INVESTIGATION OF EVOLUTIONARY RELATIONSHIPS AND HISTORY OF RYE (*SECALE* SP.) IN TURKEY WITH GLOBAL COMPARISONS

by

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To memory of my father,

Turgay MARACI

For encouraging me to discover “the little blackfish” deep inside of me

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ABSTRACT

Genus *Secale* is a small but important genus that includes cultivated rye. The genus is quite heterogeneous with annual, perennial, self-incompatible and self-compatible species and subspecies. Taxonomy of the genus is still controversial due to disagreement about the delimitation of species and intraspecific taxa, out-crossing nature of many species and lack of hybridization barriers between species and subspecies. A collection of 727 individual genotypes derived from 142 different accessions of the genus *Secale* representing *S. cereale*, *S. vavilovii*, *S. strictum* and *Secale sylvestre* were investigated from different eco-geographical areas of the world with a concentrated focus on Turkey and the Middle East. The accessions were screened with a set of seven polymorphic SSR loci, a chloroplast SNP, and a nuclear SNP, in order to understand the population structure and estimate distribution of genetic variation within the genus, and infer the genetic bases of current taxonomy and phylogeny. The results showed high levels of genetic diversity among cultivated rye populations from different geographical origins. Among these regions, the Middle East showed the highest genetic diversity, supporting the idea of the area being the center of origin for cultivated rye. The hierarchical population structure indicated that perennial *S. strictum* subspecies are genetically divergent from annual forms of the genus. A strong geographical structuring was observed within annual taxa. Existence of two distinct clusters was observed, one corresponding to Asia, and second covering samples from outside of Asia. Lack of structuring corresponding to taxonomical delimitations among annual taxa was explained by insufficient time for the evolution of isolation mechanisms or barriers between cultivated rye and its wild and weedy relatives, and consequent introgression between these taxa. Although this extensive introgression between taxa might blot out the phylogenetic signal, markers developed using next-generation sequencing might provide better resolution for understanding the exact phylogenetic relationships within the genus *Secale*.

ÖZET

Secale cinsi, kültür çavdarını da içine alan küçük ama önemli bir taksonomik birimdir. Söz konusu cins tek yıllık, çok yıllık, kendine döllenen ve yabancı döllenen tür ve alt türleri içerdiğinden oldukça heterojendir. Günümüzde *Secale* cinsin taksonomisi, tür ve alttürlerin tayinine ilişkin kriterler üzerinde uzlaşı olamaması ve pek çok tür ve alttür arasında üreme bariyerlerinin yetersizliği sonucunda gen akımı olması nedeniyle tartışmalıdır. Çalışma kapsamında Türkiye ve Orta Doğu başta olmak üzere dünyanın farklı eko-coğrafik bölgelerinden 142 farklı *S. cereale*, *S. vavilovii*, *S. strictum* and *Secale sylvestre* popülasyonundan 727 genotip incelenmiştir. Bu popülasyonlar, popülasyon yapısının ve genetik çeşitliliğin türler arası dağılımının anlaşılması ve mevcut taksonomi ve filogeninin genetik temellerinin belirlenmesi amacıyla yedi SSR, bir kloroplast SNP ve bir nükleer SNP markörü kullanılarak taranmıştır. Çalışma, farklı coğrafik bölgelerden edinilen kültür çavdarının genetik çeşitliliğinin yüksek olduğunu ortaya koymuştur. İncelenen bölgeler arasında, genetik çeşitlilik düzeyinin Orta Doğu'da en yüksek olduğu saptanmıştır. Bu durum Orta Doğu'nun kültür çavdarı için orijin merkezi olduğunu doğrulamaktadır. Hiyerarşik popülasyon yapılanması çok yıllık *S. strictum* alttürlerinin, tek yıllık türlerden genetik olarak farklı olduğunu ortaya koymuştur. Tek yıllık türlerde ise coğrafik farklılaşmaya tekabül eden iki farklı genetik grup gözlenmiştir. İlk grup Asya kökenli örneklerden oluşurken, ikinci grup Asya dışından toplanmış örnekleri içermektedir. Tek yıllık formlarda taksonomik sınırlara karşılık gelen farklılaşmanın gözlenmemesi, kültür çavdarı ve yabani akrabaları arasında izolasyon mekanizmaları ve bariyerlerinin evrimleşmesi için yeterli sürenin geçmemiş olması ve bu nedenle bu formlar arasında melezlenme aracılığıyla gen akışının olmasına bağlanmıştır. Bu gen akışı filogenetik sinyalleri azaltmakla birlikte, yeni nesil dizileme yöntemi aracılığıyla geliştirilecek markörlerin *Secale* cinsinin evrimsel ilişkilerinin tam olarak anlaşılmasını sağlayabileceği düşünülmektedir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Unit
bp	Base Pairs	
ctDNA	Chloroplast DNA	
DNA	Deoxyribonucleic acid	
dNTP	Deoxynucleosidetriphosphate	
EtBr	Ethidium Bromide	
G_{ST}	The coefficient of genetic differentiation	
H_T	Total genetic diversity	
h	Haplotype diversity	
He	Expected heterozygosity	
Ho	Observed heterozygosity	
H_S	Within population genetic diversity	
KCl	Potassium chloride	
M	Molar	Mol/L
$MgCl_2$	Magnesium Chloride	
Min	Minute	
ML	Maximum-likelihood	
µ	Micro	
Na	Number of alleles	
Ne	Number of effective alleles	
TE	Tris EDTA	
P_A	Number of private alleles	
P_R	Private allelic richness	
PCR	Polymerase Chain Reaction	
R_S	Allelic richness	
Sec	Second	
SNP	Single Nucleotide polymorphism	
SSR	Simple Sequence Repeats	
UV	Ultraviolet	

1. INTRODUCTION

Genus *Secale* belongs to the family of true grasses Poaceae (syn. Gramineae), the Pooideae subfamily in the Triticeae tribe that also comprises wheat (*Triticum* spp.) and barley (*Hordeum* spp.) (Table 1.1). The genus diverged from the other species in its family about 1.7 million years ago (Schlegel, 2013). It is a quite heterogeneous genus with annual, perennial (long or short lived), self-incompatible and self-compatible forms (Vences et al., 1987). Besides cultivated rye, genus *Secale* comprises weedy and wild species.

Table 1.1. Scientific Classification of Genus *Secale*.

Kingdom	Plantae (Plants)
Division	Magnoliophyta (Flowering plants)
Class	Liliopsida (Monocotyledons)
Order	Poales
Family	Poaceae (Grass family)
Subfamily	Pooideae
Tribe	Triticeae
Genus	*Secale*

Taxonomy of the genus is still controversial due to disagreements among scientist about the delimitation of species and intraspecific taxa. As a result, the number of species classified in the genus varies from three to 14 in different studies. However, according to the classification system adopted by Germplasm Resources Information Network (GRIN), the taxon contains four species: *Secale cereale*, *Secale vavilovii*, *Secale sylvestre*, and *Secale strictum* (synonym *Secale montanum*) (Sencers and Hawkes, 1980; De Bustos and Jouve, 2002). Among these, *Secale cereale* and *Secale strictum* are polytypic (See Table 1.2).

Table 1.2. Species and subspecies within the *Secale* Genus (Sencers and Hawkes 1980, De Bustos and Jouve, 2002).

Species	Subspecies	Life Form	Breeding Habit
Secale cereale	*S. cereale* ssp. *cereale*	Annual	Outbreeder
	S. cereale ssp. *afghanicum*	Annual	Outbreeder
	S. cereale ssp. *dighoricum*	Annual	Outbreeder
	S. cereale ssp. *segetale*	Annual	Outbreeder
	S. cereale ssp. *ancestrale*	Annual	Outbreeder
	S. cereale ssp. *tetraploidum*	Annual	Outbreeder
	S. cereale ssp. *rigidum*	Annual	Outbreeder
	S. cereale ssp. *tsitsinii*	Annual	Outbreeder
Secale strictum (Syn. *Secale montanum*)	*S. strictum* ssp. *strictum*	Perennial	Outbreeder
	S. strictum ssp. *africanum*	Perennial	Inbreeder
	S. strictum ssp. *anatolicum*	Perennial	Outbreeder
	S. *strictum* ssp. *kuprijanovii*	Perennial	Outbreeder
	S. strictum ssp. *ciliatoglume*	Perennial	Unknown
Secale vavilovii		Annual	Inbreeder
Secale sylvestre		Annual	Inbreeder

Secale cereale, is the annual outbreeder species, that contains the only cultivated subspecies, *Secale cereale* ssp. *cereale.* Like many cultivated forms of cereals, *S. cereale ssp. cereale* is characterized by large and plump grains, a non-fragile rachis that holds the seeds together by preventing shattering of the ear before harvesting, and loosely attached glumes (Zohary and Hopf, 2000) (Figure 1.1). Weedy and wild forms of *Secale cereale* can be distinguished from cultivated rye by fragility of ear rachis, smaller seeds and tightly attached glumes (Frederiksen and Petersen, 1998). *S. cereale* ssp. *afghanicum* differs from *S. cereale* ssp. *cereale* with fragility of upper ¾ of its rachis (Figure 1.2), whereas *S. cereale* ssp. *segetale* is characterized by fragility of upper ¼ part of the same character (Khush, 1963a) (Figure 1.3). *S. cereale* ssp. *ancestrale* is characterized by being longer and having fully fragile rachis (Roshevitz, 1947) (Figure 1.4). All of the non-cultivated varieties can cross with the cultivated form, *S. cereale* ssp. *cereale*, to yield vigorous hybrids with normal pollen fertility (Heemert and Sybenga, 1972; Riley, 1955; Stuz, 1972).

Figure 1.1. Ear, spikelet and seeds of *S. cereale* ssp. *cereale.*

Figure 1.2. Ear, spikelet and seeds of *S. cereale* ssp. *afghanicum.*

Figure 1.3. Ear, spikelet and seeds of *S. cereale* ssp. *segetale*.

Figure 1.4. Ear, spikelet and seeds of *S. cereale* ssp. *ancestrale*.

Secale strictum (Syn. *Secale montanum*) is a complex group containing both outbreeding (*S. strictum* ssp. *strictum, S. strictum* ssp. *kuprijanovii*, and *S. strictum* ssp. *anatolicum*) and inbreeding (*S. strictum* ssp. *africanum*) subspecies, all of which are perennial. The plants have a fully fragile rachis. The *S. strictum* ssp. *africanum* is distinct from other subspecies in having a smaller bristle and smaller anthers (Frederiksen and Petersen, 1998).

Secale vavilovii is an annual and interbreeding species characterized by being shorter than the other species (about 50-80 cm tall). Due to the brittleness of its rachis, it is considered to be wild (Figure 1.5). It is fully interfertile with the cultivated rye (Sencer and Hawkes, 1980).

Figure 1.5. Ear, spikelet and seeds of *S. vavilovii.*

Secale sylvestre, also known as Tibetan rye, is an annual, wild and self-pollinating *Secale* species. The species is morphologically most distinct from rest of the taxa. It is characterized by a short stem, slender culms, narrow dark green leaves, extremely long awns and completely fragile rachis (Figure 1.6). The spike is black-brown, and the pollens

are spherical. It has the shortest anthers of all the species. It differs from *S. cereale* and *S. strictum* by three and one chromosomal translocations, respectively, and is intersterile with both of these species (Zohary and Hopf, 2000).

Figure 1.6. Ear, spikelet and seeds of *S. sylvestre.*

The exact center of origin of the genus *Secale* is not known. However, Vavilov (1928) proposed two centers of origin for rye: the primary center is suggested to be the area between Tabriz and Black Sea, namely East Anatolia, Transcaucasia and north-west Iran, while the secondary center embraces northern Afghanistan, north-east Iran and much of Tadzhikistan, Uzbekistan and Turkmenistan. Furthermore, Turkey was also proposed to be center of origin for perennial rye and geographic origin of cultivated rye was shown to be the area around mountain Ararat and Lake Van (Sencer and Hawkes, 1980).

Although different theories exist on evolutionary history of genus *Secale,* we still do not exactly know the whole story despite availability of sophisticated molecular methods. Nearly all of wild species was proposed to be ancestor of cultivated rye in

different studies (Roshevits, 1947; Hammer et al., 1987; Hammer, 1990; Frederiksen and Petersen, 1998).

Besides phylogenetic studies, investigation of genetic diversity has occupied a distinct position in literature. Genetic diversity refers to the variation of heritable characteristics in different individuals in a population of a single species, is crucial for adaptation to the different environmental conditions, and provides raw material required for evolution. Harlan (1970) emphasized the necessity of conserving plant genetic resources by 'The varietal wealth of the plants that feed and clothe the world is slipping away before our eyes, and the human race simply cannot afford to lose it'.

Genetic diversity in crop plants has been decreasing due to genetic erosion triggered by extensive use of genetically uniform modern cultivars, destruction of habitats because of effects of urbanization, climate change and environmental degradation, changes in the food preferences of populations and natural disasters. Considering global environmental change, understanding the extent and distribution of genetic variation in crop species is extremely important not only in terms of increasing efficiency of their conservation and utilization in the present and the future breeding programs as useful sources of genetic variation, but also conserving agro-biodiversity under the condition of globalized mono-agriculture and securing food security.

Eco-geographical position of Turkey is unique in terms of plant genetic resources and agro-biodiversity. Turkey is situated on the cross road of three main phytosociological regions, Euro-Siberian, Irano-Turanian, and Mediterranean. The topographic and geomorphologic features and climatic conditions of Turkey exhibit significant variety over short distances, leading to great alterations in ecosystem dynamics within the country. Moreover, southeastern part of Turkey is located within the Fertile Crescent where agriculture is believed to have arisen in (Moore et al., 2000 and Gopher et al., 2001). Furthermore, two of the eight gene centers proposed by Vavilov (1951), Mediterranean and the Middle Eastern gene centers intersect in Turkey. De Candolle (1882) stated that genetic diversity of crop species on interspecific and intraspecific level is not evenly distributed,

and Vavilov (1926) underlined the importance of centers of domestication that is also accepted to be centers of diversity, in terms of being gene pools for plant genetic resources.

With its position adjacent to multiple domestication centers, Turkey is the centre of origin for many crop species and is endowed with a diversity of wild crop relatives and primitive landraces that contributed to evolution of modern varieties. Considering that rye is thought to have originated in Turkey (Sencer and Hawkes, 1980) and many landraces have been growing in the area, the current status of landraces should be investigated and proper measures to conserve these landraces should be undertaken in Turkey. Furthermore, Anatolia has a wealth of wild and weedy forms of *Secale* species, including *S. strictum*, *S. sylvestre* and *Secale cereale* ssp. *ancestrale* especially around the Armenia and Iran borders (Hammer and Khoshbakht, 2005). Among these *S. cereale* ssp. *ancestrale* is endemic to south-eastern part of Turkey and categorized as a rare species by International Union for Conservation of Nature (2001). Therefore, a detailed investigation of *Secale* species originating from Turkey is crucial in terms of understanding evolutionary history of the genus.

2. THEOTRETICAL BACKGROUND

2.1. General Characteristics of the Genus

2.1.1. Biology and Morphology

All of the taxa in genus *Secale* are diploids with 14 chromosomes (Jain, 1960). Although it was shown that *Secale cereale* exhibits remarkable caryotypic variation in terms of its banding patterns (Linde-Laursen et al., 1980), results of Giemsa banding assays propounded that general characteristics of chromosomes in the genus are large telemoric heterochromatin regions with several slight interstitial bands.

Genus *Secale* is characterized by an extensive root system consisting of three to four primary roots that develop during seed germination, and several secondary roots that form adjacent to tillering nod. Compared to other cereals, the genus has the most developed root system, penetrating to a depth of 90-230 cm, and laterally spreading 15-25 cm.

The genus comprises herbaceous plants that have a straw stem consisting of three to seven hollow internodes located between glabrous nodes. The stem is erect and green with a bluish-gray shade due to a waxy film. Although the height of the plant may reach up to 3 meters in some species; the average is about 1.5 meters. The leaves of the plant consist of a linear, auriculate lamina with a ligula and auricles at the base, and a sheath tightly enveloping the stem. The lamina is blue-green, 2.5–20 mm wide with a hairy lower surface and a glabrous upper surface. The ligula is located at the inner junction of the leaf sheath and lamina, and horizontally truncated with an unfringed membrane. It envelops the stem and has functions as a protective structure for insects.

The inflorescence is in the form of spike: several solitary spikelets are attached directly to a hollow rachis. Each spikelet is composed of two hermaphroditic florets and rarely a sterile male floret. The glumes are 8–18 mm long and awned. The lemmas are 10–19 mm long, and a relatively long palea is present. Each flower consists of three stamens 2.3-12 mm long, with light yellow to purple color anthers and hairy ovaries with two white stigmas. Even though flowers are hermaphrodite, most of the species in the genus are self-incompatible (Jain, 1960; Sencer and Hawkes, 1980). That is why the plants are cross-pollinating and anemophilous (i.e. it is pollinated via wind dispersed pollen). The grains of cereal rye are oblong or oval shaped and white, yellow, light-blue, violet, brown or green in color. 1000 seeds weigh 30 to 45 g.

2.1.2. Geographical Origins and Timing of Rye Cultivation and Domestication

Cultivation and domestication are two terms that are often confused with one another. Cultivation is “the deliberate planting and harvesting of either wild or domesticated forms” (Salamini et al., 2002). About 12 thousand years ago, the people living in Fertile Crescent, the area spanning Israel, Jordan, Lebanon and western Syria, into southeast Turkey and, along the Tigris and Euphrates rivers, into Iraq and the western fringe of Iran (Moore et al., 2000; Goopher et al., 2002) (Figure 2.1) shifted their hunter-gatherer life style and pioneered first deliberate farming practices. Cultivating plants enabled humans to control natural environment to some degree and created many culture changes which, led to one of the major transitions in the human history, known as the Neolithic revolution. Subsequently humankind adopted a sedentary life style. Although the exact driving forces that initiated agricultural activity are still unknown, the most convincing possibility is that a major climatic change, at the end of the last ice age might have triggered the process (Gupta, 2004). A cold, dry climate gradually spread through the earth in an episode that lasted approximately 1000 years. These dry seasons may have impaired the natural stands of edible plants and adversely affected communities who had to invest in agricultural activity and start cultivation (Bar-Yosef, 1998).

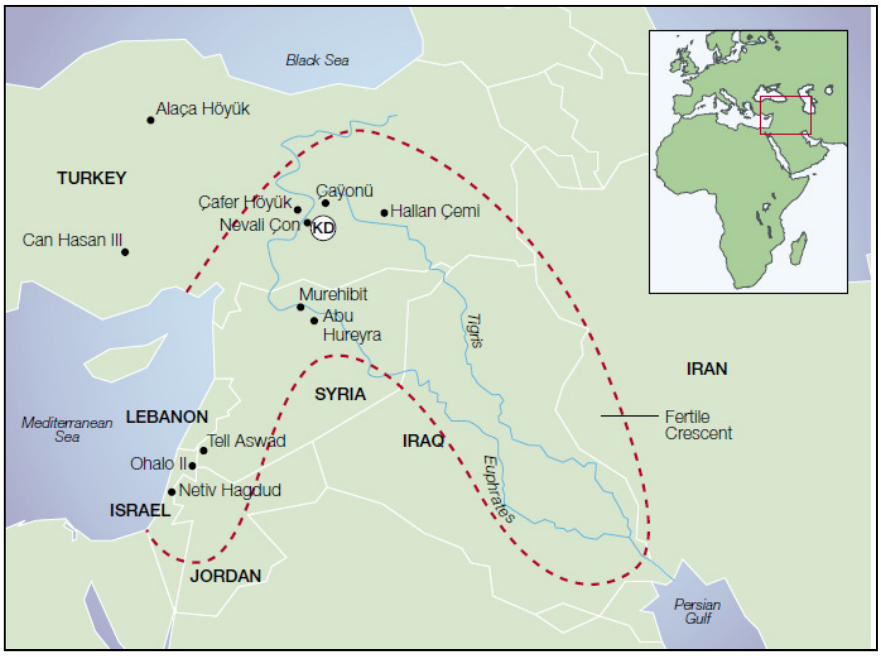

Figure 2.1. Map of the Near East**.** The Fertile Crescent is dashed red line (From Salamini et al., 2002).

Domestication refers to the process of altering specific traits of wild plants by genetic selection to obtain crop varieties having desirable characteristics with the aim of meeting human needs (Salamini et al., 2002; Doebley et al., 2006; Brown et al., 2008). Domesticated plants have bigger grains compared to wild forms. Furthermore the rachis of the spikelets is more fragile in wild forms enabling the mature seeds to disperse. On the other hand in domesticated plants the rachis stays though on maturity and holds the grains together in a harvestable and threshable ear. Thus, domestication leads to loss of seed dispersal mechanisms. Moreover the glumes, leaf-like protective structures are tightly attached to the seed in wild forms, whereas in domesticated forms it releases from the grain easily. Besides domesticated plants may lose seed dormancy, and exert synchronous tillering, flowering and ripening. Humankind started to domesticate crops and animals at the Bronze Age. Based on archaeobotanical evidence (Moore et al., 2000; Gopher et al., 2002; Zohary and Hopf, 2000) and current distribution of wild progenitors of wheat (*Triticum urartu*, *T. boeoticum* and *T. dicoccoides*), and barley (*Hordeum spontaneum*) (Gopher et al., 2002; Zohary and Hopf, 2000) in the area, the Fertile Crescent was proposed to be the center of domestication for Neolithic founder crops like wheat, barley,

pea, lentil, chickpea and vetch (Zohary, 1996). The process gradually disseminated to other parts of the world.

Exact timing and localities of beginning of rye cultivation and domestication is still a controversial issue. Many researchers from different fields like archeology and even philology have put efforts to help clarify the issue.

Although the exact center of origin is not known, the most probable region for it is considered to have been southwestern Asia. Vavilov proposed two centers of origin for rye: the primary center is suggested to be the area between Tabriz and Black Sea, namely East Anatolia, Transcaucasia and north-west Iran, while the secondary center embraces northern Afghanistan, north-east Iran and much of Tadzhikistan, Uzbekistan and Turkmenistan (Vavilov, 1928). In both places, the weedy forms of the genus still exist. Turkey was proposed to be center of origin for perennial rye and geographic origin of cultivated rye was shown to be the area around mountain Ararat and Lake Van (Sencer and Hawkes, 1980).

Although linguistic methods alone cannot be used to determine original habitats and domestication sites, they may contain informative clues. In Turkestan, Persia, Arabia, Turkey and Afghanistan rye is named “dzhoy-der” “gandum-der”, “chau-der”, or “jou-dar” meaning ‘plant preying upon’ indicating that rye was a weed contaminating wheat and barley fields and it was very difficult to get rid of this “weed” (Vavilov, 1917).

