*, *Tr. Carthlicum* and *T. polonicum*. *Triticum dicoccum* is found also in Czech Republic, Turkey, Italy and Spain. Out of about 20 wheat species known in the world in the earlier times, 14 were cultivated in Georgia including emmer and persian wheat and polish wheat [75].

MOLECULAR BREEDING

Nowadays, Biochemical markers have been used to evaluate the genetic diversity among wheat landraces, such as isozymes, seed storage proteins, DNA-based markers, like random amplified polymorphic DNA (RAPD) [76,77], and (AFLP, RFLP, ISSR, SSR) are applied to estimate the genetic diversity in different genotypes of emmer wheat like in other plant species [78,79]. [80] estimated the genetic diversity of ten diploid and tetraploid wheat species (including *Triticum dicoccum*) using random amplified polymorphic DNA (RAPD) markers, cluster analysis was used to determine genetic similarities, structural analysis was applied for eight yield components after evaluating the same genotypes in field, Their results indicated that some of genetically similar genotypes were different phenotypically which means that the phonotypic similarity in genotypes does not mean in all times that they are genetically similar. Nevertheless, diverse varieties belong to one species usually confirm similarity in the phenotypic and genotypic level. Precise control of the situation of the elite gene pool is needed as the germplasm collecting is a permanent procedure. Evaluation the variation between different collected genotypes is very essential to make conservation more effective which could be significantly maintained by applying molecular genotyping ways [81]. In modern agricultural systems most of genetic diversity has been vanished, causing a reduction in the possibility of wheat development [82]. Landraces, and other related species like primitive species can have crucial roles in breeding programs [83] as a reason of their broad variability regarding traits of quality, biotic, abiotic, phenological, and morphological traits.

In tetraploid wheats, the assessment of the genetic diversity level and structure is a requirement for the preservation of plant genetic resources and for breeders. The use of morphological and biochemical markers pedigrees and molecular markers are all several ways to evaluate the level and structure of genetic diversity [84,85]. For analysis of genome, several molecular markers could be applied, actually many of them have been used resulting good achievement regarding genetic mapping [86].

[87] used 6 morphological, 9 seed storage protein loci, twenty six SSRs and nine hundred and seventy DArT markers to investigate the levels of genetic diversity and population genetic structure of collection of two hundred and thirty accessions belong to seven subspecies: twenty accessions from each of ssp. *turanicum* and ssp. *polonicum*, nineteen accession from each of ssp. *turgidum* and ssp. *dicoccum*, twelve accession from each of ssp. *carthlicum*, and the rest 128 from ssp. *durum*, in wild and cultivated emmer, the genetic diversity of the morphological characters and seed storage proteins was all the time higher compared to durum wheat, results showed that durum wheat cultivars were distinguished from the studied tetraploid genotypes when using Bayesian clustering (K = 2), and two different subgroups were noticed in the subspecies of durum wheat, that was in accordance with their origin and releasing year. It was noticed that the improved cultivars of durum wheat registered after 1990 had all the time lower genetic diversity of seed storage proteins and morphological characters comparing to the intermediate and older cultivars. This noticeable result on diversity was not well noticed for molecular markers, but only a weak reduction. SSR markers illustrated a better level of resolution than for DArT, with their detection of more number of clusters inside each subspecies. Analysis of DArT marker variation among the subspecies of wheat specified outlier loci which are possibly related to genes monitoring some essential agronomic characters.109 markers were mapped from 211 loci which recognized under selection, and a number of these markers were clustered into definite places on chromosome arms 2BL, 3BS and 4AL, while many genes of quantitative trait loci (QTLs) are contained in the tetraploid wheats domestication, like the tenacious glumes (Tg) and brittle rachis (Br) characteristics. Depending on above results, it can be said that the population structure of the tetraploid wheat collection to some extent refers to *Triticum turgidum* L. subspecies evolution steps and the genetic value of landraces and wild accessions for the exploration of undiscovered alleles. Persian wheat *T. carthlicum* was recommended as one of the most attractive donors for the development of bread wheat for the lots of valuable characters and easiness of transferring genes to common wheat [65,66]. Based on morphology, agronomic traits, and even enzymatic and molecular markers, the genetic relationship between Persian wheat genotypes had been extensively studied [88].