According to the information given by De Candolle, there is no indication of rye cultivation in Egyptian monuments (De Candolle, 1885); suggesting rye is not among the founder crops of Neolithic Revolution. Based on archeological records of rye cultivation from several Bronze Age settlements like Alacahöyük (Hillman, 1975), and different parts of Europe, it was proposed that rye was cultivated during the Bronze Age. However, large rye grains from Aceramic Neolithic site of Can Hasan III in the Konya Basin of South-Central Anatolia dated to 6600 B.C (Hillman, 1978) and Tell Abu Hureyra in Syria were also reported (Moore et al., 2000). These relatively recent excavation reports provoked the idea that cultivation of rye occurred much earlier. Furthermore, Hillman reported wild rye seeds, assembled with wild einkorn, and barley seeds from Epipaleolithic site of northern

Syria, Tell Mureybit (Van Zeist and Casparie, 1968), indicating humans were cultivating wild rye in Neolithic Near Eastern sites, before its domestication.

Vavilov proposed that, cultivated rye is a secondary crop: its wild forms entered wheat and barley fields as a weed and humankind selected the individual weedy ryes with non-brittle rachis, bigger seed size and suitable maturity time consciously (Vavilov, 1917). During the migration out of the central Asia, the most likely center of origin, humans dispersed the seeds of this invasive plant together with wheat and barley seeds northwards or into higher altitudes. As rye tolerates lower temperatures and poor soils better than barley and wheat do, it out-competed the others in severe environmental conditions. Subsequently, the farmers realized that rye grain was as edible as wheat. Thus it underwent domestication and was adopted as a crop. Subsequently the name of the plant was intentionally or unintentionally converted from "the plant preying upon" to "better gift from gods" (Vavilov and Dorofeev, 1992).

Although its exact route of the migration is obscure, it was hypothesized that rye reached Europe mixed with other cereals, and during the climatic deterioration at the end of the Bronze Age and beginning of the Iron Age it emerged as a crop. There are different hypotheses about migration route of rye from central Asia. The first scenario is that, rye dispersed from Turkey across the Balkan Peninsula into north-central Europe as the weed of other cereals (Kornicke, 1885; Schultz, 1911; Regel, 1922**;** Popov, 1939; Deodikar 1963). Afterwards it was carried to rest of Europe and became a European crop. On the other hand, some authors proposed that rye as a weed was carried from East Anatolia and Transcaucasia through Persia and Turkestan, east of the Caspian, and finally into East and central Europe via the Ural Gap (Vavilov, 1917; Schiemann, 1932). Consistent with this hypothesis, Kuckuck and Scheibe proposed a northward route, from Asia Minor into Russia, followed by a westward route into Poland and Germany (Kuckuck, 1937; Scheibe, 1935). Another hypothesis is that cultivated rye may have emerged independently from the two gene centers: it was first cultivated in Transcaucasia, Afghanistan and Turkestan. This was followed by cultivation of the varieties in northeastern Kazakhstan, Uzbekistan and northern Transcaucasia (Khush, 1963b). Once cultivated rye reached Europe, it was carried to North America and western South America, during the sixteenth and seventeenth

centuries. Its introduction to Argentina, Brazil, Uruguay, Australia, and South Africa occurred in the twentieth century.

2.1.3. Ecology and Distribution

Compared to other taxa in Triticeae, genus *Secale* is more adaptive to different environmental and climatic conditions. The plants can grow under unfavorable conditions that the other cereals cannot tolerate. Some wild and cultivated forms are known to be winter-hardy and can tolerate very low temperatures as low as minus 30-35°C. These forms can be discriminated by some morphological and biological features like narrow leaves, thicker outer epidermis wall, and a shorter mesocotyl cell. Members of the genus are quite drought-resistant due to their extensive root system that allows them to use less water than other relatives (Evans and Scoles, 1976). In terms of soil requirements, optimal growth occurs on fertile and well-drained loams having pH of 5.6 to 5.8 or higher. However members of the genus are able to tolerate different kinds of soil including poor, sandy, rocky, and acidic. Furthermore high levels of aluminum and manganese in soil can also be well tolerated (Culvenor et al., 1985).

Due to its adaptability to a wide range of environmental and climatic conditions, genus *Secale* has a broader global distribution than the other grasses (Stutz, 1972; Lorenz, 1991; Bushuk, 2001). Although the genus is regarded as the typical representative of Mediterranean flora, *Secale* species grow in the six continents (Brink, 2006), and Southwest Asia, specifically Turkey, Lebanon, Syria, Iran, Iraq, and Afghanistan are the main areas of its distribution (Sencer and Hawkes, 1980). *Secale strictum* grows in a wide geographical range spanning the Sierra Nevada Mountains of southern Spain and the Atlas Mountains of Morocco, to Sicily and southern Italy, Greece, south-east Europe, and the Near East (De Bustos and Jouve, 2002). *Secale strictum strictum* and *Secale strictum* ssp. *anatolicum* grow in Turkey, Western Iran, and Iraq (De Bustos and Jouve, 2002), while *Secale strictum* ssp. *kuprijanovii* and *Secale strictum* ssp. *africanum* are endemic to northern Caucasus Mountains and South Africa, respectively. *Secale vavilovii* was reported to grow on sandy soils around the Aras River and montane areas of eastern Turkey and Armenia (Sencer, 1972). *Secale sylvestre* is capable of growing in sandy regions such as

river deltas, seashores, steppes, and semi-deserts (Frederiksen and Petersen, 1998). Its distribution spans Eastern Europe to central Asia (Roshevitz, 1947; Sencer, 1972). *Secale cereale ssp. ancestrale*, the subspecies endemic to Turkey, was first discovered in Aydın (Zhukovsky, 1933), and is reported to grow naturally on sandy soils, roadsides and stony fields between Turkey and Kazakhstan. Although a cultivated species, *S. cereale* ssp. *cereale*, is grown in a wide area spanning Scandinavia to Southern Chile, and due to its winter hardiness it is preferred in the cooler temperate zones of Europe.

2.1.4. Economic Value and Uses

In 2012 approximately 14.5 million tons of cereal rye was produced on 5.5 million hectares of land all over the world (FAOSTAT, 2012). Due to its winter hardiness, cultivated rye is a preferred crop in cooler parts of the Europe and its main producers are Poland, Germany, western Russia, Belarus, and Ukraine (Bushuk, 2001). On the other hand, compared to other crops like wheat, rice and maize its production is still very limited and it is considered to be a minor crop.

Cultivated rye is primarily grown for livestock feed. Rye grains have a considerable amount of feeding value and are mixed with the grains of other cereals to produce animal feed. Furthermore, immature rye is harvested as forage. Rye grains are also used for human consumption. Although rye grain does not contain true gluten, because of its high soluble fiber content, rye flour improves the volume and texture of bread, thus it is mixed with the flour of other cereals such as wheat and maize in bread production. Rye is also processed to produce alcoholic beverages like whisky, vodka and beer. Rye starch is used for industrial purposes such as production of glue, paper, and plastics. Because it is tolerant to biotic and abiotic stress conditions (Duke, 1978), it is used in breeding programs for improvement of wheat varieties. Rye (*Secale cereale*) is also used in the production of first human-made crop, triticale (*Triticosecale*) which is a synthetic amphiploid derived by crossing it with wheat *(T. durum L.* or *T. aestivum L.*). The other important features of cultivated rye are its ability to contribute to soil's organic content, reducing soil erosion and enhancing water retention and penetration. Thus it can be grown as cover crop, green manure (McLeod, 1982) or a pioneer crop for sterile soils. It was also suggested that rye

residues remaining on the soil produce some metabolites that prevents growth of weeds (Barnes and Putnam, 1983), and due to this allelopathic effect, rye could be used for weed control. Moreover studies are made to assess its potential as a biomass energy crop in Europe.

2.2. Molecular Marker Systems Used in Plant Studies

Molecular markers are commonly used for taxonomy, phlogeny, ecology, and breeding studies in higher plants (for reviews see Avise et al., 1994; Henry et al., 2012; Semagn et al., 2006). The first generation molecular markers were based on protein molecules. Two groups of proteins have been widely used in these kinds of studies: the first was isozymes, enzymes that convert the same substrate, but which are not necessarily encoded by the same gene. The second kind was allozymes, enzymes that convert the same substrate and are encoded by different alleles of the same gene. Protein markers were widely used in phylogenetic studies of cereal plants like wheat (Benito et al., 1980), oat (Marshall and Allard, 1970) and barley (Almgard and Landegren, 1974), due to their co-dominant nature, and time and cost-efficiency. However, due to their drawbacks, such as requirement of fresh tissue and limited variation, they were subsequently replaced by DNA markers.

Genetic markers, also known as DNA markers, are defined as a fragment of DNA molecule that exerts sufficient nucleotide variation to detect polymorphism at genomic level. In contrast to protein markers, this variation is not necessarily related to phenotypic expression. An ideal marker should be highly polymorphic to provide adequate resolution of nucleotide differences, have ubiquitous distribution in the genome, yield highly reproducible and simple results, and be quick and inexpensive to use. The different kinds of DNA markers that are explained in detail below have been used to address a variety of questions in plants (for a review see Varshney et al., 2007b).

2.2.1. Restriction Fragment Length Polymorphism (RFLP)

RFLPs, which can be classified as the first DNA markers, were developed by Botstein et al. (1980). The method is based on restriction of the DNA by at least one restriction enzyme that recognizes specific nucleotide sequences and subsequent comparison of the length of the obtained fragments by electrophoresis. The method detects nucleotide changes in recognition sites that lead to differences in fragment size (Tanksley et al., 1989). This co-dominant marker allows evaluation of polymorphisms in different parts of the genome (nuclear, mitochondrial, or chloroplastic) and is used in genetic diversity studies of plant populations. RFLP marker was used in many different kinds of crop plants, including rice (McCouch et al., 1988), maize (Helentjaris, 1987), wheat (Chao et al. 1989), barley (Graner et al., 1991), chickpea (Simon and Muehlbauer, 1997) and rye (Murai et al., 1989; Petersen and Doebley, 1993; Skuza et al., 2007; Işık et al., 2007).

2.2.2. Randomly Amplified Polymorphic DNA (RAPD) Markers

Randomly Amplified Polymorphic DNA, the first PCR-based DNA marker, was developed in by Welsh and McClelland (1990). The method involves amplification of discrete, random DNA fragments using a single, short, arbitrary primer, which detects nucleotide changes at the primer binding site. The main drawback of this marker is their dominant nature that does not allow discrimination of heterozygous individuals from homozygous ones. However the technique is easy and applicable with universal primers, and requires no prior sequence information. Thus it is attractive tool for genetic fingerprinting and linkage studies, genetic diversity assessments, and phylogenetic analyses of plant populations (Reiter et al., 1992; Nesbitt et al., 1995; Chikaiza et al., 2006; Mayuri et al., 2013 Harris, 1999; Stojalowski et al., 2004) and was used in the evaluation of genetic diversity studies in the *Secale* genus (Matos et al., 2001; Ma et al., 2004).

2.2.3. Amplified Fragment Length Polymorphism (AFLP)

AFLP marker combines RFLP method with PCR-based techniques, and was developed by Vos et al. (1995). The analysis involves digestion of total DNA using specific restriction enzymes, ligation of double stranded adapters to the sticky ends, and amplification of DNA fragments by PCR. The primers used in the reaction correspond to adapters and restriction sites. One to three selective bases are also added to 3' regions of the primers, so that the number of fragments is reduced to facilitate the analysis. Because the technique is reliable and also produces DNA fingerprints without prior sequence information, it has been used in phylogenetic studies, assessment of genetic diversity and distinguishing closely related individuals of the same species (Althoff et al., 2007). Because of these advantages, AFLPs have been used in many molecular studies for plants like wheat (Barrett and Kidwell, 1998; Goodwin et al., 1998; Koebner et al., 1998; Hazen et al., 2002; Roy et al., 2002; Altintas et al., 2008) barley (Badr et al., 2000; Ellis et al., 1997; Schut et al., 1997), rice (Mackill et al., 1996; Zhu et al., 1998; Nuijten et al., 2009) and rye (Chikmawati et al., 2005).

2.2.4 Simple Sequence Repeat (SSR)

Simple Sequence Repeats, also known as microsatellites or Short Tandem Repeats (STR), are loci consisting of tandem repeats of one to six base pairs that are distributed throughout nuclear and organelle genomes of eukaryotic organisms (Tautz and Renz, 1984). This marker was developed during 1990s, and was first used in plant systems by Condit and Hubel (1991). The rationale behind this method is detecting variation in the number of repeated units stemming from slippage during DNA replication. Because the rate of slippage is higher than point mutations, SSR exhibits hyper-variability.

SSRs offer a variety of advantages such as co-dominate inheritance, high levels of polymorphism and easy reproducibility to the researchers. These advantages prompted the use of SSRs for a variety of purposes including investigation of evolutionary relationships of related taxonomic units, assessment of genetic variation between and within

populations, determination of population and subpopulation structure, and marker assisted selection in plant breeding (Gupta et al., 1996).

In addition to nuclear ones, organellar SSR markers are also regarded as effective tools in the analysis of genetic structure and phylogeography of populations of different organisms. Mitochondrial DNA of plants is more complex and has a higher rate of sequence reorganization compared to those of animals (Sederoff et al., 1981). As a result it is not a preferred marker for phylogenetic analysis in plant populations. On the other hand, due to their conserved gene order, uniparental (generally maternal in angiosperms and paternal in gymnosperms) mode of inheritance, lack of recombination and heteroplasmy, availability of primers and relatively high levels of polymorphism, chloroplastic SSRs have been widely used in genetic studies of self-pollinating crops such as wheat (Röder et al., 1995; Bryan et al., 1997; Ishii et al., 2001; Hirosawa et al., 2004 and Ishii et al., 2006), barley (Becker and Heun, 1995; Liu et al., 1996), and soybean (Akkaya et al., 1992) and outcrossing crops, such as maize (Taramino and Tingey, 1996), sugar beet (Mörchen et al., 1996), and spinach (Groben and Wricke,1998) as a complementary tool.

Today, more than 100 SSR markers, developed by independent research groups are available to resolve phylogenetic relationships of the genus *Secale* (Saal and Wricke, 1999. Korzun et al., 2001; Hackauf and Wehling, 2002; Khlestkina et al., 2004; Boolibok, 2006).

2.2.5. Single Nucleotide Polymorphisms (SNPs)

Single nucleotide polymorphisms (SNPs) that provide 100-fold higher resolution than microsatellites are the most powerful DNA-based markers in crop systems (Cho et al., 1999; Rafalski, 2002). The system based is on determination of nucleotide sequence differences between individuals and allow us to understand the evolutionary relationships of different alleles. The method has also used for genetic mapping, genetic diversity assessment, and marker-assisted breeding. SNPs have been discovered for many crop plants including rice (Nasu et al., 2002; Feltus et al., 2004), maize (Tenaillon et al. 2001; Ching et al., 2002), wheat (Somers et al., 2003), barley (Kota et al., 2001; Russell et al.,

2004; Rostoks et al., 2005), soybean (Zhu et al., 2003; Van et al., 2004), and sugar beet (Möhring et al., 2004) and rye (Varshney et al., 2007a).

2.2.6. Transposon-based Markers

Transposable elements (TEs) are the mobile DNA segments that can insert themselves into new chromosomal locations. Barbara McClintock, a plant scientist first discovered TEs in maize (McClintock, 1950) and suggested that these elements might have a regulatory function in gene activation (McClintock, 1965). TEs are the largest components of most eukaryotic genomes, comprising 50–80% of some grass genomes (Meyers et al, 2001; SanMiguel and Bennetzen, 1998; Vincent et al, 1999). Functionally speaking, transposable elements can affect gene structure and expression, and contribute to evolutionary events.

Based on their structure and transposition, intermediate TEs can be classified in two categories (Craig et al., 2002): The first group of TEs is Class 2 Elements, also known as DNA transposons. They directly cut themselves from DNA molecule and then reintegrate themselves at another position on the DNA molecule.

The other group is Class 1 Elements, also known as retrotransposones, which function by copying themselves into an RNA transcript and inserting their reverse-transcribed cDNA copies back into target region of the genome. As a result, they amplify to high copy numbers and contriburte significantly to genome size (Alix and Heslop-Harrison, 2004; Boyko et al., 2002; Ellis et al., 1998; Hedges and Batzer, 2005; Schnable et al., 2009). Furthermore they lead to intra-chromosomal recombination, and higher genetic variability, and they constitute more than half of the repetitive DNA in crop plants (Schnable et al., 2009). There are two kinds of retrotransposons: the first kind is long terminal repeat (LTR) retrotransposons, which contain a long terminal repeat region with a conserved sequence at the end. Ty1-copia retrotransposons and Ty3-gypsy retrotransposons are contained in this group. Second is non-long terminal repeat retrotransposons (non-LTR), which contains Long Interspersed Elements (LINEs) and Short Interspersed Elements (SINEs).

LTR retrotransposons were shown to be the predominating class of transposable elements in higher plants (Schnable et al., 2009). Due to their pervasive nature, widespread distribution in the genome, and high copy numbers, retrotransposons were evaluated as an important source in development of new molecular markers (Kalendar and Schulman, 2006; Schulman et al., 2004; Shedlock and Okada, 2000), all of which depend on detecting the insertion of these long elements.

2.2.6.1. Sequence-Specific Amplified Polymorphism (SSAP). SSAP is a retrotransposon-based method developed by modification of the AFLP system (Waugh et al., 1997). It is based on cleavege of the DNA molecule with restriction enzymes to generate a binding site for a specific primer and subsequent amplification of the region between restriction sites and conserved LTR region of retrotransposon by PCR. The method allows to determine distribution and structure of retro elements in different organisms of a species and has been used to determine the rate of genetic variability and evolutionary relationships in different crop plant populations like oat (Yu and Wise, 2000), barley (Waugh et al., 1997, Watkins et al., 1997, Ellis et al., 1997, Leigh et al., 2003), and wheat (Gribbon et al., 1999; Queen et al., 2004; Bento et al., 2008; Charles et al., 2008, Wicker et al., 2009, Ragupathy et al., 2010).

2.2.6.2. Interretrotransposon Amplified Polymorphism (IRAP). The IRAP method is based on isolation of DNA fragments between two nearby retroelements using one or two primers annealing to the outward parts of the LTR sequences by PCR (Kalendar et al., 1999; Kalendar and Schulman, 2006). Thus it detects insertional polymorphisms and fingerprinting patterns. Because this technique is easy and cost-effective, it provides remarkably polymorphic results, and it has been increasingly used in plant studies (Branco et al., 2007, Kalendar et al., 2000). The technique was also used to resolve evolutionary relationships of *Secale* genus (Achrem et al., 2014).

2.2.6.3. Retrotransposon Microsatellite Amplification Polymorphisms (REMAP). The rationale behind the REMAP method is amplification of DNA fragments between retrotransposon and microsatellites using primers specific to conserved LTR sequence and a SSR motif, so that the marker can detect polymorphisms stemming from both insertion of retrotransposons and mutations in the microsatellite regions. The method has been used for

variety of purposes like gene mapping, measurement of genetic diversity, and determination of details of evolutionary history of *Aegilops* species (Boyko et al., 2002) and barley (Manninen et al., 2000; Vicient et al., 2001).

2.2.6.4. Inter PBS Amplification (iPBS) Analysis. Kalender et al. (2010) developed a retrotransposon based, universal marker that can be used in any organism without previous sequence knowledge. It was shown that LTR retrotransposons utilize cellular tRNAs as primers for reverse transcription to replicate themselves, and tRNA recognizes the primer binding site (PBS) located on the 5' region of an LTR (Kelly et al., 2003; LeGrice, 2003). Based on this information, it is possible to designate a set of primers that match conserved domains of PBS sequences. This promising marker has been used to DNA fingerprinting and genetic diversity studies of *Cicer* species (Andeden at al., 2012).

2.3. Taxonomy and Phylogeny of the Genus *Secale*

2.3.1. Taxonomy of the Genus

In early taxonomic studies ecology, morphology, life cycle and distribution of plants were used to discriminate various *Secale* species. Vavilov (1917; 1926) classified four species within the genus: *S. africanum*, *S. cereale*, *S. fragile*, and *S. strictum*. Three subspecies of *S. cereale* were distinguished by Zhukovsky (1928): these were *S. cereale ssp. cereale*, *S. cereale* ssp. *ancestrale* and S. *cereale* ssp. *segetale*. Afterwards, Zhukovsky (1933) raised subspecies *ancestrale* to species level. Roshevitz (1947) stated that the genus contains 14 species constituting three major groups. Group Kuprijanovia contained *S. strictum* and entire perennial species (*S. kuprijanovii, S. dalmaticum, S. ciliatoglume, S. daralagesi, S. anatolicum,* and *S. africanum*). Group Cerealia, the evolutionarily youngest group derived from Group Kuprijanovia, was composed of *S. cereale* and all weedy annual relatives (*S. vavilovii, S. dighoricum, S. afghanicum, S. ancestrale,* and *S. segetale*). The third one was Group Silvestria consisting of the annual species *S. sylvestre* only.

Using cytogenetic data, Khush and Stebbins (1961) proposed that the perennial taxa (*S. strictum*, *S. africanum*, *S. kuprijanovii*) should not be classified as separate species, but instead be evaluated as subspecies of *S. strictum*. They also classified weedy forms (*S. ancestrale*, *S. afghanicum*, *S. dighoricum*, and *S. segetale*) as subspecies of *S. cereale*. Sencer (1975) discriminated three species; *S. cereale* containing annual, wild, weedy, and cultivated forms, the wild and perennial *S. strictum*, a wild and annual species (*S. sylvestre*) that is isolated from *S. strictum*, and *S. cereale*. This classification was supported by enzymatic studies (Jaaska, 1975; 1999). Furthermore, using morphological and genetic data Frederiksen and Petersen (1998) concluded that there are three species of *Secale,* including *S. sylvestre, S. strictum*, and *S. cereale*. They also recognized two subspecies for *S. strictum* (*S. strictum* ssp. *strictum* and *S. strictum* ssp. *africanum*). Similarly, using AFLP data, Chikmawati et al. (2005) showed that there were three clearly separated species groups within genus *Secale*, whicn included perennial *S. sylvestre* and *S. strictum*, and annual *S. cereale*.

On the other hand, the findings of isoenzymes studies by Vences et al. (1987) were consistent with Vavilov's idea that there were four species in the genus *Secale* (Vavilov, 1926). This view was supported by other studies (Hammer et al., 1987; Hammer, 1990).

2.3.2. The Evolutionary Origin of Cultivated Rye and the Phylogeny of the Genus

The origin of cultivated rye and evolutionary history of genus *Secale* have been studied by researchers from various disciplines. There is a general agreement on Vavilov's hypothesis that rye is a secondary crop, and non-brittle weedy forms of Southwest and Central Asia are direct progenitors of cultivated rye (Vavilov, 1917; Vavilov, 1926 and Schiemann, 1948). On the other hand, the identity of the ancestral species from which cultivated rye evolved from is still a matter of debate.

Some investigators offered that *S. vavilovii*, which evolved from *S. strictum*, is the ancestor of *S. cereale* (Vavilov, 1926; Roshevits, 1947). After considering its brittle rachis, Zohary and Hopf (2000) also supported the hypothesis that *S. vavilovii* being the wild progenitor of the cultivated crop. *S. ancestrale* was also proposed as the progenitor of modern rye varieties and *S. ancestrale* and S. *strictum* were suggested to be descendants of a common ancestor (Schiemann, 1948; Zhukhovsky, 1933).

It was also reported that *S. cereale* originated from *S. strictum* (Rilley, 1955). Based on ecological and morphological properties Khush and Stebbins (1961) suggested that *S. strictum* progressively differentiated to *S. cereale*; *S. strictum* and *S. sylvestre* were the oldest, and *S. cereale* was the youngest of *Secale* species. Depending on the data from his subsequent studies, Khush (1962) suggested that all species were differentiated from *S. strictum* and differentiation occurred by two and one translocation event for *S. cereale* and rest of the genus, respectively. He broadened his studies by investigating phylogenetically important morphological characters such as brittleness of the rachis and caryopsis size, and concluded that domestication of rye occurred in distinct places and at different times (Khush, 1963a; 1963b), which is consistent with Vavilov's hypothesis. Investigating the rye populations from Turkey, Zohary (1971) proposed that all weedy and cultivated ryes evolved from *S. strictum* by sympatric speciation stemming from disruptive selection.

Whyte (1975) explained that because being annual is more advantageous especially at dry climates and poor soils, annual species should have derived from perennial forms. Furthermore, Stebbins (1957) offered that open pollination should be considered as an evolutionarily older feature than self pollination. Because of two characteristics, being open-pollinated and perennial, the most possible ancestor of the genus is thought to be *S. strictum*. On the basis of isoenzyme studies Vences et al. (1987) affirmed that S. *strictum* is the most ancient species and *S. sylvestre* was the most distinct species. This proposal was confirmed by ecological studies (Hammer et al., 1987; Hammer, 1990), and analysis of chromosomal distribution of a defined repeated-DNA sequence by Jones and Flavell (1982) who stated that the progenitors of *S. strictum* and *S. sylvestre* were separated first, then *S. africanum* evolved from *S. strictum*, and *S. vavilovii* and *S. cereale* originated most recently from *S. strictum*.

Stutz (1957) offered that *S. cereale* derived from a weedy relative, which formed as a result of introgression of *S. strictum* into *S. sylvestre*. Having studied primitive collections from Iran, Kranz (1957) proposed a polyphyletic origin for rye and explained the variation between the populations studied by adaptation, isolation and hybridization. Based on further studies, he also considered *S. vavilovii* as the intermediate form between perennial wild ryes and cultivated rye (Kranz, 1961).

Development of DNA markers has provided a direct means to assess phylogenetic relationships, genome diversity and evolutionary history of the genus *Secale*. Murai et al. (1989) conducted an RFLP analysis of chloroplast DNAs (ctDNA) of five important *Secale* species (*S. cereale*, *S. vavilovii*, *S. africanum*, *S. strictum*, and *S. sylvestre*) to reveal interspecific relationships in the genus and phylogenetic relations between the genus and two related genera, *Triticum* and *Aegilops*. They reported that chloroplast genomes of both *S. sylvestre* and other *Secale* species were closely related to those of *Triticum* and *Aegilops*. Furthermore, the degree of diversification of the chloroplast genome in *Secale* was nearly the same as in *Triticum* and *Aegilops*. The analysis also revealed observable differences in ctDNA of *S. sylvestre* compared to those of the other *Secale* species, indicating an early separation. Similarly rDNA spacer length variation analysis (Reddy et al., 1990) and RFLP analysis based on plastid genome (Petersen and Doebley, 1993) revealed that *S. sylvestre* was differentiated from rest of the taxa. This finding is consistent to the data obtained by

Skuza et al. (2007) who, by using a mitochondrial RFLP analysis, showed that *S. sylvestre*, the first species that differentiated from others, diverged from *S. strictum* during pliocene epoch or later and *S. vavilovii*, *S. strictum* ssp. *africanum*, *S. strictum* and *S. strictum* ssp. ssp. *kuprijanovii* are evolutionarily the youngest species in the genus. They also grouped genus *Secale* into two groups: the first one includes two genetically very similar species, *S. sylvestre* and *S. cereale* ssp. *segetale*, whereas the second group includes *S. vavilovii*, *S. cereale*, *S. strictum*, *S. strictum* ssp. *africanum* and *S. strictum* ssp. *kuprijanovii*.

Pozo et al. (1995) performed a PCR based analysis using 22 different accessions of *Secale* genus. Their findings were consistent with those proposed by Khush (1962). On the other hand *S. cereale* ssp. *anatolicum* was found to be closer to *S. cereale* than to *S. strictum*, while *S. strictum* ssp. *kuprijanovii* was found to be closest to *S. sylvestre*.

Cuadrado and Jouve (1997) conducted a fluorescence in situ hybridization assay in wild and cultivated *Secale* species. They identified two separate groups as well. In this study the first group included the autogamous annuals *S. sylvestre* and *S. vavilovii*. The second group comprised the perennials S. *strictum*, *S. anatolicum*, *S. africanum*, and *S. kuprijanovii*, and annual, cross-pollinated species *S. cereale* and its related weedy forms. They concluded that *S. sylvestre* is the most distant species, and the most probable ancestor of cultivated rye is wild and weedy forms of *S. cereale* and *S. ancestrale*.

Chikmawati et al. (2005) conducted a study to reveal phylogenetic relationships among *Secale* genus by using the AFLP method. The obtained data led to (i) classification of investigated species into three groups: The first group includes first separated species, *S. sylvestre* and *Secale ciliatoglume*, an isolated weedy population endemic to Mardin, southeastern Turkey. The second group consisted of annual taxa *S. ancestrale,* S. *afghanicum*, *S. cereale, S. dighoricum, Secale turkestanicum*, S. *segetale*, and *Secale vavilovii*, and third group of *S. strictum, Secale anatolicum*, *S. kuprijanovii* and *S. africanum*. Moreover *S. ciliatoglume* was found to be the most distant relative of the other species. In contrast to Roshevitz's idea that *S. strictum* was the most ancient species, this study revealed that *S. sylvestre* was evolutionarily oldest species, while *S. cereale* was the youngest. The results are consistent with the Roshevitz's (1947) hypothesis that there are three major groups in the genus. Furthermore the study emphasized that life-cycle is a

critical characteristic determining evolutionary relationships. Besides, the obtained data was concordant with the idea that *S. cereale* and *S. vavilovii* are separate but closely related species (Khush, 1962; Del Pozo et al., 1995; Cuadrado and Jouve, 1997).

The phylogenetic relationship of cultivated *Secale* species from different geographical regions from all over the world was investigated using 24 microsatellite markers by Shang et al (2006). The results of the study revealed that (a) the highest and lowest within-species diversity was observed for *S. sylvestre* and *S. strictum*, respectively (b) microsatellite polymorphism in the cultivated rye is lower (c) *S. vavilovii* was found to be closely related to *S. cereal*, and *S. sylvestre* was more divergent from the other species.

Investigating different repetitive DNA sequences, Cuadrado and Jouve (2002) stated that *S. strictum* is the ancestral species from which *S. sylvestre*, the most diverse group within the taxon was diverged from during Miocene, not supporting the hypothesis that a common ancestor for both species had existed (Khush and Stebbins, 1961). This is followed by relatively recent separation of inbreeding subspecies *S. strictum* ssp. *africanum*; during Pleistocene a population of *S. stictum* migrated to South Africa and has evolved separately from the rest of the genus. On the basis of close relationship between *S. cereale* and *S. vavilovii* they also stated that these two forms are subspecies of a single species. Moreover, the authors postulated that *S. strictum* is a complex group consisting of subspecies that emerged due to independent evolution and subsequent isolation of populations.

In another study, De Bustos and Jouve (2002) studied evolutionary relationships of the *Secale* species by direct sequencing of internal transcribed spacer of 18S-5.8S-26S rDNA region. They did not observe sequence differences between the subspecies of *S. cereale* and explained this situation by suggesting that these taxa evolved recently and sufficient time had not passed for concerted evolution to end. The results obtained from the study indicated that *S. strictum* was the precursor of all other species with great heterogeneity, and that *S.sylvestre* was the most diverse group.

Shang et al. (2007) investigated non-transcribed spacer of 5S ribosomal DNA from wild and cultivated *Secale* taxa to elucidate the extent of molecular diversity; to determine

whether the sequence polymorphisms of NTS can be used to as molecular markers to reveal insights about evolutionary history of genus *Secale* and related taxa. Although they grouped *Secale* species into two groups, the resolution provided by 5S DNA fragments were not fully sufficient.

Zhou et al. (2010) investigated evolutionary relationships of the genus using genomic in-situ hybridization method. They propounded that *S. sylvestre* was the first species that diverged from *S. strictum*. This was followed by separation of *S. strictum* ssp. *africanum* from the rest of genus. Furthermore their studies showed similarity of *Secale cereale* and *S. vavilov*ii.

A recent study by Ren et al. (2011) emphasized that *S. strictum* ssp. *africanum*, *S. strictum* ssp. *anatolicum*, *S. sylvestre*, and *S. strictum* ssp. *strictum* descended from a common ancestor, and subsequently they were separated geographically leading to genetic differentiation. They also suggested that cultivated rye emerged as a result of domestication of an annual weedy form that evolved from *S. strictum* ssp. *strictum*. Moreover their findings supported the idea that there are three species in genus *Secale*: annual and wild *S. sylvestre*, the perennial wild *S. strictum*, and *S. cereale*.

Achrem et al. (2014) used ISSR and IRAP markers to assess evolutionary relationships among *Secale* species. Their findings revealed three different clusters: First cluster comprised *Secale strictum* and *Secale sylvestre,* the most distinct species. The second contained *Secale strictum* ssp. *africanum* and *Secale strictum* ssp. *kuprijanovii*. The remaining taxa were contained in the third cluster and in which the similarity between *Secale vavilovii* and *Secale cereale* was found to be higher than that between *Secale cereale* and its subspecies, indicating *Secale vavilovii* might be considered as subspecies of *Secale cereale*. Furthermore the similarity between subspecies of *Secale strictum* and *Secale strictum* was found to be low, implying that the subspecies of *S. strictum ssp. strictum* evolving independent of its subspecies. This supports the Khush and Stebbins' (1961) idea that *S.strictum*, *S. strictum* ssp. *kuprijanovii* and *S. strictum* ssp. *africanum* evolved separately, which was also supported by rDNA polymorphism analysis (De Bustos and Jouve, 2002).

The different hypotheses on the evolutionary history of *Secale* genus are summarized in Table 2.3. There is a general agreement on *S. strictum*, the perranial species, being the ancestral species and rest of the taxa having originated from it (Khush and Stebbins, 1961; Khush, 1962; Zohary, 1971; Vences et al., 1987; Hammer et al., 1987; Hammer, 1990; Jones and Flavell, 1982). The first species that diverged from *S. strictum* is *S. sylvestre* during the Pliocene epoch (Stutz, 1972; Skuza et al., 2007; Achrem et al., 2013). Thus *S. sylvestre* is morphologically and genetically the most distinct species (Vences et al., 1987; Murai et al., 1989; Reddy et al., 1990; Petersen and Doebley, 1993; Skuza et al., 2007; Cuadrado and Jouve, 1997; Shang et al., 2006). *S. strictum* ssp. *africanum* diverged from *S. strictum* during early Pleistocene epoch and evolved separately (Jones and Flavell, 1982; Achrem et al., 2014). Although there is an ongoing debate on whether *S. cereale* and *S. vavilovi* are distinct species (Khush, 1962; Cuadrado and Jouve, 1997; Hammer et al., 1987; Hammer, 1990; Del Pozo et al., 1995; Chikmawati et al., 2005; De Bustos and Jouve, 2002), or *S. vavilovii* is a subspecies of *S. cereale* (Frederiksen and Petersen, 1998; Shang et al., 2006; Cuadrado and Jouve, 2002; Achrem et al., 2014; Ren et al., 2011), this two species are evolutionarily the youngest (Jones and Flavell, 1982; Skuza et al., 2007; Chikmawati et al., 2005). Furthermore, *S. cereale* shows high molecular similarity to its subspecies (De Bustos and Jouve, 2002; Shang et al., 2006; Hammer, 1990; Ren et al., 2011), whereas *S. strictum* is significantly different from its subspecies (Shang et al., 2006; Hammer, 1990; Cuadrado and Jouve, 2002), indicating that *S. strictum* has been evolving independend from its subspecies (Acherem et al., 2014). Finally for the origins of cultivated rye, different researchers have different opinions: *S. vavilovii* (Vavilov, 1926; Roshevits, 1947; Zohary and Hopf , 2000), *S. ancestrale* (Schiemann, 1948; Zhukhovsky, 1933), and *S. strictum* (Rilley, 1955; Khush and Stebbins, 1961) were suggested to be the progenitor of the cultivated rye.

Table 2.1. The different hypotheses on the evolutionary history of *Secale* genus.

Hypothesis	**References**
S. vavilovii, evolved from *S. strictum*, is the ancestor of *S. cereale*	Vavilov, 1926; Roshevits, 1947; Zohary and Hopf , 2000
S. ancestrale is the progenitor of cultivated rye	Schiemann, 1948; Zhukhovsky, 1933
S. ancestrale and S. *strictum* share a common ancestor	Schiemann, 1948; Zhukhovsky, 1933
S. cereale originated from *S. strictum*	Rilley, 1955;Khush and Stebbins, 1961
All species originated from S. strictum	Khush and Stebbins, 1961; Khush, 1962; Zohary, 1971; Vences et al., 1987; Hammer et al., 1987; Hammer, 1990; Jones and Flavell, 1982
S. sylvestre separeted from *S. strictum* during Pliocene epoch	Stutz, 1972; Skuza et al., 2007; Achrem et al., 2013
S. sylvestre was the most distinct species	Vences et al., 1987; Murai et al., 1989; Reddy et al., 1990; Petersen and Doebley, 1993; Skuza et al., 2007; Cuadrado and Jouve, 1997; Shang et al., 2006
S. africanum evolved from *S. strictum* during early Pleistocene	Jones and Flavell, 1982; Achrem et al., 2014
S. vavilovii and *S. cereale* are the youngest species	Jones and Flavell, 1982; Skuza et al., 2007; Chikmawati et al., 2005
S. vavilovii is an intermediate form between perennial wild taxa and cultivated rye	Kranz, 1961
S. cereale ssp. *anatolicum* was found to be closer to *S. cereale* than to *S. strictum*, while *S. strictum* ssp. *kuprijanovii* was closest to *S. sylvestre*	Pozo et al., 1995
S. cereale and *S. vavilovii* are separate, but closely related species	Khush, 1962; Cuadrado and Jouve, 1997; Hammer et al., 1987; Hammer, 1990; Del Pozo et al., 1995; Chikmawati et al., 2005 De Bustos and Jouve, 2002;
Secale vavilovii might be considered as a subspecies of *Secale cereale*	Frederiksen and Petersen, 1998; Shang et al., 2006; Cuadrado and Jouve, 2002; Achrem et al., 2014; Ren et al., 2011
S. strictum ssp. *kuprijanovii* is closest to *S. sylvestre*	Pozo et al., 1995
S. sylvestre is evolutionarily oldest species	Chikmawati et al., 2005
S. strictum is a heterogeneous complex, with large differences between subspecies	Shang et al., 2006; Hammer, 1990; Cuadrado and Jouve, 2002
S. strictum has been evolving independent from its subspecies	Acherem et al., 2014
*S. cereale s*how high molecular similarity to its subspecies	De Bustos and Jouve, 2002; Shang et al., 2006; Hammer, 1990; Ren et al., 2011
Annual weedy form evolved from *S. strictum* ssp. *strictum,* which is the ancestor of *S. cereale*	Ren et al., 2011

2.4. Genetic Diversity and Conservation of Crop Relatives and Landraces

2.4.1. The Concept of Genetic Diversity

Genetic diversity is the variation of heritable characteristics in different individuals in a population of a single species. These differences stem from variation of coding or non-coding DNA sequences, or differences in their location in the genome. The potential evolutionary responses of plant populations depends on the degree of genetic diversity maintained in that population. As the genetic diversity increases, a populations ability to adapt to biotic and abiotic stress (resistance to disease, tolerability of poor soil conditions, etc.) also increses.

One of the greatest environmental changes and challenges of modern times is global climate change. During the last decade, a 0.5 ºC increase in the temperature of the world was observed due to greenhouse gas emissions (Cox et al., 2000). Because of the increased level of CO_2, the climatic conditions of the globe are projected to change dramatically within the next 50 years: a 3 to 5 ºC increase in temperature and accompanying alterations in hydrological balance is expected (IPCC, 2007). Global warming does not affect all regions of the world uniformly: there will be major shifts in the current temperatures at some locations (Battisti and Naylor, 2009). Climatic factors such as temperature change, alterations in the precipitation regimes, floods, and droughts directly affect productivity and composition of agricultural ecosystems (Fuhrer, 2003). Thus considering changing climatic conditions, conservation of plant genetic diversity is extremely important (Etterson, 2004a, b).

Landraces are strains of a crop species that have been selected and cultivated by traditional agricultural practices for many generations, which have become locally adapted to various environments (Zeven, 1998). Having been grown in a particular region each year, landraces have become adapted to the conditions in a given area, by changing the frequencies of phenotypes and of alleles, absorbing new genotypes or accumulating new mutations (Myers, 1994). As a result, contrary to genetically uniform and stable modern cultivars, the landrace populations are genetically diverse (Tang and Knapp, 2003; Reif et

al., 2004; Yamasaki et al., 2005; Warburton et al., 2008). Thus, when the environmental conditions change, at least one genotype within the landrace population will survive and yield satisfactorily. Furthermore gene flow between landrace populations and improved varieties or wild relatives of open pollinated crops is a common phenomenon (Bellon et al., 2006). Because gene flow leads to dispersal of potentially adaptive alleles between populations, it increases variation and facilitates adaptation to environmental change. Therefore landraces and wild relatives should be regarded as genetic resources reservoirs for new niches and future breeding programs.

2.4.2. Genetic Erosion in Crop Plants

Genetic erosion refers to gradual loss of variability from crop populations. From a agricultural point of view, genetic erosion is defined as reduction in richness or absolute loss of a crop, a variety (landrace or cultivar) or allele. As early as in 1930s, before the intensive breeding techniques were developed, two plant explorers, Harlan and Martini (1936) first emphasized the problem of genetic erosion in crops. The first human related activities related to erosion of plant genetic resources are domestication syndrome and Colombian exchange (Ceccarelli, 2009). Neolithic humans selected and used a limited number of wild progenitors bearing desirable traits meeting their needs. As a result the progenitor populations experienced a severe bottleneck effect and only a subset of the diversity in the progenitor is contained in the domesticated species. Moreover, the traits associated with those selected against during domestication may have been lost due to directional selection, reducing the genetic diversity even further. Thus domestication syndrome reduced genetic variability. The second cause of the early genetic erosion, as mentioned above is Columbian Exchange. After the voyages of Christopher Columbus in 1492, exchange of plants, animals, foods, and human populations between the Eastern and Western hemispheres across the oceans became possible. It is considered to be first example of globalization of agriculture; the selected crops were transported from the place of domestication to new environments and climates. In this case, only the limited number of individuals carrying small proportion of alleles that the original population has was transported. Considering that only a few plants were disseminated, this second bottleneck effect leaded to even a more remarkable decrease in genetic diversity (Zeder et al., 2006).

After the discovery of Mendel's laws in 1886, modern breeding techniques were developed. This progress provided a basis for the breeding strategy of a transformation process in agriculture, known as "green revolution", in 1960s. Green revolution was a development program based on mechanization, extensive use of agricultural inputs (fertilizers, pesticides), highly developed irrigation systems and new crop cultivars. These new cultivars were developed by utilization of only a limited number of landraces and the predominating philosophy behind the process was selection for "wide adaptation". In this case, the crops are able to adapt to wide geographic areas rather than wide environmental conditions (Ceccarelli, 1989). Indeed they are genetically uniform and perform well only in the created optimal conditions using high levels of fertilizers and extensive irrigation. As a result, a dramatic decline in agricultural biodiversity was observed due to extensive use of genetically similar varieties and elimination of hundreds of genetically diverse local varieties selected by traditional farmers over centuries for specific adaptation to their own environment. This phenomenon is also known as modernization bottleneck (Ceccarelli, 1989). Other reasons of genetic erosion include destruction of habitats to which the landraces are adapted to, due to the effects of urbanization, climate change and environmental degradation, changes in the food preferences of population and natural disasters.

The extent of genetic erosion varies considerably between countries and crops. Most of the landraces were replaced by modern cultivars in the regions which have highly developed agricultural systems like North America and North-Western Europe in the 1970s (Hammer et al., 2003). On the other hand in the centers of diversity located on subtropical and tropical regions, genetic erosion is less progressed. Furthermore the problem of genetic erosion exert its effect more apparently for the economically important crops that were subjected to international and national breeding programs such as wheat, rice and maize. The degree of genetic erosion also depends on the degree of agricultural industrialization. In the countries having modern plant breeding methods, highly developed agriculture, and specialized seed industry, original diversity of landraces are rapidly lost (Altieri, 1999). On the other hand in the countries in which seed industries are less developed, and many farmers are practicing traditional farming with very little external input, or in the areas where modern cultivars have not been able to sustain the requirements

of the many marginal or stressful environments, cultural preferences, landraces continue to exist (Hammer et al., 2003).

2.4.3. Conservation of Plant Genetic Resources

Today there are two kinds of conservation strategies that should be considered as complimentary to each other.

2.4.3.1. Ex Situ Conservation. Ex situ conservation refers to maintenance of genetic resources in gene banks, botanical gardens, and agricultural research stations (Plucknett et al., 1987). Ex situ conservation strategies are based on keeping small number of individuals outside their natural habitat with the aim of maintaining the genetic material in the state in which it was collected, to avoid loss or degeneration. As a result, only a small proportion of alleles are stored. Furthermore, these kinds of artificial environments are usually free from parasites, predators, and related species. Thus, they do not involve agro-ecological interrelationships that are fundamental parts of crop evolutionary systems, like genetic exchange between different populations and species, and adaptation and selection to biotic and abiotic stress. On the other hand, ex situ conservation, being an important back-up and source, for re-introduction and restoration of genetic diversity is still an indispensible element of conservation strategies (Cohen et al., 1991).