Eighty-seven Persian wheat (*T. carthlicum*) genotypes were obtained from fifteen countries represent the majority of the environmental display of persian wheat globally, were chosen with the purpose of estimate genetic diversity depending on the adaptation toeco-conditions southwestern China, based on EST-SSR markers and also to provide information for wheat breeding and improvement in Southwest China. Genetic variations were detected by 14 EST-SSR primers on all seven wheat homologous chromosome groups, results showed that higher genetic variations in the 87 persian wheat accessions were observed and the results revealed that there was a distinctive banding profile for every genotype, and all genotypes at the average GS value of 0.602 were clustered to four main groups, first group contained four Turkish genotypes, three genotypes from Former Soviet and one Iraqi genotype, second group contained three Turkish genotypes. Third group contained thirty-eight genotypes are all from United States but the origin from eight countries as most of them from Turkey and others were from Canada, China, Former Soviet, Russian, Armenia and Albania, the forth group contained thirty-eight genotypes, which all Iranian genotypes, and genotypes from Georgia, England, Poland, and Hungry, as well, the left genotypes were from Canada, Albania, Russian and Turkey, it was not valuable to cluster together all the genotypes from Iran, Georgia, Poland, England, United States and nearly all genotypes from Turkey, since it was suggested that most of the genotypes with neighboring geographic origins had the tendency to cluster with each other, for that reason, additional awareness has to be concentrated on the setting up a genotype catalogue applying suitable and efficient pointers such as EST-SSR, and therefore watching the lively variation in gene pools and as a reference for germplasm managing and breeding policies [89].

[90] recognized a novel allele of the Vrn-A3 gene that is connected to the trait of early flowering in wheat, this trait is caused by a cis-element GATA box in Vrn-A3, they studied days from germination to heading (DGH) in tetraploid wheat accessions in order to recognize novel flowering genes, they found that the variety(TN26) (emmer wheat) which is tetraploid harbors unidentified genes that improve on the earliness influence of the early flowering allele Ppd-A1a harbored by TN28 (*T. turgidum* L. ssp. *turgidum*). After crossing both TN26 and TN28, the resulted inbred lines were used after recombination, they achieved a quantitative trait locus (QTL) analysis for days from germination to heading, they identified a QTL for earliness in TN26 on chromosome 7AS, the chromosome on which Vrn-A3 is located, they furthermore identified a 7-bp insertion (by sequence analysis for the Vrn-A3 locus in both TN26 and TN28) that included a cis-element GATA box sequence at the promoter region of the Vrn-A3 locus of TN26,researchers suggested that the GATA box in Vrn-A3 is the cause of early flowering trait of TN26 based on an expression analysis using sister lines for Vrn-A3,as well they considered tetraploid wheat as a valuable genetic resource for breeders to improve wheat. [91] investigated the genetic diversity and genetic relationships using microsatellite (SSR) markers among 48 accessions of *Triticum turgidum* L., including thirty accessions of *Triticum turgidum* L. ssp. *turgidum*, and seven accessions of *Triticum turgidum* L. ssp. *durum*, while four accessions of *Triticum turgidum* L. ssp. *carthlicum*, and three accessions of *Triticum turgidum* L. ssp. *paleocolchicum*, and finally two accession of *Triticum turgidum* L. ssp. *turanicum*, and also two accessions of *Triticum turgidum* L. ssp. *polonicum*, all of them were examined, ninety-seven alleles were noticed at 16 SSR loci, number of alleles varied from two to fourteen at each locus, the average was 6.1, value of genetic similarity (GS) varied between 0.20 and 0.92 with the mean of 0.59,in cluster analysis, it was found the 48 *Triticum turgidum* L. accessions could be distinguished easily by SSR markers, whereas the six subspecies taxonomic entities of *T. turgidum* L. could not differentiate with each other indicating that the morphological differences presented among the six subspecies could not be reflected by the SSR markers. Their results suggested that SSR markers had superiority in detecting the genetic diversity of *T. turgidum* L. while they were not good for studies of the phylogenic relationships among the subspecies of *T. turgidum*.