2.4.3.2. In Situ Conservation. In situ conservation was defined as “the maintenance of variable populations in their natural or farming environment, within the community of which they form a part, allowing the natural processes of evolution to take place" (Qualset et al., 1997). In situ conservation has many advantages over ex situ conservation. First of all, in situ conservation allows supporting greater number of alleles and different genotypes compared to accessions in seed banks (Marshall, 1989). Besides, considering the fact that crop plants are not evolutionarily discrete from their wild relatives, and gene flow between the wild and domesticated taxa may have a substantial impact on their evolution (Kuckuck, 1974): conserving cereal fields together with wild relatives in a manner that allows genetic interchange is the best strategy especially in gene centers where the specific crops originated from.

2.4.4. Genetic Diversity of *Secale* Genus

Like the other minor crops, the number of studies on genetic diversity of cereal rye and its wild relatives is limited. In small number of studies, genetic diversity of *Secale* species with a concentrated focus on cultivated varieties and landraces of *S. cereale*, have been investigated using different approaches.

Perez De La Vega and Allard (1984) studied four populations of *Secale cereale* and one population of *S. vavilovii* by using nine enzyme systems. They observed that *Secale cereale* was extensively variable whereas *S. vavilovii* was invariant. Ramirez and Pisabarro (1986) conducted an isoenzyme assay by using Cathodal Peroxidase System to investigate polymorphism rate of eight rye cultivars. The percentage of polymorphic loci and the average heterozygosity values ranged from 53.84% to 92.3% and 0.179 to 0.378, respectively. Genetic distance values were similar to the expected values for plants with little diversification.

Persson and Bothmer (2000) investigated genetic diversity of landraces from Sweden, and genetic relationships between landraces and cultivar by using nine isozyme loci in seven improved varieties and 19 landraces. They observed that although genetic diversity of the improved varieties was comparable to the observed values for landraces, the two groups separated in the dendogram. The group also conducted a RADP analysis to resolve patterns of genetic diversity in the rye and reported that landraces and improved cultivars clustered separately and the variation observed within accessions are higher than between accessions (Persson et al., 2001). This is concordant with the pattern expected for a cross pollinated crop. The same authors conducted another study (Persson and Bothmer, 2002) to estimate genetic variation in three commercial varieties and 18 landraces of *Secale cereale*, originating from the northern Europe by using different enzymes, and compared the results with those of the previous study. The data revealed that the genetic variation in landraces is high and all of the Finnish landraces clustered with 11 of the Swedish landraces.

Matos et al. (2001) compared the genetic diversity of landraces and cultivars by using 20 isozyme loci, 169 Random-Amplified Polymorphic DNA (RAPD) markers and 342 Inter Simple Sequence Repeat markers. It was shown that Portuguese landrace rye populations have a large genetic variability compared to other landrace and cultivated accessions.

Ma et al. (2004) studied genetic diversity and phylogenetic relationships of spring and winter varieties of *Secale cereale* using RAPD markers. They observed two major clusters as spring and winter. Cultivars within the spring group assigned to a North European and an American-Chinese group, whereas cultivars of winter rye assigned to four groups: Northern European, Russian, American and Chinese lines. The clusters were consistent with the geographical locations of the cultivars, indicating isolation is a significant factor in the evolution of rye. They concluded that observed genetic diversity stemmed from temporal isolation, rather than geographical isolation.

Using allozyme and SSR markers, Burger et al. (2006) evaluated the origin of feral rye. They found genetic diversity within populations was incomparably higher in feral populations than in cultivars and mountain rye. The results also suggested a common ancestry or past introgression between cultivars and the feral populations. Moreover compared to mountain rye or ancestral rye, feral populations were found to be closer relatives of cultivated rye.

Işık et al. (2007) conducted chloroplast and mitochondrial DNA PCR-RFLP analysis by using 96 accessions of improved varieties originating from diverse geographical areas, in order to obtain new information on organellar genome diversity. Data obtained from the study revealed that the level of organellar polymorphism was low among the cultivated rye genotypes, compared to wheat and triticale.

3. RESEARCH OBJECTIVES

As can be seen above, there is no consensus regarding taxonomy of the genus *Secale.* Furthermore phylogenetic relationships of the species in the genus are still obscure. The primary objective of present multidisciplinary research is to obtain a deeper understanding of taxonomy, phylogeny and genetic structure of *Secale* species and identify the underlying factors forming these structures. For this reason, 142 accessions belonging to different species or subspecies from different geographical origins, with a detailed focus on Turkey and Middle East were investigated using nuclear microsatellites, and chloroplast and nuclear SNPs. The more specific aims of the study are:

1- To determine any structuring among *Secale* populations and evaluate its correspondence with taxonomic or spatial delimitation.

2- To determine level and distribution of genetic diversity of cultivated rye populations from different geographical regions of the world, investigate areas that show high levels of diversification.

4. MATERIALS AND METHODS

4.1. Obtaining and Planting the Samples

In this study, a total of 729 samples belonging to 140 different accessions of cultivated varieties, landraces and wild populations of *Secale* genus were investigated from different eco-geographical areas of the world, but with a concentrated focus on Turkey and Middle East (Figure 4.1-4.4). These accessions were kindly provided by National Small Grains Collection of United States Department of Agriculture and nstitute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. Detailed information about the collection sites, seed sources and sample sizes of the accession used in the study are given in Appendix A.

The obtained seeds were planted in trial fields of Çukurova University, Department of Field Crops from December 2009 to June 2010. The seeds of each accession were sown into a depth of about two cm on 01.12.2009.

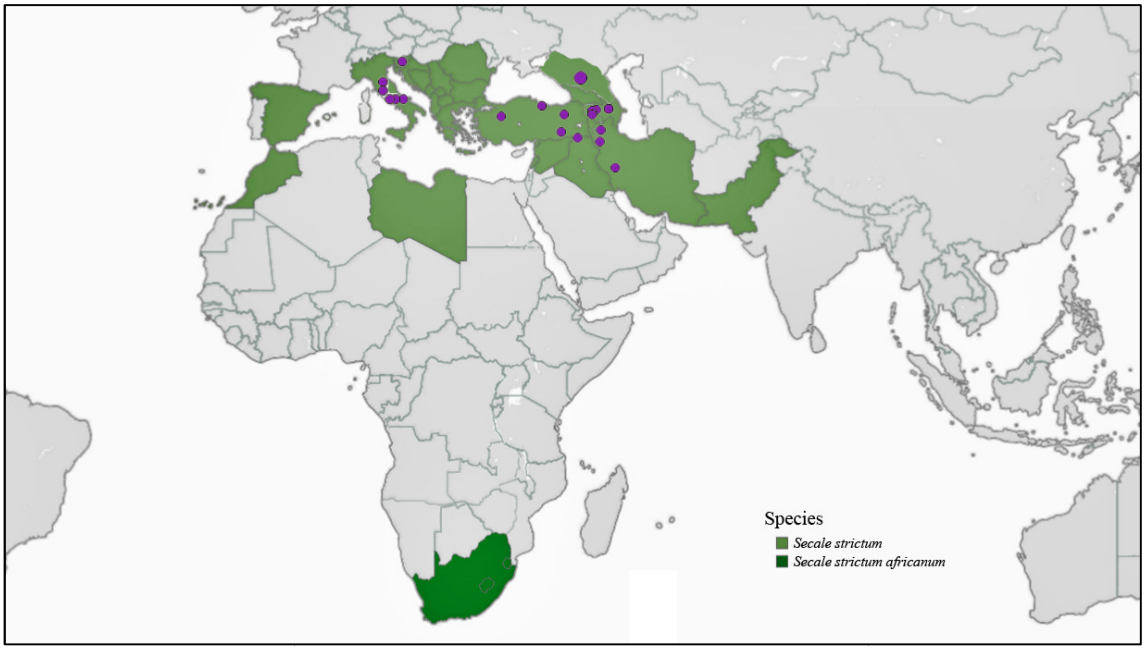

Figure 4.1. Distribution (based on Barcode of Life Data Systems) and sampling localities of *Secale strictum.*

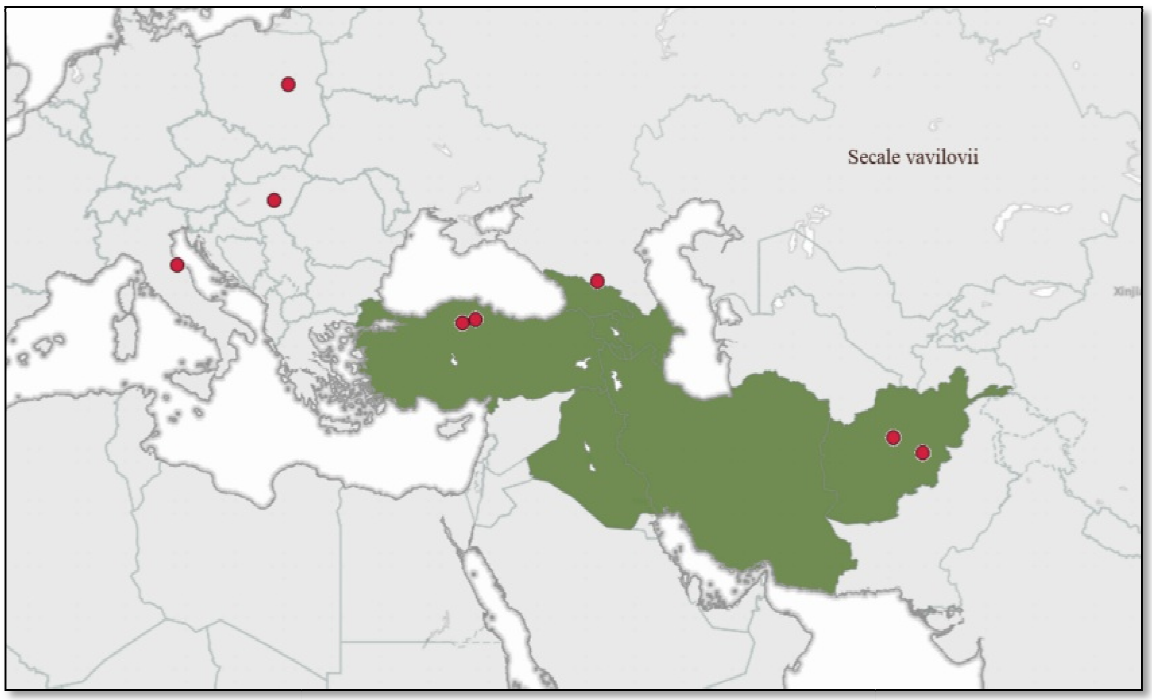

Figure 4.2. Distribution (based on Barcode of Life Data Systems) and sampling localities of *Secale vavilovii.*

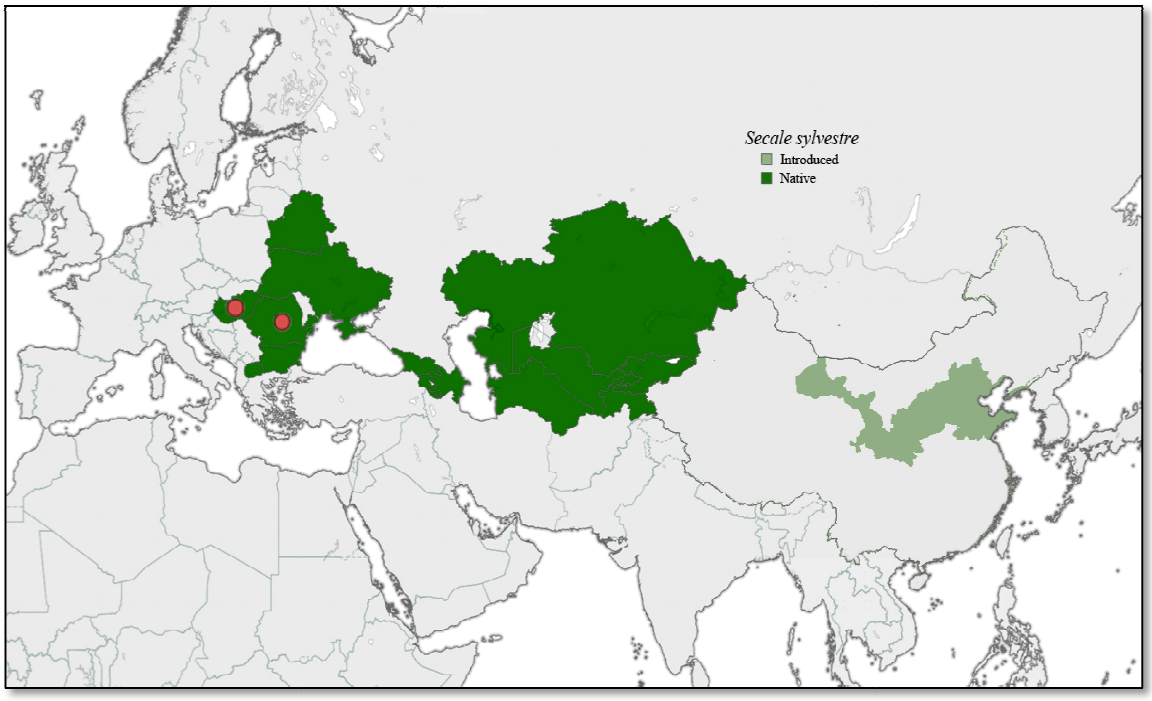

Figure 4.3. Distribution (based on Barcode of Life Data Systems) and sampling localities of *Secale sylvestre.*

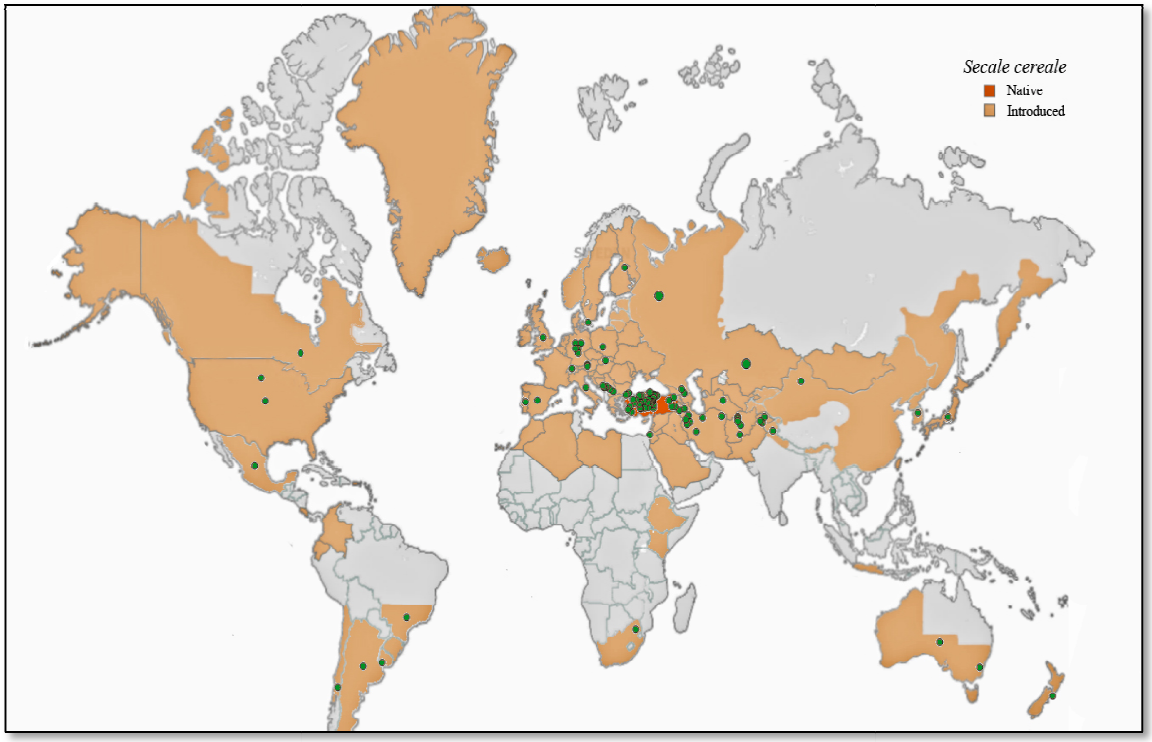

Figure 4.4. Distribution (based on Barcode of Life Data Systems) and sampling localities of *Secale cereale.*

4.2. Evaluation of Morphological Characters

The samples were regularly evaluated for some phenotypic characters during all developmental stages. These evaluations are performed following Frederiksen and Petersen (1997) and Descriptoars for Rye and Triticale prepared by Biodiversity International explained in detail below. These characteristics were used to confirm taxonomic delimitations of used accessions. Furthermore, morphological polymorphism observed in cultivated rye accessions from different geographical regions was evaluated. Detailed information on investigated morphological characters is given in Appendix B.

4.3. Molecular Studies

4.3.1. DNA Isolation

The DNA was extracted from each individual plant according to the method described by Doyle and Doyle (1990). The plant material was first washed by distillated water and dried. About 10 grams of plant material from each individual plant was cut into small pieces and put in a 2 ml eppendorf tube. Metal balls were also placed into the eppendorf tube. The tubes were exposed to liquid nitrogen and then homogenized by a Tissue Llyser (Qiagen, Germany). 900 µl of 2X CTAB solution was added into the tubes and subsequently the tubes were incubated for 1 hour at 65 °C in a water bath with occasional shaking. Next, the tubes were held at room temperature for 10 minutes. Subsequently 900 µl of chloroform-isoamyl alcohol mixture (24:1) was added into the tubes and the tubes were mixed by inverting for 15 minutes and centrifuged for 15 minutes at 14000 rpm. Upper phase was transferred into a new 1.5 ml tube. Subsequently, 500 µl of ice-cold isopropanol was added and the tubes were inverted slowly for 10 minutes to precipitate the DNA. If DNA pleets appeared, the tubes were centrifuged for 3 minutes at 6000 rpm and the supernatant was discarded. If DNA pellets did not appear, the tubes were held at -25 °C overnight or at -70 for 3 hours before centrifugation at 6000 rpm for 3 minutes. Following precipitation, the DNA pellet was washed by adding 1 ml of 7.5 molar ammonium acetate solution in 70% ethanol and shaking for 15 minutes. The tubes were centrifuged at 3000 rpm for 1 minute and supernatant was discarded. The pellet was

allowed to stand at room temperature overnight for drying. DNA was resuspended in 40 µl of sterile DNase free water. DNA samples were stored at -20 °C.

4.3.2. Determination of DNA Concentration

To determine the concentration of total DNA, a 0.8% agarose solution was prepared by melting 0,64 grams of agarose 80 ml of 1X TAE buffer (0.04 M Tris – Acetate 0.001 M EDTA) in a microwave oven. The gel was allowed to cool at room temperature for a few minutes, and then 3 µl Ethidium Bromide was added. The gel was casted by using supplied tray and comb, and was allowed to set for 20 minutes at room temperature, and then 20 minutes at 4°C. One µl of each DNA sample and 50ng/µl, 100 ng/µl, and 200ng/µl aliquots of λ DNA was mixed with 5 µl loading buffer and loaded into separate wells. The gel was run for 30 minutes at 110 V and visualized by Gel-Doc program quantity one (Biorad, USA). The concentration of each DNA sample was visually determined by comparing the DNA sample with λ DNA with a known concentration on a gel.

3.3.3. Microsatellite Analysis

Twenty nuclear SSR primers previously used in different studies were tested. Among these a set of ten microsatellite primers yielding good PCR products were selected and used for the analysis of 729 samples (Table 4.1) (Khlestkina et al., 2004, Saal and Wricke, 1999; Bolibok et al., 2006). PCR reactions were performed in a 12.5 µL volume containing approximately 30 ng genomic DNA, 1.5 µL of 10X Taq buffer containing KCl, 3 mM of $MgCl_2$, 0.4 mM of each primer, 0.4 mM of each deoxyribonucleotide triphosphate (dNTP) and 1 U Taq DNA polymerase (Thermo Scientific). The PCR program was as follows: an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of 1 minute at 94°C, 90 seconds at annealing temperature, 90 seconds at 72°C, with a final extension step of 5 minutes at 72°C. To control amplification success, PCR products were tested on a 1.5 % agarose gel containing 3 µl Etidium Bromide. Fragment analysis of PCR products were performed using ABI 3130xl Genetic Analyzer (Applied Biosystems) and the alleles were sized using Genemarker V1. 95 (Softgenetics).

Table 4.1. SSR primers used in the study.

NAME	SSR	Exp. Lenght	Chromosome	Forward and Reverse Primer (5'-3')	Label	Anneal (°C)	REFERENCE
REMS1303	$(CTC)_5$	309	1R	TAGCACCACTCGCTCTCTCA TTTCCCATCAGAAAAATCGC	NED	55	Khlestkina et al., 2004
REMS1238	$(CGG)_5$	286	2R	TACGTGGACGAGGAGGAGAC TACCTACCATCACCACCCTG	FAM	60	Khlestkina et al., 2004
REMS1254	(CGG)5	311	3R	AAAATACGGAGGAGGCAGGT ACATCAACAAGATCGTGGGC	FAM	65	Khlestkina et al., 2004
REMS1323	$(CT)_7$atctatcta$(TC)_7$	292	3R	GCAATCTCAGATCCTACGGC GGTGATCCATGAACAAACCC	VIC	65	Khlestkina et al., 2004
REMS1160	$(TAG)_7$	228	4R	GCAATCTCAGATCCTACGGC ACCAGAGGAATCGCAAACAC	VIC	60	Khlestkina et al., 2004
REMS1205	$(ACAT)_6$	281	5R	GCAATCTCAGATCCTACGGC TCACATCATGGAGGAACCAA	PET	62	Khlestkina et al., 2004
REMS1264	$(CGTC)_5$	282	5R	GCAATCTCAGATCCTACGGC GAACTCGCTCTTCATCCTCG	NED	56	Khlestkina et al., 2004
REMS1259	$(CGT)_5$	271	6R	GCAATCTCAGATCCTACGGC ATTTAGATCGACGACACGGG	PET	52	Khlestkina et al., 2004
SCM 180	$(GT)_6(GA)_7$	158	6RL	GCAATCTCAGATCCTACGGC ACGTGTCGCTTTCCATTGCCC	PET	65	Saal and Wricke, 1999
REMS1187	$(CAA)_5$	215	7R	GCAATCTCAGATCCTACGGC CACGTGTTGTTTCCCTTCCT	FAM	60	Khlestkina et al., 2004

4.3.4. Chloroplast SNP Analysis

To investigate phylogenetic relationships of diploid *Triticum* and *Aegilops* species, Yamane and Kawahara (2005) analyzed sequence variations in non-coding regions of chloroplast DNA. For this purpose, they amplified four chloroplast fragments based on chloroplast sequence of wheat and they included two *Secale* species because of the close relationship between the two genera. The possibility of using these three markers to reveal phylogenetic relationships of genus *Secale* was evaluated (Table 4.2) and based on amplification profile *ndhF-rpl32* intergenic region was included in the analysis.

Table 4.2. Chloroplastic SNP markers tested and used in the study.

Region	Primer Name	5'-3' Sequence	Annealing
ndhF-rpl32	ndhF/139F	GACGAAGATTTTTTGTTGCTG	65°C
	IGR 643R	TATGGTATGGAAGCCTATCC	
atpI-atpH intergenic region	atpI/643F	CCGGTCATGTTTCTTGGATT	64°C
	atpH/18R	CAATAACAGAAGCAGCAGCA	
rpl32-trnL (CUA)	rpl32-trnLF	ATCCGCATTAGACAAAATGAAG	60°C
	rpl32-trnLR	GTGTTTCTATTGGGCAAAGCA	

A total of 178 samples representing four species of the genus *Secale* were included in the study. The PCR reactions were performed in a 25 µL volume containing 30 ng DNA, 0.25 µM of each primer, 0.2 µM of each dNTP, 2 µM of $MgCl_2$, 2.5 µL Taq buffer containing KCl and one unit of Taq Poylmerase (Thermo Scientific, USA). Amplification was carried out according to the following temperature profile: an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of 30 (sec) at 94°C, 30 sec at 65°C 30 sec at 72°C and 8 min at 72°C. The amplified fragments were subsequently sequenced at Macrogen Europe. The amplified DNA fragments were edited visually and aligned using Sequencher 4.5 (Gene Code Corp).

4.3.5. Nuclear SNP Analysis

Varshney et al. (2007a) assessed existing resource of barley EST markers to develop nuclear SNPs in rye. Four of these markers were tested (Table 4.3) and GBS0551, which gave the best results, was selected and included in our study. A total of 66 samples representing four species of *Sacale* gunus were included in the study. The PCR reactions were performed in a 25 µL volume containing 30 ng DNA, 0.25 µM of each primer, 0.2 µM of each dNTP, 2 µM of $MgCl_2$, 2.5 µL Taq buffer containing KCl and one unit of Taq Poylmerase. Amplifications were carried out according to the following temperature profile: an initial denaturation step at 94°C for 5 minutes, followed by 35 cycles of 30 sec at 94°C, 30 sec at 63°C, 30 sec at 72°C and 8 min at 72°C. The amplified fragments were commercially sequenced at Macrogen Europe. The amplified sequences were edited visually and aligned using Sequencher 4.5 (Gene Code Corp).

Table 4.3. Nuclear SNP markers tested and used in the study.

Region	Primer Name	5'-3' Sequence	Annealing
GBS0186	GBS0186F	CAACTGCAGCTTATTCGGGAT	63°C
	GBS0186R	ACCTTGGAGATTGGTCCCAC	
GBS0456	GBS0456F	TCACTGCAATGCAGATCACG	60°C
	GBS0456R	CGGGTACGAGGTGATCAAGAG	
GBS0526	GBS0526F	AGACAGAATCCTCACAGGTGCC	62°C
	GBS0526R	CCATGCCGAAGCAGATCC	
GBS0551	GBS0551F	GTGCAGCCTTGCCTTCATAA	63°C
	GBS0551R	CGTCGGATTCAACGTCTCCA	

4.4. Statistical Analyses

4.4.1. Nuclear Microsatellite Analyses

Ten microsatellite markers were amplified as described section 3.3.3. The alleles were automatically binned using FlexiBin, a macro developed in Microsoft Visual Basic for Excel (Amos et al., 2007). The genotyping errors stemming from null alleles, large allele dropout or the scoring of stutter peaks that can potentially lead to deviations from Hardy–Weinberg proportions were detected using Micro-checker (Oosterhout et al., 2004).

Allelic frequencies were tested for the deviations from Hardy–Weinberg equilibrium (HWE) using an exact test with a Markov chain (10000 steps) and 1000 dememorisation steps in Genepop version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008) following Guo and Thompson (1992). Linkage disequilibrium (i.e. presence of significant association between pairs of loci), was also tested between all loci using Genepop version 4.0.10 (Raymond and Rousset, 1995; Rousset, 2008). The number of alleles (Na), the effective number of alleles (Ne), the expected heterozygosity (the proportion of heterozygosity expected under random mating) (He), observed heterozygosity (the proportion of heterozygous samples to sample size at a given locus) (Ho), number of private alleles (Pa), and Shannon's information index (I) were calculated using GenAlEx v6.4 (Peakall and Smouse, 2006). The sample sizes in different accessions/regions used in the study were different from each other. Therefore, to compensate for this sampling bias that may lead to inaccurate estimation of allelic richness per locus, allelic richness (RS) and private allele richness (PR) independent from sample size were computed by the rarefaction method (Hulbert, 1971) as implemented in HP-RARE software (Kalinowski, 2005).

In addition to these genetic diversity parameters, the overall gene diversity (H_T), the within-population genetic diversity (H_S), the amount of gene diversity among populations (D_{ST}), and the coefficient of genetic differentiation between populations (G_{ST}) were calculated by FSTAT 2.9.3 (Goudet, 2001). To avoid any misinterpretation stemming from sampling bias D_{ST}, H_T and G_{ST} values were also calculated independently of sample size using the same program.

F-statistics are crucial components of population genetic structure analyses (Wright 1931). Three F-statistic indices are the within-population inbreeding coefficient (F_{IS}), the measure of reduction in heterozygosity of an individual due to non-random mating within its subpopulation, the inbreeding coefficient of an individual relative to the total population (*Fit)*, and the effect of subpopulation compared to the total population (F_{ST}) (Weir and Cockerham, 1984). Pairwise F_{ST} values between each population were calculated using GenAlEx v6.4 (Peakall and Smouse, 2006).

Population structure of each species, *S. cereale*, *S. vavilovii* and *S. strictum* (*S. sylvestre* accessions were not included in the pilot analysis due to the low sample size) were analyzed separately using Bayesian clustering algorithm implemented by the software Structure 2.3.3 (Pritchard et al., 2000). Admixture model of ancestry and correlated allele frequency were allowed. The length of the burn-in was set to 30,000 and data were collected over 300,000 Markov Chain Monte Carlo (MCMC) replications in each run (K = 1–5). In some cases, the signal obtained from Structure was relatively weak. To alleviate this problem the analysis was repeated applying LOCPRIOR model using population information as prior to assist clustering (Hubisz et al., 2009). In most of the cases, the optimum number of clusters (*K*), was determined as described by Evanno et al. (2005) with the help of Structure Harvester (Earl and Von Holdt, 2012). However, in some cases, the number of clusters suggested by structure harvester did not reflect the real structure of the data due to the low number of individuals that can be assigned to some clusters. In these cases, the smallest values of K that captures the major structure of the data was chosen as suggested by Pritchard et al. (2000). Each individual with an ancestry value equal to or larger than 0.7 was assigned to the corresponding cluster, while the individuals with a smaller ancestry value were considered to have mixed ancestry fallowing Coulon et al. (2008). In order to infer population structure of whole genus, the same analysis was conducted for entire set of genotypes.

Finally an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) tree was constructed using Poptree2 (Takezaki et al., 2010) based on Nei's genetic distance (D_A) (Nei et al., 1983) with 10,000 bootstrap iterations.

4.4.2. Chloroplast DNA Phylogenetic Analysis

The sequences were aligned using Sequencher 4.5 (Gene Code Corp), and a haplotype network was constructed using TCS (Clement et al., 2000). In order to evaluate genetic heterogeneity of genus *Secale* several estimates of genetic diversity such as number of haplotypes, haplotype diversity (h), nucleotide diversity (π) number of segregating sites (Nei, 1987) were calculated using with DnaSP 5.0 (Librado and Rozas, 2009). The pairwise F_{ST} values were computed using the same program.

4.4.3. Nuclear DNA Phylogenetic Analysis

The nuclear sequences were first edited visually using Sequencher 4.5 (Gene Code Corp). However, the discrimination of the alleles of heterozygote samples, especially with multiple differences is not straightforward. Therefore these sequences were edited by DNAsp version 5.0 (Librado and Rozas, 2009) using the coalescent-based Bayesian algorithm of the PHASE software (Stephens et al., 2001). Maximum-likelihood (ML) and Maximum parsimony trees were constructed using MEGA 5 (Tamura et al., 2011). Reliability of the phylogenetic relationships was tested by bootstrapping (1000 replicates).

4.4.4. Genetic Diversity of Cultivated Rye

To describe patterns of genetic diversity, a total of 443 samples from 54 different accessions originating from various geographical regions were used (Appendix A, Table A1), representing the 10 main gene pools: Africa, Australia, Europe, Balkans, Caucasus, East Asia, South and Central Asia, Middle East, North America and South America.

The number of alleles (N_A), effective number of alleles (Ne), number of private alleles, observed heterozygosity (Ho), expected heterozygosity (He), and Shannon's information index (I) were calculated using GenAlEx v6.4 (Peakall and Smouse 2006). The sample sizes in different accessions used in the study were different from each other, therefore RS and PR were used to correct for this issue, as described above. In addition to these genetic diversity parameters, the total genetic diversity (H_T), the within-population genetic diversity (H_S), the amount of gene diversity among populations (D_{ST}), and the coefficient of genetic differentiation between populations (G_{ST}) were calculated by FSTAT 2.9.3 (Goudet, 2001). To avoid any misinterpretation stemming from sampling disparity D_{ST}, H_T and G_{ST} values were also calculated independent of sample size as D'_T, H'_T and G'_{ST} respectively, using the same program. Pairwise F_{ST} values between each geographical population were calculated.

Principle Component Analysis (PCA) is a statistical method, which reduces the large datasets to a small number of dimensions by implementing multivariate analysis between factors, keeping the data variation at a maximum. The method represents each population as a single value, so that it simplifies visualization of data and detection of pattern and outliers. PCA analysis performed using GenAlEx v6.4 (Peakall and Smouse, 2006).

5. RESULTS

5.1. *Secale strictum*

5.1.1. Nuclear Microsatellite Diversity and Population Structure in *S. strictum*

The microsatellite analyses were conducted in 88 samples belonging to three subspecies of *Secale strictum* (one accession of *S. strictum* ssp. *anatolicum*, one accession of *S. strictum* ssp. *kuprijanovii*, and eight accessions of *S. strictum* ssp. *strictum*). Twenty nuclear microsatellite loci were tested and seven of these, REMS1187, REMS1254, REMS1323, REMS1264, REMS1205, REMS1238, REMS1160 (Khlestkina et al., 2004; Saal and Wricke, 1999; Bolibok et al., 2006) were amplified successfully. These loci were tested for genotyping errors using Micro-checker software v.2.2.3 (Oosterhout et al., 2004) and any of these markers did not show any evidence for presence of null alleles, large allele dropout or scoring of stutter peaks that can potentially lead to deviations from Hardy–Weinberg proportions. Seven microsatellite loci were also tested for linkage disequilibrium using Fisher exact test. No significant association between alleles was found. Allelic frequencies of each locus can be found in Appendix C.

Observed mean heterozygosity ranged between 0.71 and 0.94 and the expected mean heterozygosity ranged between 0.66 and 0.73 (Table 5.1). The seven microsatellite loci did not vary significantly in terms of number of alleles generated and total number of observed alleles (65). A total of 14 private alleles were observed.

Table 5.1. Levels of genetic variability at seven microsatellite loci for the three *S. strictum* subspecies. N, sample size; N_a, number of alleles; N_e, number of effective alleles; H_o, observed heterozygosity; He, expected heterozygosity; uHe, unbiased expected heterozygosity.

Subspecies	Locus	N	Na	Ne	Ho	He	uHe
S. strictum* ssp. *anatolicum	**REMS1187**	11	5	3.14	0.91	0.68	0.71
	REMS1254	4	2	1.88	0.75	0.47	0.54
	REMS1323	11	8	4.03	0.82	0.75	0.79
	REMS1264	11	4	2.81	0.82	0.64	0.68
	REMS1205	13	5	2.50	0.77	0.60	0.62
	REMS1238	11	4	2.57	0.91	0.61	0.64
	REMS1160	13	10	6.90	0.92	0.86	0.89
	Av.	10.57	5.43	3.41	0.84	0.66	0.70
S. strictum* ssp. *kuprijanovii	**REMS1187**	6	3	2.57	1	0.61	0.67
	REMS1254	3	3	2.57	1	0.61	0.73
	REMS1323	5	7	5.56	1	0.82	0.91
	REMS1264	4	3	2.67	1	0.63	0.71
	REMS1205	4	3	2.13	0.75	0.53	0.61
	REMS1238	6	4	2.88	1	0.65	0.71
	REMS1160	5	6	5.56	0.80	0.82	0.91
	Av	4.71	4.14	3.42	0.94	0.67	0.75
S. strictum* ssp. *strictum	**REMS1187**	55	6	2.38	0.75	0.58	0.59
	REMS1254	14	7	3.60	0.79	0.72	0.75
	REMS1323	56	16	6.62	0.89	0.85	0.86
	REMS1264	44	5	2.60	0.41	0.62	0.62
	REMS1205	57	8	3.61	0.79	0.72	0.73
	REMS1238	57	7	4.42	0.63	0.77	0.78
	REMS1160	58	12	6.81	0.72	0.85	0.86
	Av.	48.71	8.71	4.29	0.71	0.73	0.74

The STRUCTURE analysis revealed two clusters (Figure 5.1). The first cluster was composed of 20 samples belonging to two accessions of *S. strictum* ssp. *strictum*, while cluster 2 contained 68 samples of *S. strictum and S. strictum* subspecies (Figure 5.2). Geographical origin of the samples in the first cluster corresponded to Iran, whereas samples in cluster 2 had a wide distribution (Figure 5.3).

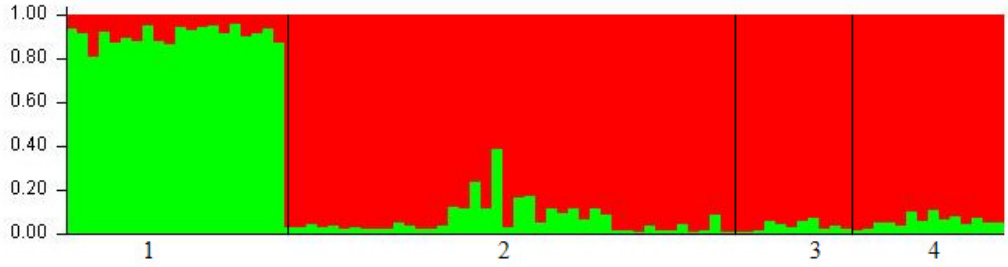

Figure 5.1. Structure results at K=2 for *S. strictum* subspecies. 1, *S. strictum spp. strictum* accessions originating from Iran, 2, *S. strictum* ssp. *strictum* accessions having diverse geographical origins, 3, *S. strictum* ssp. *kuprijanovii*; 4, *S. strictum* ssp. *anatolicum*.

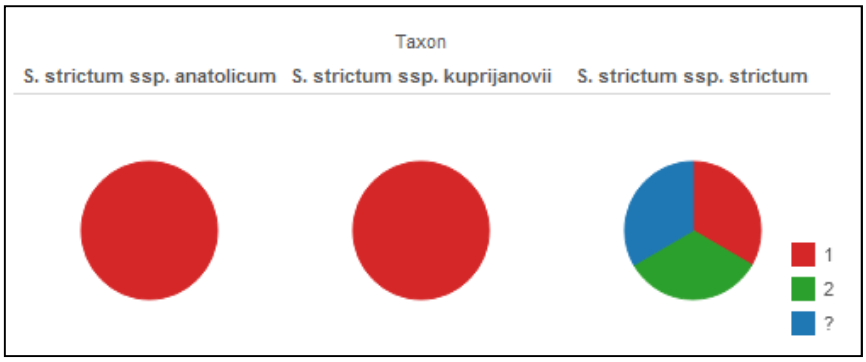

Figure 5.2. Partitioning of two clusters among *S. strictum* subspecies. The question mark designates unassigned individuals.

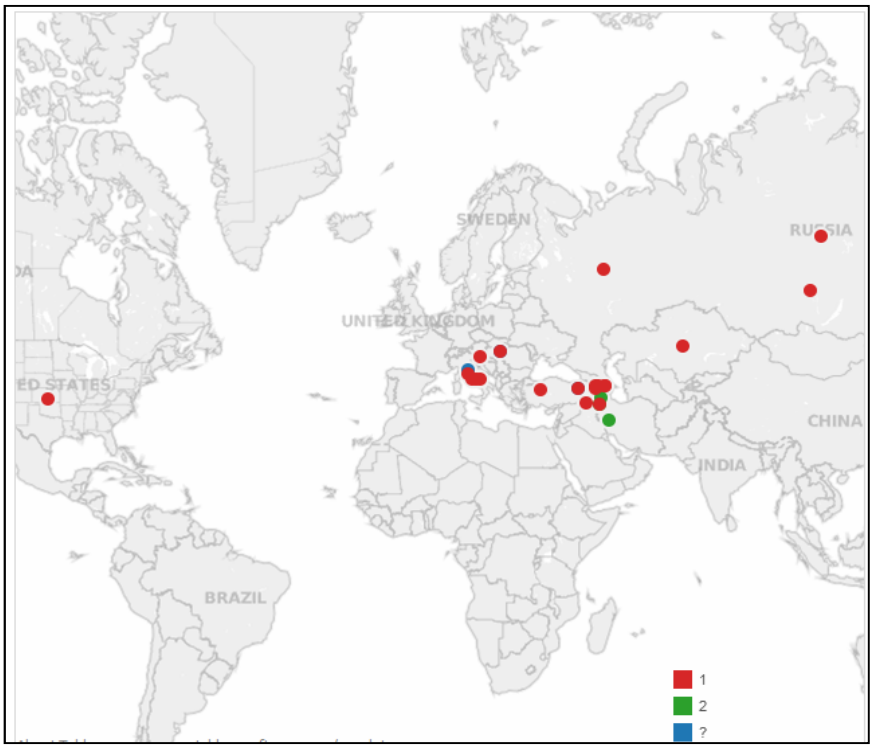

Figure 5.3. Geographical origins of *S. strictum* samples in each cluster. The question mark designates unassigned individuals.

5.1.2. Chloroplast DNA Diversity of *S. strictum* Subspecies

A 667 bp length fragment of *ndhF-rpl32* intergenic region of chloroplast DNA was amplified in 27 samples from different geographical origins (Table 5.2). Eight nucleotide substitutions and nine insertion-deletion events were observed in 36 polymorphic sites. The number of haplotypes was 16. Haplotype diversity and average number of differences was found to be 0.944 and 4.613, respectively. Nucleotide diversity was calculated as 0.00266. The haplotype network constructed considering the insertions and deletion as fifth character is shown in Figure 5.4. The network shows several ambiguous connections involving all subspecies, with no obvious differentiation of any.

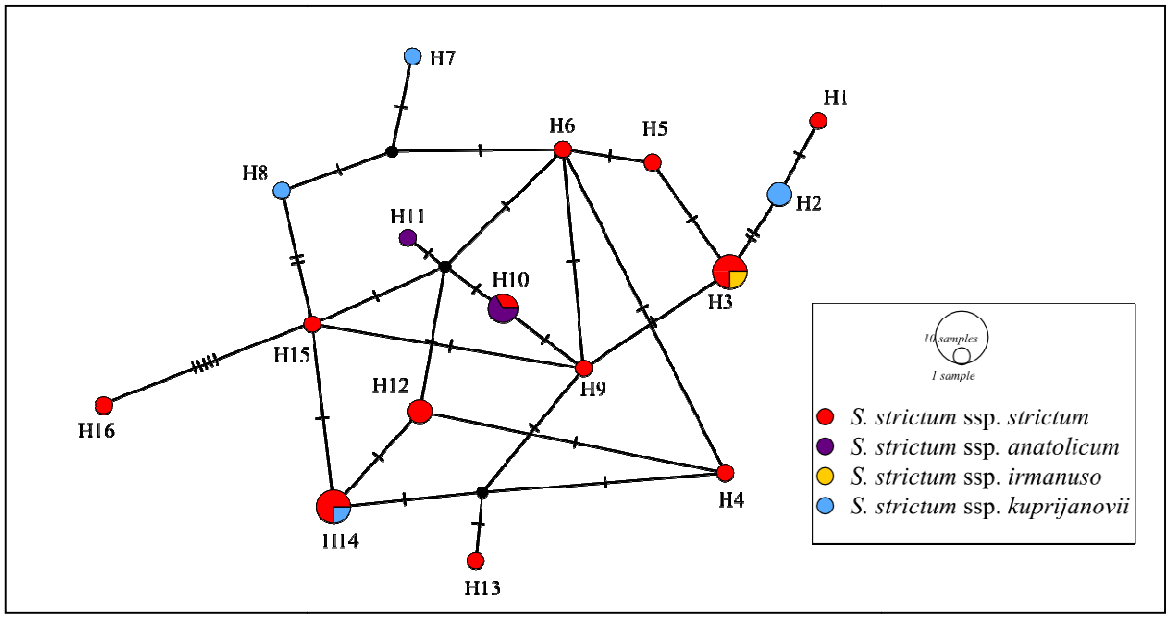

Figure 5.4. The haplotype network based on *ndhF-rpl32* intergenic region from *S. strictum* subspecies. The pie charts represent distinctive haplotypes and their frequencies in each subspecies. The sizes of the circles are proportional to the frequency of each haplotype. Each line represents a single mutational change. Black circles represent hypothetical haplotypes

Table 5.2. Haplotypes identified in chloroplast DNA analysis of *S. strictum* subspecies.