TISSUE CULTURE

By applying technologies of genetic engineering, an important genes found in ancient wheats (which considered as valuable resources) that could be analyzed and introduced into new varieties. Successful in vitro plant regeneration is the first stage of biotechnological crop improvement. The ability of routine regeneration of complete plants from *in vitro* tissue cultures considers as an important factor in the improvement of wheat species transformation techniques, more than twenty cultivated species are included in wheat [92], but extensive investigations efforts have been given mostly to bread and durum wheat, due to their dominance in the world production [93, 94]. Many wheat species till latterly were not examined for the ability of plant rejuvenation in vitro, however, the concentration in this researches nowadays is increasing [95,96].The opportunity to regenerate plants from diverse tissues of tetraploid emmer wheat was confirmed by many researches over years [97, 98, 99, 95], rejuvenation of plantlet throughways of somatic embryogenesis consider a big problem for several germplasm, such as persian wheat and polish wheat after testing various tissues, culture media and environmental conditions for many years of research, the screening of *in vitro* response of genotypes is still important for biotechnological applications. Professional *in vitro* culture of the embryos of mature wheat was well-known via two most important methods. Embryos ought to be isolated from mature seeds in the same way as from immature seeds, followed by placing the entire or longitudinally separated or multiple divided embryos [97, 96, 100, 101, 102] on callus induction medium, instead, placing the whole seed with the wounded mature embryo on induction medium may achieve *in vitro* plant regeneration under endosperm-supported [103]. [104] examined twelve wheat germplasm with different genetic formulas diploid ($2n=2x=14$), tetraploid ($2n=4x=28$) and hexaploid, they studied from the tetraploid each of: emmer wheat *T. dicoccum*(Runo) (AABB), Persian wheat *T. carthlicum* (AABB), cone wheat *T. turgidum* (AABB), Polish wheat *T. polonicum*(AABB), and timopheevii wheat *T. timophevii* (AAGG) through the use of two explants types (immature vs. mature embryos).Even though that the morpho-genic ability of immature embryos was higher, all of the tested germplasm were able to regenerate plants. In immature embryo cultures of tetraploid species (*T. polonicum*, *T. turgidum*, *T. carthlicum*, and *T. dicoccum*), the highest rate of embryogenic structure formation was found in addition to spelt wheat which is hexaploid. At the same time, the diploid *T. monococcum* which is einkorn wheat and polyploidy species having chromosomes Glike *T. timophevii* were described by low embryogenesis and by the attendance of albino plantlets between shoots.

MUTATION BREEDING

Since the 1970s, γ-rays, and ethyl methane sulfonate (EMS) have been used for wheat breeding [105]. Inducing mutations with60Co γ-ray is an effective way and had bred some hexaploid wheat cultivars. Inducing mutation with 60Co γ-ray was also used for the breeding of tetraploid wheat, howeverjust2local varieties were bred from durum wheat AABB the first one from Bulgaria named Yavor and the second one from Turkey called Implus, besides dissimilar frequencies of induced mutations were detected under 100Gy60Co γ-ray [106, 107].Tetraploid wheat with AB genomes is important natural resource for breeding [108], valuable genes contributing to the grains per spike in *Triticum carthlicum* Nevski [109], dwarf genes in *Triticum polonicum*L. [110], therefore, creating novel mutation through radiation in tetraploid or hexaploid wheat may be an effective way for wheat breeding. Ten accessions of tetraploid wheat (including Persian and Polish wheat) were irradiated with 100Gy 60Co γ-ray by [111], many traits were detected such as energy and rate of germination, other traits (secondary tillers, wax powder on stalk and dwarf), meiotic process, and high-molecular-weight glutenin subunits (HMWGSs). Diverse species have dissimilar radiation sensibility, the most sensitive to this dose of radiation was (PI434999) which is emmer wheat when seed germinated (5%), while both of polish wheat(As304) and persian wheat (As293) were affected by this dose when seed germination rate was 35% and 40%. Both of (As2255) 253-10 *T. turgidum* and (As302) 224-14*T. polonicum* were the two distinguished dwarf mutant plants. In comparing to the non-irradiated seed of polish wheat(As304) CK, a novel HMW-GS was identified in kernels of polish wheat(As304) 230-7 and its Electrophoretic mobility was in the range of 1By8 to 1Dy12 which were the HMW-GSs of Chinese Spring, their results showed that these mutant materials would be resources for wheat breeding. It is well Known that making progress in any the strategy of transformation is depending mostly on the capability of regeneration of the studied explants, a research works on leaf basal segments to attain great rejuvenation reaction by somatic embryogenesis. Basal segments of 5-day-old seedlings of *emmer wheat* variety DDK1001 and bread wheat variety CPAN1676 were cultured on callusing medium for three weeks at temperature of twenty six (± 1) °C, discontinuous light followed by a culture period of fifteen days at twenty-one (± 1)°C in continuous light. The calli then were moved to a medium free of auxin used for regeneration in discontinuous light at twenty-six (± 1) °C. Regeneration via somatic embryogenesis was detected during two weeks in *bread wheat* varietyCPAN1676 and *emmer wheat* variety DDK1001 (68 and 82% respectively). This embryo genic calli were exposed to particle bombardment to attain resistant of hygromycin *in* emmer wheat and bread wheat. The efficiency of transformation of 8.6, 7.5 and 4.9% was achieved in both of bread wheat variety CPAN1676, PBW343 and emmer wheat DDK1001, respectively. Attendance of the transgene *hpt*II (hygromycin) in T_0 plants was completed by Southern hybridization [50].