Hap.	ID.	Ac. No.	*Taxon*	Origin
H1	R0834	PI 253956	*S. strictum ssp. strictum*	Dahuk, Iraq
H2	R1028	R1151	*S. strictum ssp. kuprijanovii*	Kazakhistan
H2	R1029	R590	*S. strictum ssp. kuprijanovii*	Russian Federation
H3	R0884	PI 568257	*S. strictum ssp. strictum*	Russian Federation
H3	R0885	PI 568257	*S. strictum ssp. strictum*	Russian Federation
H3	R1003	R1047	*S. strictum ssp. strictum*	Armenia
H3	R1006	R1055	*S. strictum ssp. anatolicum*	Armenia
H3	R1022	R858	*S. strictum ssp. irmanuso*	Italy
H4	R0823	PI 253956	*S. strictum ssp. strictum*	Dahuk, Iraq
H5	R0825	PI 253956	*S. strictum ssp. strictum*	Dahuk, Iraq
H6	R0981	R1000	*S. strictum ssp. strictum*	Italy
H7	R0980	R579	*S. strictum ssp. kuprijanovii*	Azerbaijan
H8	R1023	R1154	*S. strictum ssp. kuprijanovii*	Russian Federation
H9	R0815	PI 205222	*S. strictum ssp. strictum*	Eskisehir, Turkey
H10	R0989	R939	*S. strictum ssp. strictum*	Italy
H10	R0892	PI 445973	*S. strictum ssp. anatolicum*	United States
H10	R0893	PI 445973	*S. strictum ssp. anatolicum*	United States
H11	R0887	PI 445973	*S. strictum ssp. anatolicum*	United States
H12	R0835	PI 253956	*S. strictum ssp. strictum*	Dahuk, Iraq
H12	R0841	PI 401402	*S. strictum ssp. strictum*	Lorestan, Iran
H13	R0856	PI 401404	*S. strictum ssp. strictum*	East Azerbaijan, Iran
H14	R0838	PI 401402	*S. strictum ssp. strictum*	Lorestan, Iran
H14	R0866	PI 531829	*S. strictum ssp. strictum*	Armenia
H14	R0867	PI 531829	*S. strictum ssp. strictum*	Armenia
H14	R0994	R1056	*S. strictum ssp. kuprijanovii*	Azerbaijan
H15	R1021	R914	*S. strictum ssp. strictum*	Italy
H16	R1007	R920	*S. strictum ssp. strictum*	Italy

5.2. *Secale vavilovii*

5.2.1. Identification of *S. vavilovii*

The *S. vavilovii* accession used in the study were initially assessed to confirm they had been identified correctly by the provider prior to further analyses. For this purpose, morphological characters offered by Frederiksen and Petersen (1997) was used. *S. vavilovii* is characterized by being shorter than *S. cereale* ssp. *cereale*: the plant height rarely exceeds 1.5 m (Tzvelev, 1976) and has partly fragile rachis (Roshevitz, 1947). Two accessions classified as *S. vavilovii*, (PI 284842 and R243), were more similar to cultivated rye with their considerably high plant height (av. 191 and 174.8 respectively), large and plump grains, and non-fragile rachis. Considering the possibility that these populations might have been misidentified, it was concluded that they need to be used with caution in further analyses.

5.2.2. Nuclear Microsatellite Diversity and Population Structure in *S. vavilovii*

The microsatellite analysis was conducted in 45 of *S. vavilovii* samples using the same seven primer sets described in 5.1.2. Fisher exact test revealed no significant association between alleles.

Observed and expected mean heterozygosity was 0.81 and 0.71, respectively (Table 5.3). The seven microsatellite loci did not vary significantly in terms of number of alleles generated. Total number of observed alleles and private alleles were 55 and 11, respectively.

Table 5.3. Levels of genetic variability at seven microsatellite loci for *S. vavilovii*. N, sample size; N_a, number of alleles; N_e, number of effective alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; uHe, unbiased expected heterozygosity.

Locus	N	Na	Ne	Ho	He	uHe
REMS1187	39	7	2.83	0.79	0.65	0.66
REMS1254	30	9	3.80	0.83	0.74	0.75
REMS1323	36	9	2.86	0.83	0.65	0.66
REMS1264	42	8	2.73	0.69	0.63	0.64
REMS1205	44	7	3.91	0.77	0.74	0.75
REMS1238	42	4	3.60	0.79	0.72	0.73
REMS1160	43	11	6.35	0.95	0.84	0.85
Av	39.43	7.86	3.73	0.81	0.71	0.72

In the STRUCTURE analysis, the optimal K offered by Structure Harvester (Earl and Von Holdt, 2012) was two. However the two clusters did not reflect the real structure of the data due to the absence of samples that can be assigned to the second cluster (Figure 4.5). Following Pritchard et al. (2000), it was concluded that there is no structuring among *S. vavilovii* accessions from different geographical regions (Figure 5.6).

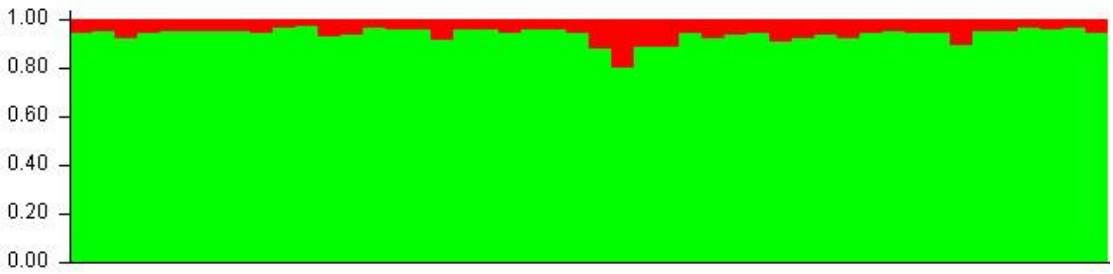

Figure 5.5. Structure results at K=2 for *S. vavilovii.*

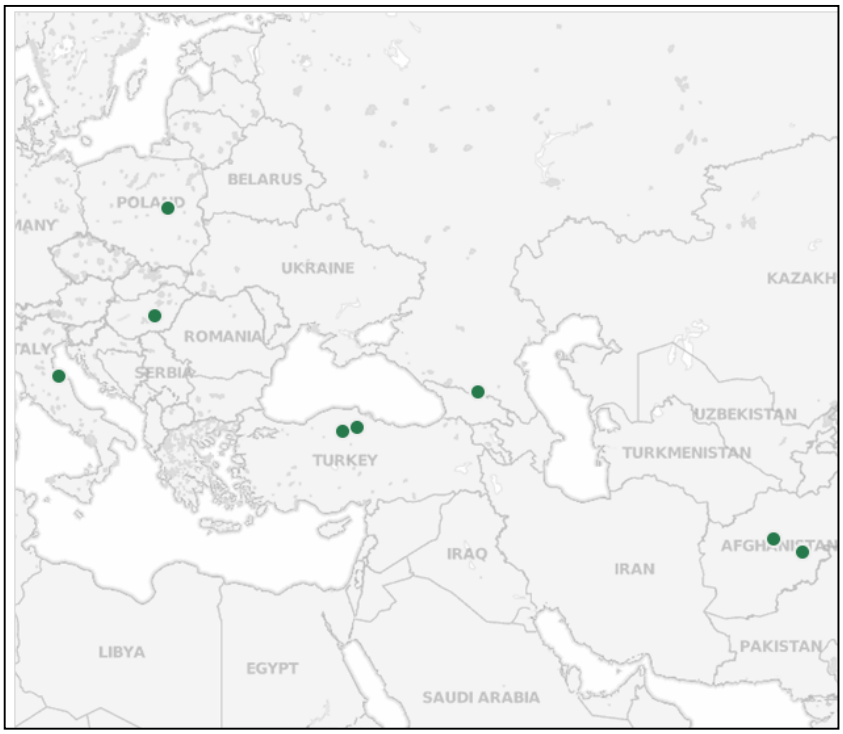

Figure 5.6. Geographical distribution of *S. vavilovii* samples.

4.2.3. Chloroplast DNA Diversity of *S. vavilovii*

The *ndhF-rpl32* intergenic region of chloroplast DNA was amplified in 12 samples from different geographical origins (Table 5.4). Eight nucleotide substitutions and nine insertion deletion events were observed in 10 polymorphic sites. A total of eight haplotypes were identified. Haplotype diversity and average number of differences were found to be 0.893 and 3.818, respectively. The nucleotide diversity was 0.00263. The haplotype network constructed considering the insertions and deletions as fifth character is shown in Figure 5.7. The network also did not show any breaks within *S. vavilovii*.

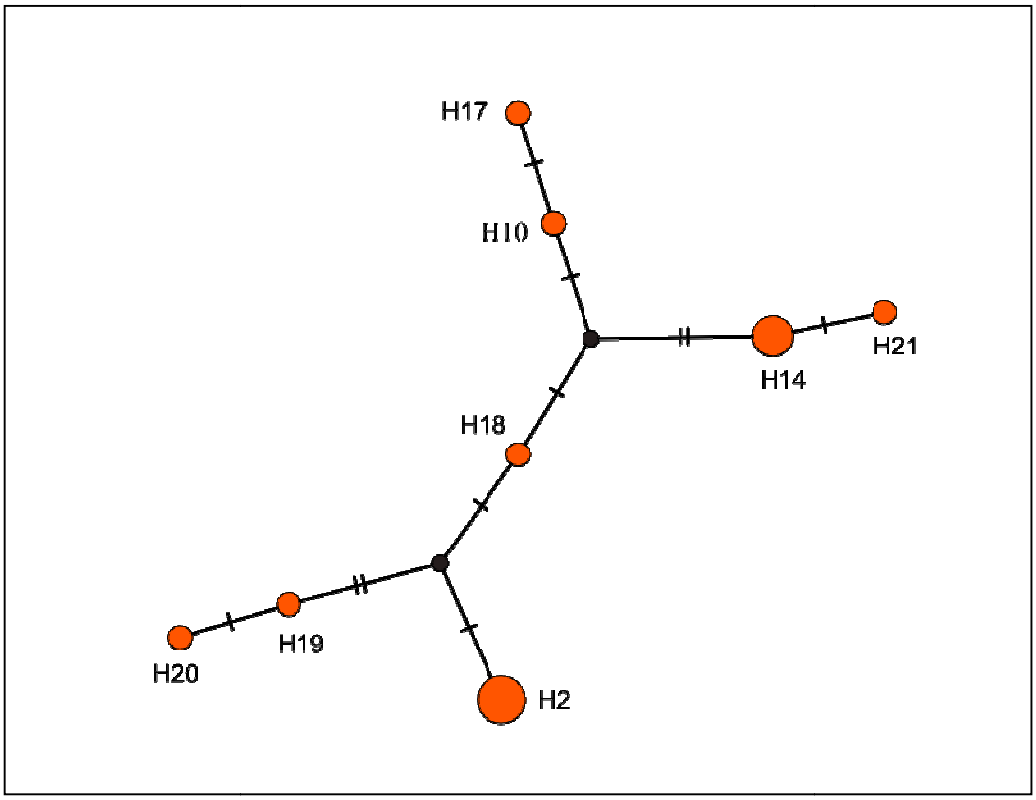

Figure 5.7. The haplotype network based on *ndhF-rpl32* intergenic region from *S. vavilovii.* The pie charts represent distinctive haplotypes and their frequencies in each subspecies. The sizes of the circles are proportional to the frequency of each haplotype. Each line represents a single mutational change. Black circles represent hypothetical haplotypes.

Table 5.4. Haplotypes identified in chloroplast DNA analysis of *S. vavilovii*.

Hap.	ID.	Ac. No.	*Taxon*	Origin
H2	R0936	PI 573649	*Secale vavilovii*	Afghanistan
H2	R0937	PI 573649	*Secale vavilovii*	Afghanistan
H2	R0996	R1125	*Secale vavilovii*	Turkey
H2	R1005	R1027	*Secale vavilovii*	Italy
H10	R0926	PI 573648	*Secale vavilovii*	Russian Federation
H14	R0917	PI 284842	*Secale vavilovii*	Hungary
H14	R0918	PI 284842	*Secale vavilovii*	Hungary
H14	R1027	R1126	*Secale vavilovii*	Unknown
H17	R0924	PI 573648	*Secale vavilovii*	Russian Federation
H18	R1010	R1126	*Secale vavilovii*	Turkey
H19	R0904	PI 253957	*Secale vavilovii*	Ghazni, Afghanistan
H20	R0901	PI 253957	*Secale vavilovii*	Ghazni, Afghanistan
H21	R0982	R1063	*Secale vavilovii*	Poland

5.3. *Secale cereale*

5.3.1. Identification of *S. cereale* Subspecies

The morphological features of *S. cereale* accessions were assessed following Frederiksen and Petersen (1997), to confirm that accessions were identified correctly by the provider. *S. cereale* ssp. *cereale* is characterized by a though rachis, big seeds and loosely attached glumes. *S. cereale* ssp. *afghanicum* differs from *S. cereale* ssp. *cereale* with fragility of upper ¾ of its rachis, whereas *S. cereale* ssp. *segetale* is characterized by fragility of upper ¼ part of the same character (Khush, 1963a). *S. cereale* ssp. *ancestrale* is characterized by being longer and having fully fragile rachis (Roshevitz, 1947). It was observed that three accessions classified as *S. cereale* ssp. *cereale* in GRIN (PI 168176, PI 429371 and PI 330526) had fragile rachis, small seed sizes, and tightly attached glumes. Considering the possibility that these accessions might have been misidentified, it was concluded that they need to be used in the further analysis with caution. Except for these three accessions, the morphological characteristics of investigated accessions were consistent with their identities.

4.3.2. Nuclear Microsatellite Analyses and Population Structure in *S. cereale*

The microsatellite analyses were conducted in a total of 578 samples belonging to four subspecies using the same seven primer sets described in 5.1.2. Fisher exact test revealed no significant association between alleles. Observed mean heterozygosity ranged between 0.67 and 0.80 (Table 5.5), and expected mean heterozygosity ranged between 0.58 and 0.72. The seven microsatellite loci did not vary significantly in terms of number of alleles.

Table 5.5. Levels of genetic variability at seven microsatellite loci for *S. cereale* subspecies. N, sample size; N_a, number of alleles; N_e, number of effective alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; uHe, unbiased expected heterozygosity.

Subspecies	Locus	N	Na	Ne	Ho	He	uHe
S. cereale* ssp. *afghanicum	**REMS1187**	3	3	2	0.67	0.50	0.60
	REMS1254	3	2	1.38	0.33	0.28	0.33
	REMS1323	3	3	2.57	1	0.61	0.73
	REMS1264	3	3	2.57	0.33	0.61	0.73
	REMS1205	3	3	2.57	0.67	0.61	0.73
	REMS1238	3	3	3	0.67	0.67	0.80
	REMS1160	3	5	4.50	1	0.78	0.93
	Av.	3	3.14	2.66	0.67	0.58	0.70
S. cereale* ssp. *ancestrale	**REMS1187**	9	4	2.08	0.56	0.52	0.55
	REMS1254	4	3	2.46	0.75	0.59	0.68
	REMS1323	11	6	3.41	0.91	0.71	0.74
	REMS1264	9	5	3.95	0.67	0.75	0.79
	REMS1205	10	7	2.56	0.70	0.61	0.64
	REMS1238	11	4	3.61	0.64	0.72	0.76
	REMS1160	10	7	5.41	0.80	0.82	0.86
	Av.	9.14	5.14	3.35	0.72	0.67	0.72
S. cereale* ssp. *cereale	**REMS1187**	504	8	3.22	0.85	0.69	0.69
	REMS1254	273	16	3.10	0.67	0.68	0.68
	REMS1323	502	20	3.69	0.84	0.73	0.73
	REMS1264	503	10	2.44	0.66	0.59	0.59
	REMS1205	435	11	2.96	0.71	0.66	0.66
	REMS1238	500	7	3.79	0.78	0.74	0.74
	REMS1160	409	19	8.12	0.87	0.88	0.88
	Av.	446.57	13	3.90	0.77	0.71	0.71
S. cereale* ssp. *segetale	**REMS1187**	28	5	2.47	0.61	0.60	0.61
	REMS1254	7	7	4.67	0.86	0.79	0.85
	REMS1323	32	10	4.06	0.97	0.75	0.77
	REMS1264	26	4	2.16	0.62	0.54	0.55
	REMS1205	32	7	3.87	0.84	0.74	0.75
	REMS1238	31	6	4.25	0.81	0.76	0.78
	REMS1160	31	13	8.94	0.87	0.89	0.90
	Av.	26.71	7.43	4.34	0.80	0.72	0.74

The STRUCTURE analysis of a total of 578 samples belonging to four different taxa revealed two clusters (Figure 5.8). Partition of clusters among species is shown in Figure 5.9, and geographical distribution of all clusters is shown in Figure 5.10-5.11. The first cluster consisted of 102 samples of all taxa, while the second cluster comprised 349 samples of *S. cereale* ssp. *cereale* and *S. cereale* ssp. *segetale.* Seventy nine samples of *S.*

cereale ssp. *cereale* and *S. cereale* ssp. *segetale* having an ancestry value smaller than 0.7 for both of the clusters were considered to have mixed ancestry. Majority of the samples in cluster one (were observed to originated from outside of Asia basically Europe and South America) (Figure 5.12). On the other hand, spatial distribution of the majority of samples in the second cluster corresponds to Middle East, South and Central Asia or Australia (Figure 5.13).

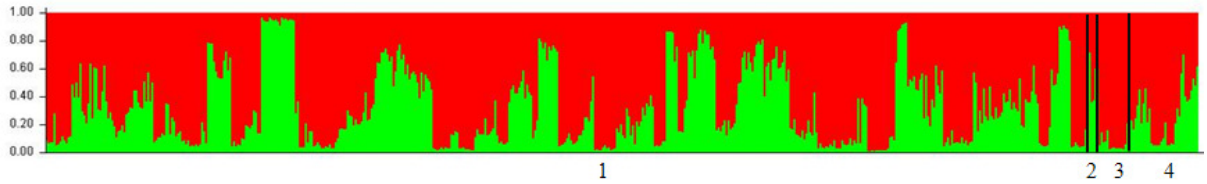

Figure 5.8. STRUCTURE results for *S. cereale* subspecies at K=2 (1, *S. cereale* ssp. *cereale;* 2, *S. cereale* ssp. *afghanicum;* 3, *S. cereale* ssp. *ancestrale*; 4, *S. cereale* ssp. *segetale*).

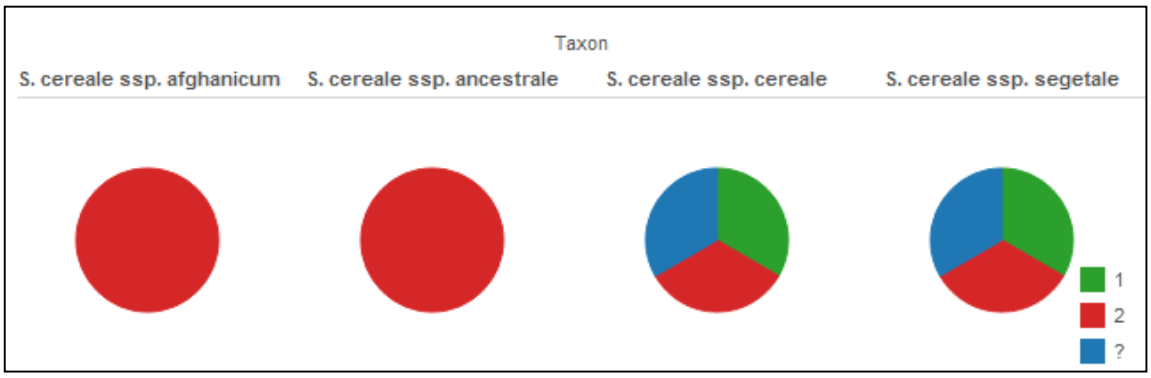

Figure 5.9. Distribution of two clusters among *S. cereale* subspecies. Clusters (Green, cluster 1; red, cluster 2; Blue, unassigned).

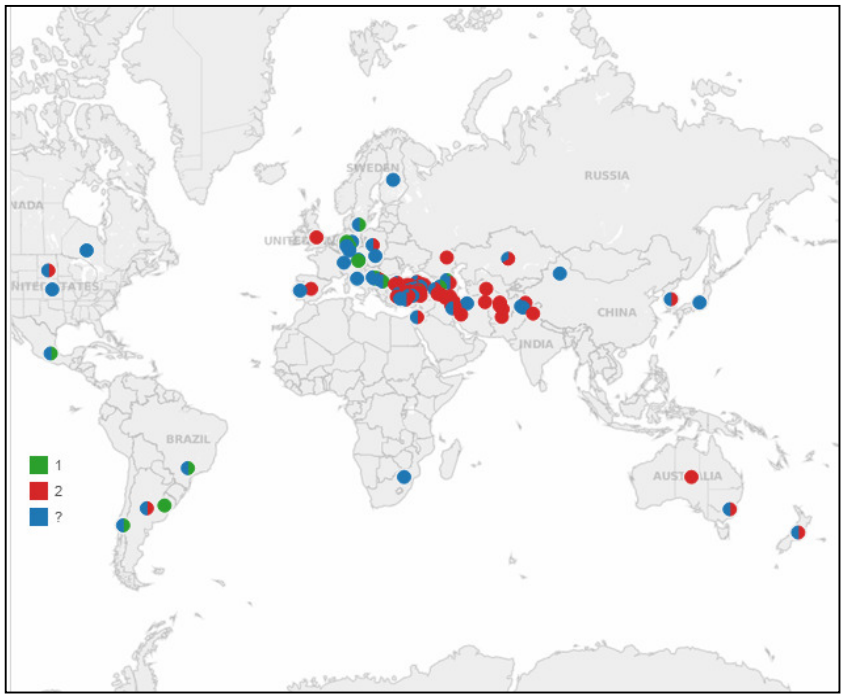

Figure 5.10. Geographical distribution of two clusters of *S. cereale* subspecies. The question mark designates unassigned individuals.

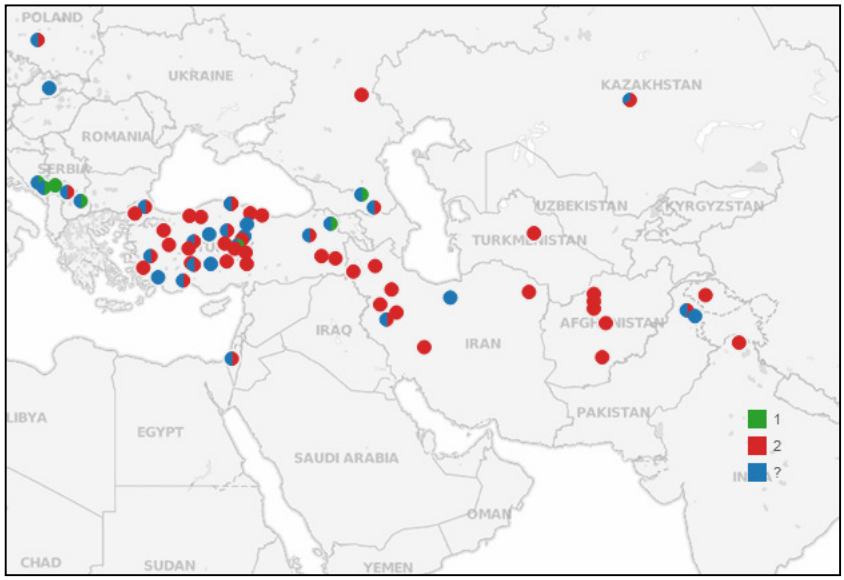

Figure 5.11. Geographical distribution *S. cereale* subspecies in Middle East The question mark designates unassigned individuals.

Figure 5.12. Geographical distribution of *S. cereale* subspecies in cluster I.

Figure 5.13. Geographical distribution of *S. cereale* subspecies in cluster II.

5.3.3. Microsatellite Based Genetic Diversity of Cultivated Rye (S*ecale cereale* ssp. *cereale*)

To determine patterns of genetic diversity and differentiation in S*ecale cereale* ssp. *cereale*, a total of 517 samples from 63 different accessions were pooled into 10 main geographical regions (Africa, Australia, Europe, Balkans, Caucasus, East Asia, South and Central Asia, Middle East (including Anatolia), North America and South America) based on their origins. These samples were analyzed using the same seven microsatellite primers used in the previous sections.

The number of alleles (N_A), allelic richness (R_S), effective number of alleles (N_E), number of private alleles (P_A), corrected private allelic richness (P_R), observed heterozygosity (H_O) expected heterozygosity (H_E), and Shannon's information index (I) were calculated for seven microsatellite loci and ten gene pools (Table 5.6). Furthermore Nei's heterozygosity estimates including H_S, the within-population genetic diversity; H_T, the total genetic diversity; H_T', the total genetic diversity independent of sample size; D_{ST} among-populations genetic diversity; D_{ST}' among-populations genetic diversity independent of sample size; G_{ST} the coefficient of genetic differentiation; G_{ST}' the coefficient of genetic differentiation independent of sample size were calculated for nine geographical regions (The single population from Africa was not included in the analysis) (Table 5.7).

Table 5.6. Levels of genetic variability at seven microsatellite loci for cultivated rye. N, sample size; N_A, number of alleles; R_S, allelic richness; N_E, number of effective alleles; P_R, number of private alleles independent of sample size; I, Shannon's information index; H_O, observed heterozygosity; H_E, expected heterozygosity; P_A, number of private alleles; P_R, private allelic richness.

Pop.	Par	**R1187**	**R1254**	**R1323**	**R1264**	**R1205**	**R1238**	**R1160**	**Av.**
	N	6	2	6	4	4	6	5	4.7
	NA	4	2	4	1	4	3	4	3.14
	RS	3.02	2	3.55	1	3.75	2.91	3.1	2.76
	Ne	2.06	1.6	3	1	2.91	2.67	1.92	2.17
	I	0.98	0.56	1.24	0	1.21	1.04	0.94	0.85
	Ho	0.67	0.5	1	0	0.75	1	0.6	0.65
	He	0.51	0.38	0.67	0	0.66	0.63	0.48	0.47
	PA	0	0	0	0	0	0	0	0
Africa	**PR**	0.45	0	0.33	0	0.41	0	0.06	0.18
	N	28	1	28	28	27	23	24	22.71
	Na	5	2	6	5	6	4	13	5.86
	RS	2.92	2	2.87	2.83	2.67	3.29	5.33	3.13
	Ne	2.69	2	2.63	2.46	1.92	3.22	8.73	3.38
	I	1.16	0.69	1.17	1.12	1.01	1.27	2.33	1.25
	Ho	0.75	1	0.96	0.5	0.56	0.65	0.75	0.74
	He	0.63	0.5	0.62	0.59	0.48	0.69	0.89	0.63
	PA	0	0	0	1	0	0	1	0.29
Australia	**PR**	0.05	0	0.19	0.44	0.13	0	0.59	0.2
	N	57	16	59	55	53	60	53	50.43
	Na	8	5	9	5	8	5	10	7.14
	RS	3.22	2.64	3.76	2.58	3.72	3.41	4.75	3.44
	Ne	3.17	2.39	3.9	2.37	3.47	3.67	6.89	3.69
	I	1.34	1.04	1.62	1.02	1.58	1.37	2.05	1.43
	Ho	0.88	0.75	0.76	0.65	0.77	0.95	0.83	0.8
	He	0.68	0.58	0.74	0.58	0.71	0.73	0.85	0.7
	PA	0	1	0	0	0	1	0	0.29
Balkans	**PR**	0.15	0.22	0.34	0.16	0.35	0.06	0.17	0.21

Table 5.6. Levels of genetic variability at seven microsatellite loci for cultivated accessions (Cont.).

Pop.	Par	R1187	R1254	R1323	R1264	R1205	R1238	R1160	Av.
	N	13	6	14	14	13	14	10	12
	Na	5	6	6	3	7	4	6	5.29
	RS	3.33	3.92	3.4	2.66	3.93	3.26	4.17	3.53
	Ne	3.13	2.67	3.02	2.43	3.6	3.29	4.55	3.24
	I	1.3	1.35	1.34	0.98	1.57	1.26	1.61	1.34
	Ho	0.92	0.67	0.93	0.71	0.85	0.64	0.9	0.8
	He	0.68	0.63	0.67	0.59	0.72	0.7	0.78	0.68
	PA	0	0	0	0	0	0	0	0
Caucasus	**PR**	0.05	1.64	0.44	0.03	0.22	0	0.01	0.34
	N	28	5	28	30	30	31	26	25.43
	Na	4	5	5	5	6	4	11	5.71
	RS	2.71	4.03	2.35	2.23	3	3.3	4.74	3.19
	Ne	2.39	3.13	1.85	1.81	2.28	3.43	5.75	2.95
	I	1.02	1.36	0.85	0.8	1.18	1.3	2.06	1.22
	Ho	0.86	0.8	0.61	0.4	0.6	0.9	0.92	0.73
	He	0.58	0.68	0.46	0.45	0.56	0.71	0.83	0.61
	PA	0	0	0	0	0	0	0	0
East Asia	**PR**	0.04	0.45	0.1	0.29	0.21	0	0.38	0.21
	N	33	16	35	36	30	34	28	30.29
	Na	7	5	7	4	7	4	13	6.71
	RS	3.48	2.73	3.77	2.57	3.33	3.35	4.99	3.46
	Ne	3.08	2.18	4	2.42	2.97	3.5	6.94	3.58
	I	1.43	1.03	1.56	1	1.36	1.32	2.22	1.42
	Ho	0.79	0.69	0.89	0.75	0.9	0.74	0.86	0.8
	He	0.68	0.54	0.75	0.59	0.66	0.71	0.86	0.68
	PA	0	0	0	0	0	0	0	0
Europe	**PR**	0.23	0.05	0.28	0.1	0.13	0	0.68	0.21
	N	220	190	210	214	163	216	170	197.57
	Na	8	15	20	12	10	5	17	12.43
	RS	3.56	3.39	3.81	2.96	3.58	3.41	5.02	3.68
	Ne	3.62	3.18	3.76	2.71	3.2	3.74	8.18	4.05
Middle East	**I**	1.51	1.52	1.78	1.29	1.55	1.38	2.33	1.62
	Ho	0.89	0.65	0.84	0.74	0.68	0.68	0.89	0.77
	He	0.72	0.69	0.73	0.63	0.69	0.73	0.88	0.72
	PA	0	6	7	3	1	1	2	2.86
	PR	0.17	0.4	0.55	0.41	0.38	0.06	0.47	0.35

Table 5.6. Levels of genetic variability at seven microsatellite loci for cultivated accessions (Cont.)

Pop.	Par	R1187	R1254	R1323	R1264	R1205	R1238	R1160	Av.
North America	N	22	6	21	22	22	20	17	18.57
	Na	5	4	8	4	5	4	10	5.71
	RS	3.25	3.42	3.54	2.1	2.97	3.33	4.64	3.32
	Ne	2.92	3.13	3.03	1.58	2.51	3.46	5.78	3.2
	I	1.29	1.24	1.46	0.68	1.15	1.3	1.95	1.3
	Ho	0.73	0.83	0.9	0.45	0.77	0.9	0.82	0.77
	He	0.66	0.68	0.67	0.37	0.6	0.71	0.83	0.65
	PA	0	0	1	0	0	0	0	0.14
	PR	0.08	0.15	0.59	0.08	0.05	0	0.21	0.17
South America	N	37	6	43	42	41	40	35	34.86
	Na	5	5	6	4	7	4	11	6
	RS	3.1	3.97	3.41	2.27	3.54	3.26	4.38	3.42
	Ne	2.79	3.6	3.39	1.88	3.25	3.26	4.85	3.29
	I	1.23	1.42	1.4	0.81	1.47	1.28	1.93	1.36
	Ho	0.78	0.83	0.86	0.52	0.88	1	0.89	0.82
	He	0.64	0.72	0.7	0.47	0.69	0.69	0.79	0.67
	PA	0	0	0	0	0	0	0	0
	PR	0.05	0.17	0.14	0.04	0.35	0	0.22	0.14
South and Central Asia	N	40	10	41	42	36	37	31	33.86
	Na	4	5	6	6	8	4	12	6.43
	RS	2.77	2.8	2.92	2.76	2.95	3.12	5.09	3.2
	Ne	2.59	1.92	2.61	2.44	2.24	3.22	7.84	3.27
	I	1.06	0.98	1.19	1.11	1.19	1.24	2.23	1.29
	Ho	0.85	0.5	0.85	0.64	0.61	0.86	0.87	0.74
	He	0.61	0.48	0.62	0.59	0.55	0.69	0.87	0.63
	PA	0	0	0	0	1	0	0	0.14
	PR	0.01	0.06	0.08	0.31	0.27	0	0.28	0.14

Table 5.7. Levels of genetic diversity for *Secale cereale* ssp. *cereale* accessions from different origins. He, expected heterozygosity; H_S, the within population genetic diversity; H_T, the total genetic diversity; H_T', the total genetic diversity independent of sample size; D_{ST} among-populations genetic diversity; D_{ST}' among-populations genetic diversity independent of sample size; G_{ST} the coefficient of genetic differentiation; G_{ST}' the coefficient of genetic differentiation independent of sample size.

Region	**He**	**Hs**	**H_T**	**H_T'**	**D_{ST}**	**D_{ST}'**	**G_{ST}**	**G_{ST}'**
Africa	0,51	Nd	Nd	Nd	Nd	Nd	Nd	Nd
Australia	0,63	0.643	0.652	0.657	0.009	0.014	0.014	0.021
Balkans	0,68	0.632	0.699	0.711	0.067	0.079	0.096	0.111
Caucasus	0,68	0.699	0.714	0.729	0.015	0.030	0.021	0.041
East Asia	0,58	0.616	0.620	0.622	0.004	0.007	0.007	0.010
Europe	0,68	0.647	0.680	0.688	0.033	0.041	0.048	0.059
M. East	0,72	0.696	0.738	0.739	0.042	0.043	0.057	0.059
N. America	0,65	0.586	0.640	0.667	0.054	0.081	0.084	0.121
S. America	0,67	0.652	0.683	0.693	0.031	0.041	0.045	0.060
S. C. Asia	0,63	0.641	0.655	0.658	0.014	0.017	0.021	0.026

The number of alleles generated ranged from 20 (REMS 1160) to five (REMS1238) across seven loci and the total number of observed alleles was 96. A total of 26 private alleles were observed. Among these, 20 (%76.9) were observed in the Middle Eastern populations. The highest mean corrected allelic richness was found in Middle East populations (3.68) and lowest in Africa population (2.71). Private allelic richness was highest the Middle Eastern populations (35) and lowest in South America and South-Central Asia populations (0.14) (Figure 5.15). The observed heterozygosity levels were found to be higher than expected heterozygosity levels in all geographical regions. The mean expected heterozygosity (also known as gene diversity) levels ranged between 0.474-0.72 with an average of 0.64. Shannon's information index (I) was found to be highest in the Middle Eastern populations (1.62), and lowest in African population (0.85) (Figure 5.16).

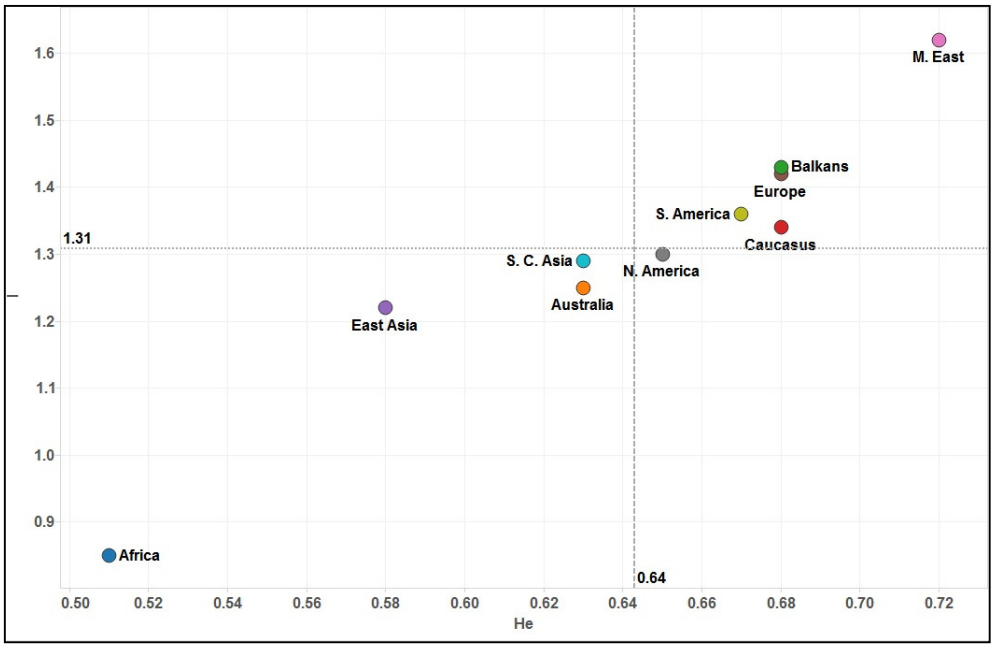

Figure 5.14. Shannon's information index (I) and expected heterozygosity (He) in *S. cereale* ssp. *cereale* populations from different geographical regions.

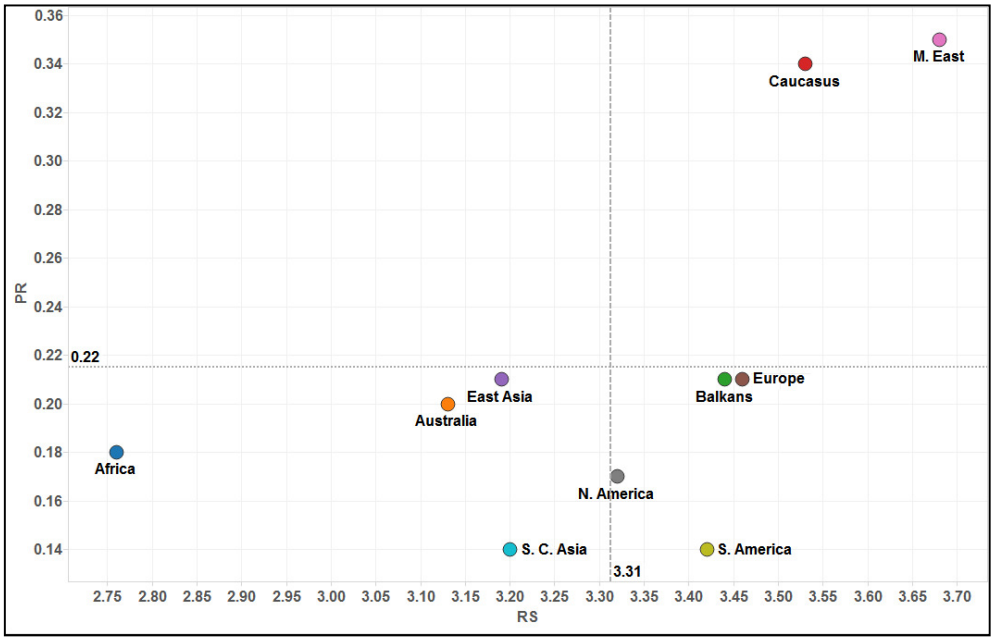

Figure 5.15. Private allelic richness (PR) and allelic richness (RS) and in S. *cereale* ssp. *cereale* populations from different geographical regions

Intra-population genetic diversity (H_S), the measure of average differences within populations was found to be highest in Caucasus populations (0,699) and lowest in North American populations (0.586). The corrected total genetic diversity (H_T') in cereal rye showed variations from region to region, ranging from 0,622 to 0,739 (Figure 5.16).

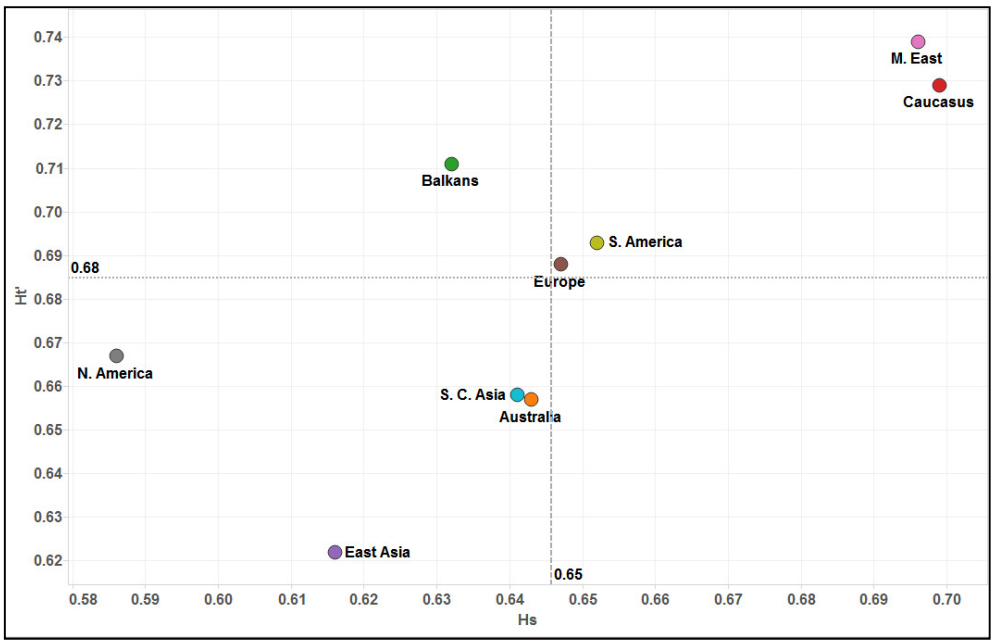

Figure 5.16. Comparison of H_T' and H_S values from different geographical regions. H_T', the corrected total genetic diversity; H_S, Intra-population genetic diversity.

To analyze gene differentiation between populations in each region, coefficient of among-populations genetic diversity independent of sample sizes (D_{ST}'), and coefficient of gene differentiation independent of sample sizes (G_{ST}') were compared (Figure 5.17). Genetic differentiation was found to be highest in North America (D_{ST}'=0.081, G_{ST}'= 0.121) and lowest in East Asia (D_{ST}'=0.007, G_{ST}'= 0.01). Furthermore, to assess the degree of genetic differentiation between geographical regions, pairwise F_{ST} genetic distances (Nei 1972; Nei, 1978) were computed. This analysis showed the African gene pool to be the most different from remaining gene pools (Table 5.8), with the highest genetic distance when compared to the South-Central Asian populations (0.96) (Table 5.8). The genetic differentiation among other gene pools was low.

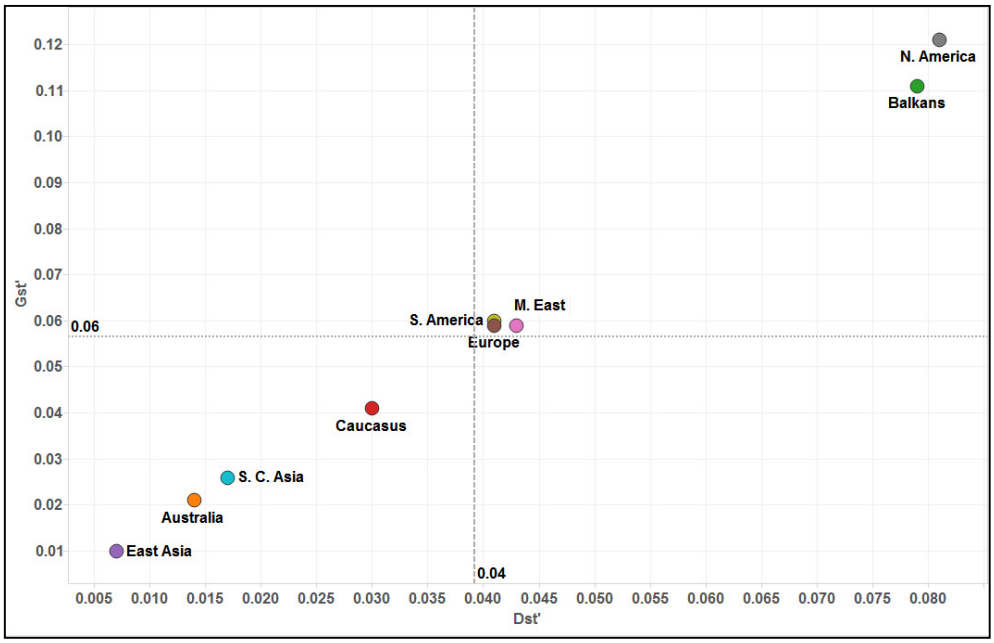

Figure 5.17. Comparison of G_{ST}' and D_{ST}' values from different geographical regions. G_{ST}', coefficient of gene differentiation independent of sample size; D_{ST}', coefficient of among-populations genetic diversity independent of sample size.

Table 5.8. The pairwise Fst vales of each geographical region calculated using seven microsatellite markers.

Africa	**Australia**	**Balkans**	**Caucasus**	**East Asia**	**Europe**	**M. East**	**N. America**	**S. America**	**S. C. Asia**	
0.000										**Africa**
0.079	0.000									**Australia**
0.081	0.019	0.000								**Balkans**
0.092	0.037	0.018	0.000							**Caucasus**
0.057	0.025	0.034	0.045	0.000						**East Asia**
0.091	0.020	0.010	0.016	0.040	0.000					**Europe**
0.083	0.015	0.010	0.016	0.028	0.012	0.000				**M. East**
0.068	0.021	0.020	0.031	0.023	0.027	0.019	0.000			**N. America**
0.062	0.038	0.018	0.033	0.027	0.028	0.028	0.024	0.000		**S. America**
0.096	0.016	0.020	0.018	0.034	0.013	0.011	0.021	0.041	0.000	**S. C. Asia**

To explore pattern of relationship between cultivated rye populations from different geographical regions with the microsatellite data, a PCA analysis was also performed. The first, second and third components explained %45.44, 23.37 and 12.54 of variance, respectively. Three distinct clusters were observed (Figure 5.18). The first cluster was dominated by samples from the Middle East, whereas the other clusters contain samples from diverse geographical areas.