HYBRIDIZATION

After the evaluation of numerous accessions of primitive wheats for yield traits and morph-physiological characters which linked to the tolerance of drought(such as enhancement of high relative water content, RWC, chlorophyll loss (chl) and photochemical quenching of chlorophyll fluorescence (qQ) under moisture stress conditions), many genotypes from species of (*Triticum polonicum*, *Triticum carthlicum* and *Triticum dicoccum*) were crossed with Cham1 and Om Rabi5 whish are improved durum wheat varieties. A direct selection (F_2 offspring) for yield and an indirect selection for physiological trait were used on interspecific populations of durum wheat × *emmer wheat*, durum wheat × *polish wheat*, and durum wheat × *persian wheat*. In order to validate the possible use of morpho-physiological traits (photochemical quenching, RWC, content of proline, and discrimination of carbon isotope, root parameters) and to estimate the resultant influences on yield, divergent selection was used. Selection reaction of these characters and heritability was estimated, and the influence of divergent selection for agronomic and morphological traits was examined under field conditions, in France, Syria, and Yemen, the divergent populations were estimated under different environmental circumstances. Selection for Morpho-physiological characters linked to moisture stress was probable because of high h2 values and effective like carbon isotope discrimination, RWC, and root parameters, causing high genetic gains. But, the selection effect of studied characters on the stability of yield requires to be more investigated. Additionally, a modified bulk method (F_2 'progeny method') was improved. In France, Syria, and Tunisia, the direct selection for grain yield per plant in F_2 was studied and yield per line in F_3 was estimated under different ecological situations. Results showed that some lines of F_3 were higher in yield in comparison to Cham1 and Om Rabi5 which are improved durum wheat varieties under both stressed environmental conditions in Syria/Aleppo and France/Montpellier. Lines were estimated in introductory yield trials at Montpellier, Aleppo and Constantine in Algeria. Results specified that the usage of correlated species jointly with the usage of the improved bulk breeding method is encouraging not just for improving yield of *Triticum durum* in environments disposed to drought, but moreover to develop yield stability of durum wheat indifferent environments. Outcomes of breeding policies are obtainable, and the probable benefits of usage of associated tetraploid species in durum wheat breeding for drought tolerance are conferred [112].

CONCLUSION AND PROSPECTS

An Overview of the Current Status

Regarding the current status, we can find that in Georgia, serious investigation of wheat landraces, varieties and species was done during the first half of 20th century in which some are still found in 2017. A huge number was collected of seed samples and conserved in gene bank and collections of live-plant of various research centers on the national and international level like IPK center at Gatersleben in Germany, Lomouri Research Institute of Crops Science, Mtskheta Breeding Station, Agricultural University of Georgia, Vavilov All-Russian Institute of Plant Industry, USDA/ARS (USA) etc. [113]. In turkey production of wheat landraces is done by farmers in usually remote areas for maintenance agriculture. Unfortunately, there is a reduction of farmers number who produce wheat landraces and diverse wheat landrace populations. There is a good strategy to conserve them in gene banks (*ex situ* conservation), but there is a need to improve conservation under grower's environments (*in situ* preservation) and to have sustainability plans using practices of organic farming, geographical indicators, production practices in mountainous, and importance on local products, and to increase public awareness of the importance of genetic resources [114].

Research Initiatives to Combat Global Climate Change

Climate change (higher temperatures, drought, changes in precipitation, soil erosion, salt water, floods, forest fires and more others) will be the main cause of high food prices and low food production, it is expected that climate change will decrease the crop yield by 2050 [115].In the farming sector, the importance of genetic resources of neglected species of crops is somehow rising due to their ability to adapt to climate change and different environmental conditions provoked worldwide [116]. Crosses with landraces originated from heat and drought stressed environments are adapted to abiotic stress. Theoretical models have been established for tolerance of stress [117] and applied to select complementary parents based on physiological characters. Modern cultivars of wheat are lower than landraces in adaptation to changeable climate and to stressed environments because of the genetic structure of their population, defending capacity, and a combination of Morpho-physiological traits ensuring adaptability to stress environments [118].

Recommendations for Future Research

Enhance collection mission of targeted species globally and evaluate it in all available ways to exploit genetic variation not only for yield, but also for other traits related to biotic and abiotic stress resistant and improve quality of food. It is highly recommended to conserve these materials for future food security and to emphasize global cooperation between scientists and researcher to share benefits of knowledge and results with local farmers.

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