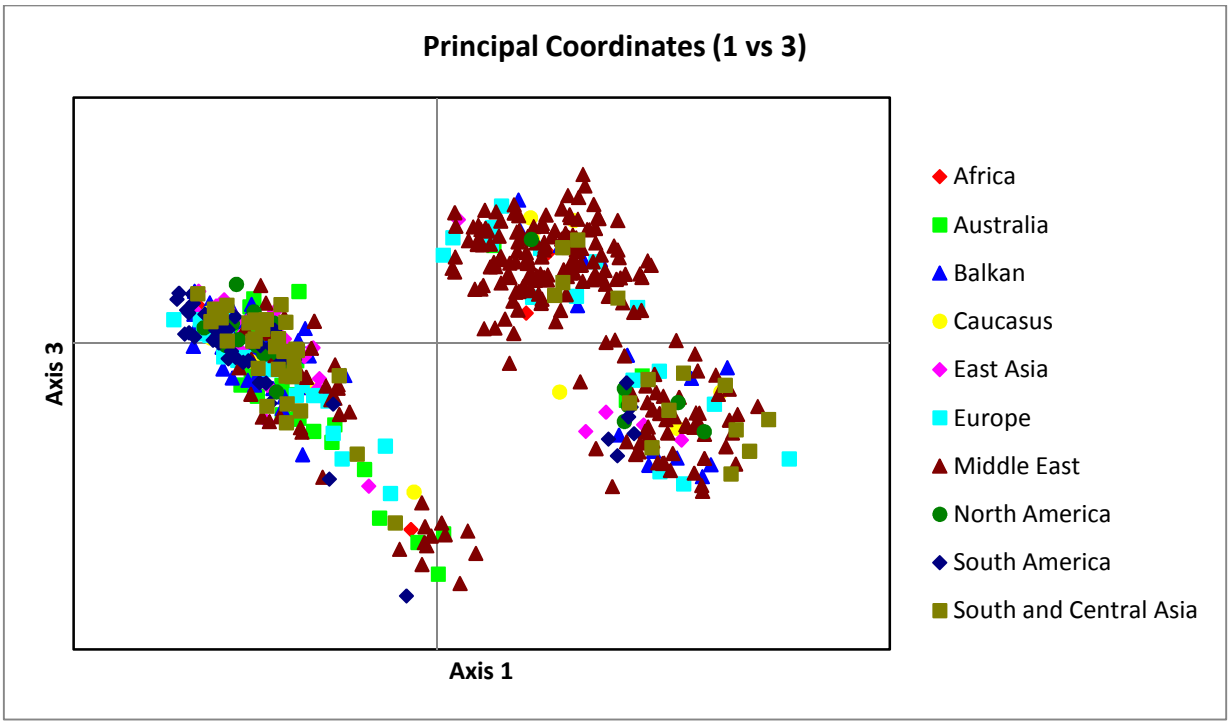

Figure 5.18. PCA analysis of *S. cereale* ssp. *cereale* samples with different geographical origins.

4.3.4. Chloroplast DNA Diversity of *S. cereale* subspecies

The *ndhF-rpl32* intergenic region of chloroplast DNA was amplified in a total of 136 samples from different subspecies of *S. cereale* samples from different geographical origins (Table 5.9). Ten nucleotide substitutions and 13 insertion deletion events in 95 polymorphic sites were observed. The number of haplotypes was 23. Haplotype diversity and average number of differences were found to be 0.733 and 4.028 respectively. The nucleotide diversity was 0.00248. The haplotype network constructed considering the

insertions and deletions as fifth character is shown in Figure 5.19. The network revealed that 12 of the haplotypes were shared among different subspecies.

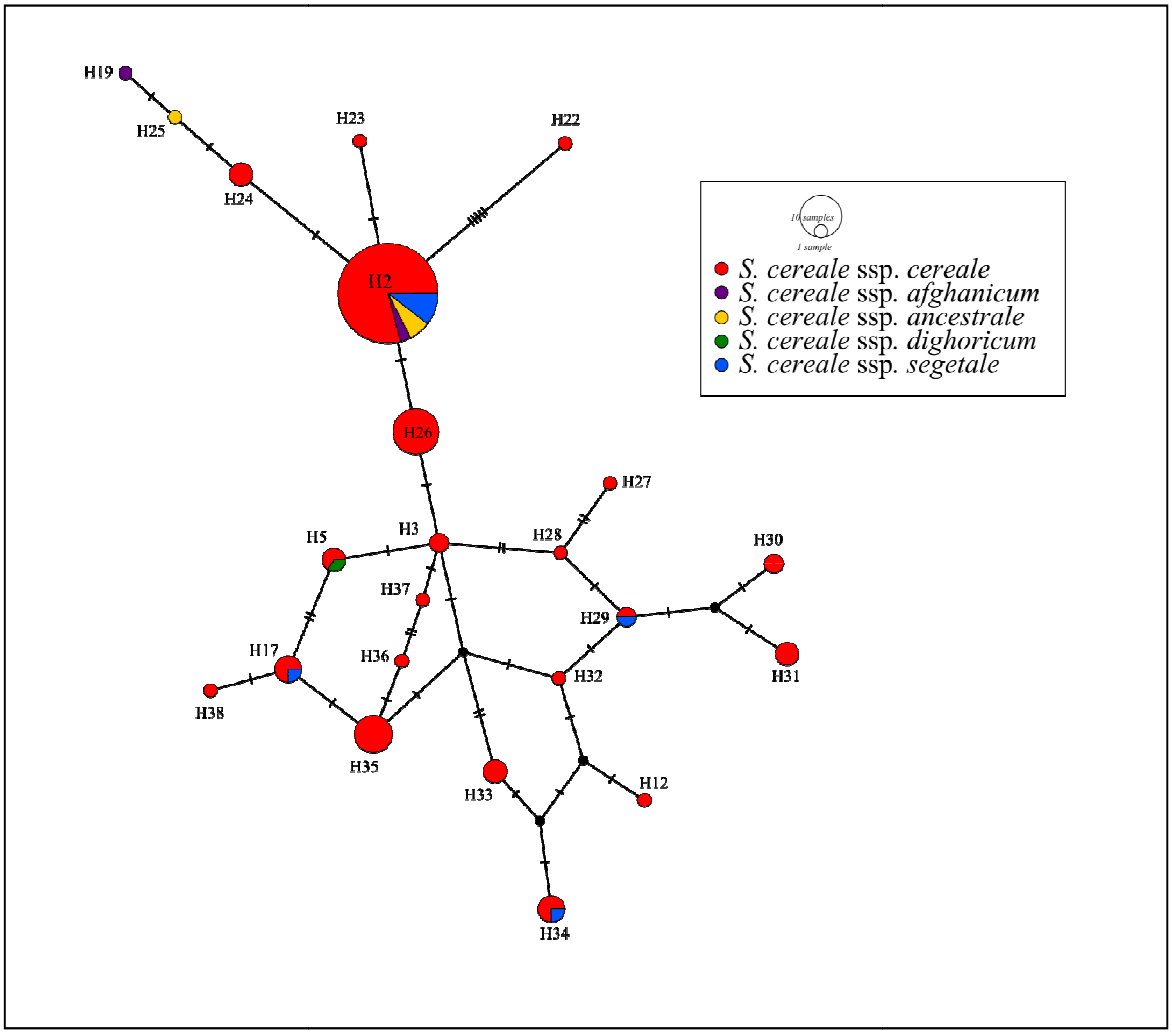

Figure 5.19. The haplotype network based on *ndhF-rpl32* intergenic region from *S. cereale* subspecies. The pie charts represent distinctive haplotypes and their frequencies in each subspecies. The sizes of the circles are proportional to the frequency of each haplotype. Each line represents a single mutational change. Black circles represent hypothetical haplotypes.

Table 5.9. Haplotypes identified in chloroplast DNA analysis of *S. cereale* subspecies.

Hap.	ID.	Ac. No.	*Taxon*	Origin
H2	R0013	PI 205221	*S. cereale ssp. cereale*	Ankara, Turkey
H2	R0072	PI 561796	*S. cereale ssp. cereale*	Bolu, Turkey
H2	R0107	PI 168205	*S. cereale ssp. cereale*	Corum, Turkey
H2	R0119	PI 168196	*S. cereale ssp. cereale*	Edirne, Turkey
H2	R0122	PI 561806	*S. cereale ssp. cereale*	Erzurum, Turkey
H2	R0147	PI 252002	*S. cereale ssp. cereale*	Eskisehir, Turkey
H2	R0161	PI 168199	*S. cereale ssp. cereale*	Isparta, Turkey
H2	R0168	PI 168136	*S. cereale ssp. cereale*	Izmir, Turkey
H2	R0173	PI 168218	*S. cereale ssp. cereale*	Kayseri, Turkey
H2	R0179	PI 168218	*S. cereale ssp. cereale*	Kayseri, Turkey
H2	R0189	PI 168195	*S. cereale ssp. cereale*	Kirklareli, Turkey
H2	R0214	PI 168209	*S. cereale ssp. cereale*	Kutahya, Turkey
H2	R0224	PI 561793	*S. cereale ssp. cereale*	Manisa, Turkey
H2	R0228	PI 561793	*S. cereale ssp. cereale*	Manisa, Turkey
H2	R0237	PI 168220	*S. cereale ssp. cereale*	Nigde, Turkey
H2	R0259	PI 173589	*S. cereale ssp. cereale*	Ordu, Turkey
H2	R0314	PI 168194	*S. cereale ssp. cereale*	Yozgat, Turkey
H2	R0332	PI 561809	*S. cereale ssp. cereale*	Pakistan
H2	R0413	PI 429371	*S. cereale ssp. cereale*	Semnan, Iran
H2	R0420	PI 429373	*S. cereale ssp. cereale*	Zanjan, Iran
H2	R0426	PI 429373	*S. cereale ssp. cereale*	Zanjan, Iran
H2	R0501	PI 344975	*S. cereale ssp. cereale*	Montenegro
H2	R0505	PI 344990	*S. cereale ssp. cereale*	Serbia
H2	R0515	PI 405812	*S. cereale ssp. cereale*	Macedonia
H2	R0522	PI 445996	*S. cereale ssp. cereale*	Malmohus, Sweden
H2	R0526	PI 445996	*S. cereale ssp. cereale*	Malmohus, Sweden
H2	R0545	PI 240675	*S. cereale ssp. cereale*	Colonia, Uruguay
H2	R0546	PI 240675	*S. cereale ssp. cereale*	Colonia, Uruguay
H2	R0563	PI 584782	*S. cereale ssp. cereale*	Georgia
H2	R0601	PI 543398	*S. cereale ssp. cereale*	Argentina
H2	R0622	PI 261400	*S. cereale ssp. cereale*	Ontario, Canada
H2	R0630	CIse 37	*S. cereale ssp. cereale*	Afghanistan
H2	R0632	CIse 37	*S. cereale ssp. cereale*	Afghanistan
H2	R0666	PI 218110	*S. cereale ssp. cereale*	Pakistan
H2	R0670	PI 220118	*S. cereale ssp. cereale*	Afghanistan
H2	R0672	PI 234655	*S. cereale ssp. cereale*	Kazakhstan
H2	R0673	PI 234655	*S. cereale ssp. cereale*	Kazakhstan

Table 5.9. Haplotypes identified in chloroplast DNA analysis of *S. cereale* subspecies (Cont.)

Hap.	**ID.**	**Ac. No.**	***Taxon***	**Origin**
H2	R0691	PI 410534	*S. cereale ssp. cereale*	Pakistan
H2	R0693	PI 410534	*S. cereale ssp. cereale*	Pakistan
H2	R0703	PI 430003	*S. cereale ssp. cereale*	India
H2	R0708	PI 447337	*S. cereale ssp. cereale*	Xinjiang, China
H2	R0729	PI 330407	*S. cereale ssp. cereale*	South Africa
H2	R0730	PI 330407	*S. cereale ssp. cereale*	South Africa
H2	R0735	PI 330407	*S. cereale ssp. cereale*	South Africa
H2	R0776	PI 326284	*S. cereale ssp. segetale*	Azerbaijan
H2	R0788	PI 326286	*S. cereale ssp. segetale*	Kazakhstan
H2	R0800	PI 618671	*S. cereale ssp. segetale*	Turkey
H2	R0809	PI 618673	*S. cereale ssp. segetale*	Turkey
H2	R0969	TR2	*S. cereale ssp. cereale*	Kars/Turkey
H2	R0976	R1133	*S. cereale ssp. cereale*	Portugal
H2	R0983	R62	*S. cereale ssp. ancestrale*	Turkey
H2	R0984	R2022	*S. cereale ssp. cereale*	Finland
H2	R0987	R1054	*S. cereale ssp. ancestrale*	Turkmenistan
H2	R0988	R1756	*S. cereale ssp. cereale*	Austria
H2	R0993	R767	*S. cereale ssp. ancestrale*	Turkey
H2	R0995	R788	*S. cereale ssp. segetale*	Spain
H2	R1000	R2199	*S. cereale ssp. cereale*	Italy
H2	R1001	R1151	*S. cereale ssp. cereale*	Switzerland
H2	R1002	R1480	*S. cereale ssp. cereale*	Germany
H2	R1009	R1148	*S. cereale ssp. cereale*	Turkey
H2	R1011	R569	*S. cereale ssp. afghanicum*	Afghanistan
H2	R1012	R1038	*S. cereale ssp. afghanicum*	Afghanistan
H2	R1014	R61	*S. cereale ssp. segetale*	Russian Federation
H2	R1016	R1658	*S. cereale ssp. cereale*	USA
H2	R1018	R29	*S. cereale ssp. ancestrale*	Russian Federation
H2	R1024	R191	*S. cereale ssp. cereale*	Germany
H3	R0705	PI 430003	*S. cereale ssp. cereale*	Himachal Pradesh, India
H3	R0299	PI 561804	*S. cereale ssp. cereale*	Van, Turkey
H5	R0307	PI 561804	*S. cereale ssp. cereale*	Van, Turkey
H5	R0446	PI 445977	*S. cereale ssp. cereale*	Israel
H5	R1013	R1092	*S. cereale ssp. cereale*	Austria
H5	R1026	R250	*S. cereale ssp. dighoricum*	Unknown
H12	R0367	PI 243741	*S. cereale ssp. cereale*	West Azerbaijan, Iran
H17	R0455	PI 323450	*S. cereale ssp. cereale*	Lublin, Poland

Table 5.9. Haplotypes identified in chloroplast DNA analysis of *S. cereale* subspecies (Cont.)

Hap.	**ID.**	**Ac. No.**	***Taxon***	**Origin**
H17	R0459	PI 330526	*S. cereale ssp. cereale*	England/UK
H17	R0664	CIse 110	*S. cereale ssp. cereale*	South Korea
H17	R0990	R1490	*S. cereale ssp. cereale*	Germany
H17	R0998	R607	*S. cereale ssp. segetale*	Slovakia
H19	R0997	R567	*S. cereale ssp. afghanicum*	Afghanistan
H22	R0728	CIse 79	*S. cereale ssp. cereale*	Australia
H23	R0502	PI 344975	*S. cereale ssp. cereale*	Montenegro
H24	R0347	PI 227870	*S. cereale ssp. cereale*	Bakhtiari va Chahar, Iran
H24	R0474	PI 344970	*S. cereale ssp. cereale*	Bosnia and Herzegovina
H24	R0475	PI 344970	*S. cereale ssp. cereale*	Bosnia and Herzegovina
H25	R0765	PI 618666	*S. cereale ssp. ancestrale*	Turkey
H26	R0085	PI 168168	*S. cereale ssp. cereale*	Bursa, Turkey
H26	R0160	PI 168199	*S. cereale ssp. cereale*	Isparta, Turkey
H26	R0188	PI 168195	*S. cereale ssp. cereale*	Kirklareli, Turkey
H26	R0256	PI 173589	*S. cereale ssp. cereale*	Ordu, Turkey
H26	R0380	PI 268281	*S. cereale ssp. cereale*	Bakhtaran, Iran
H26	R0383	PI 268281	*S. cereale ssp. cereale*	Bakhtaran, Iran
H26	R0428	PI 429377	*S. cereale ssp. cereale*	Hamadan, Iran
H26	R0435	PI 429377	*S. cereale ssp. cereale*	Hamadan, Iran
H26	R0572	PI 314964	*S. cereale ssp. cereale*	Sao Paulo, Brazil
H26	R0599	PI 543398	*S. cereale ssp. cereale*	Argentina
H26	R0613	CIse 12	*S. cereale ssp. cereale*	South Dakota, US
H26	R0614	CIse 12	*S. cereale ssp. cereale*	South Dakota, US
H27	R0241	PI 168220	*S. cereale ssp. cereale*	Nigde, Turkey
H28	R0125	PI 561806	*S. cereale ssp. cereale*	Erzurum, Turkey
H29	R0280	PI 561798	*S. cereale ssp. cereale*	Sinop, Turkey
H29	R0805	PI 618673	*S. cereale ssp. segetale*	Turkey
H30	R0079	PI 561797	*S. cereale ssp. cereale*	Bolu, Turkey
H30	R0164	PI 168136	*S. cereale ssp. cereale*	Izmir, Turkey
H31	R0372	PI 250745	*S. cereale ssp. cereale*	East Azerbaijan, Iran
H31	R0399	PI 289814	*S. cereale ssp. cereale*	Khorasan, Iran
H31	R0401	PI 289814	*S. cereale ssp. cereale*	Khorasan, Iran
H32	R0053	PI 561802	*S. cereale ssp. cereale*	Bitlis, Turkey
H33	R0002	PI 168211	*S. cereale ssp. cereale*	Afyon, Turkey
H33	R0193	PI 168213	*S. cereale ssp. cereale*	Kirsehir, Turkey
H33	R0273	PI 168181	*S. cereale ssp. cereale*	Samsun, Turkey

Table 5.9. Haplotypes identified in chloroplast DNA analysis of *S. cereale* subspecies (Cont.).

Hap.	ID.	Ac. No.	*Taxon*	Origin
H34	R0206	PI 168176	*S. cereale ssp. cereale*	Konya, Turkey
H34	R0292	PI 561799	*S. cereale ssp. cereale*	Sinop, Turkey
H34	R0955	TR1	*S. cereale ssp. cereale*	Gökova/Turkey
H34	R0956	TR1	*S. cereale ssp. cereale*	Gökova/Turkey
H34	R0992	R279	*S. cereale ssp. segetale*	Turkey
H35	R0009	PI 173587	*S. cereale ssp. cereale*	Amasya, Turkey
H35	R0587	PI 446023	*S. cereale ssp. cereale*	Mexico
H35	R0640	CIse 108	*S. cereale ssp. cereale*	Japan
H35	R0641	CIse 108	*S. cereale ssp. cereale*	Japan
H35	R0663	CIse 110	*S. cereale ssp. cereale*	South Korea
H35	R0738	PI 345739	*S. cereale ssp. cereale*	Capital Terr., Australia
H35	R0748	PI 446027	*S. cereale ssp. cereale*	New Zealand
H35	R0750	PI 446027	*S. cereale ssp. cereale*	New Zealand
H35	R0979	R1489	*S. cereale ssp. cereale*	Germany
H35	R1019	R1653	*S. cereale ssp. cereale*	Germany
H36	R0581	PI 436190	*S. cereale ssp. cereale*	Los Lagos, Chile
H37	R0571	PI 584782	*S. cereale ssp. cereale*	Georgia
H38	R0461	PI 330526	*S. cereale ssp. cereale*	England/UK

5.4. Genetic Diversity and Structure of Genus *Secale*

5.4.1. Nuclear SSR Diversity of Genus *Secale*

For a better understanding of the population structure and relationships of the species in all *Secale* species examined, a comprehensive analysis was conducted on the whole data set including 727 samples used in the previous analyses and two samples of *S. sylvestre*. The results obtained from STRUCTURE analysis revealed three separate clusters (Figure 5.20). The first one contained 33 samples of *S. strictum* ssp. *strictum* and one *S. strictum* ssp. *irmanuso* sample (Figure 5.21). Among these, seven samples originated from Europe, 20 samples originated from Iran, and the remaining samples originated from other parts of the Middle East. The second cluster included 246 samples of *S. cereale* ssp. *cereale* and other subspecies of *S. cereale* subspecies, and seven samples of *S. strictum* ssp.

strictum originating from Russia. Geographical origins of majority of the samples (72%) corresponded to the Middle East or South-Central Asia. The third cluster was composed of *S. cereale* ssp. *cereale* and *S. vavilovi* samples, most of which originated from out of Asia. The remaining 392 samples could not be assigned to any of these three clusters, and was considered to have mixed ancestry.

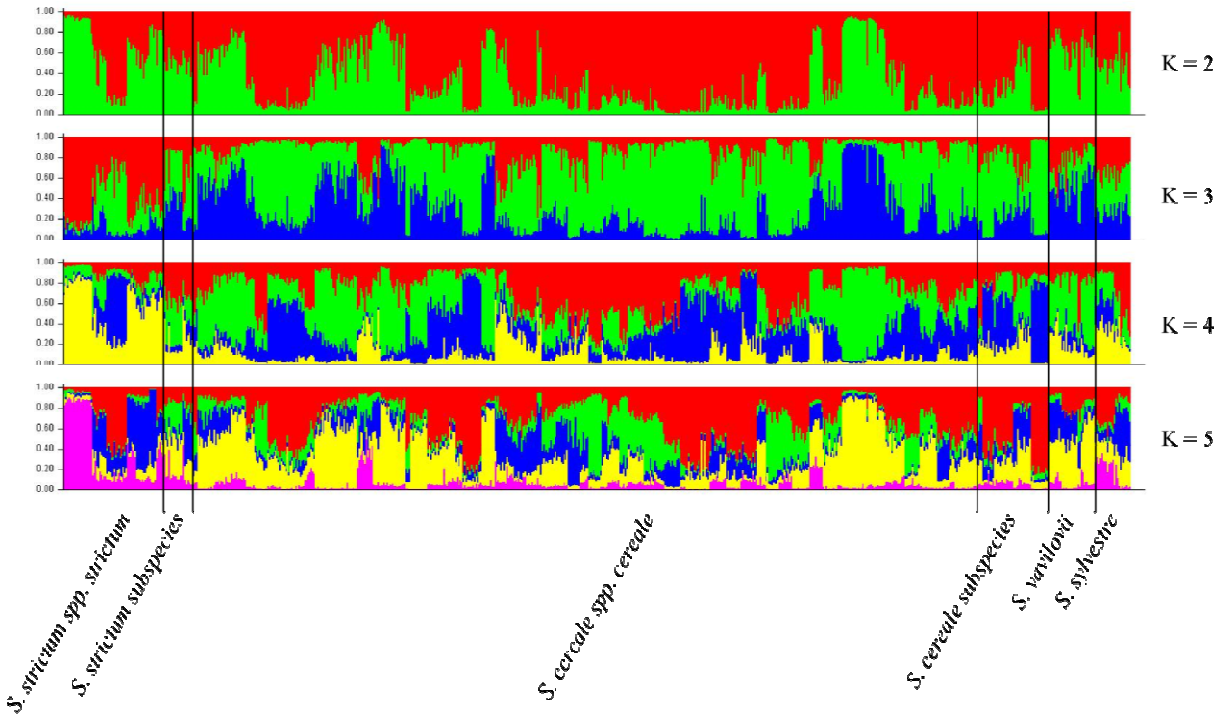

Figure 5.20. STRUCTURE results at K=2 to K=5

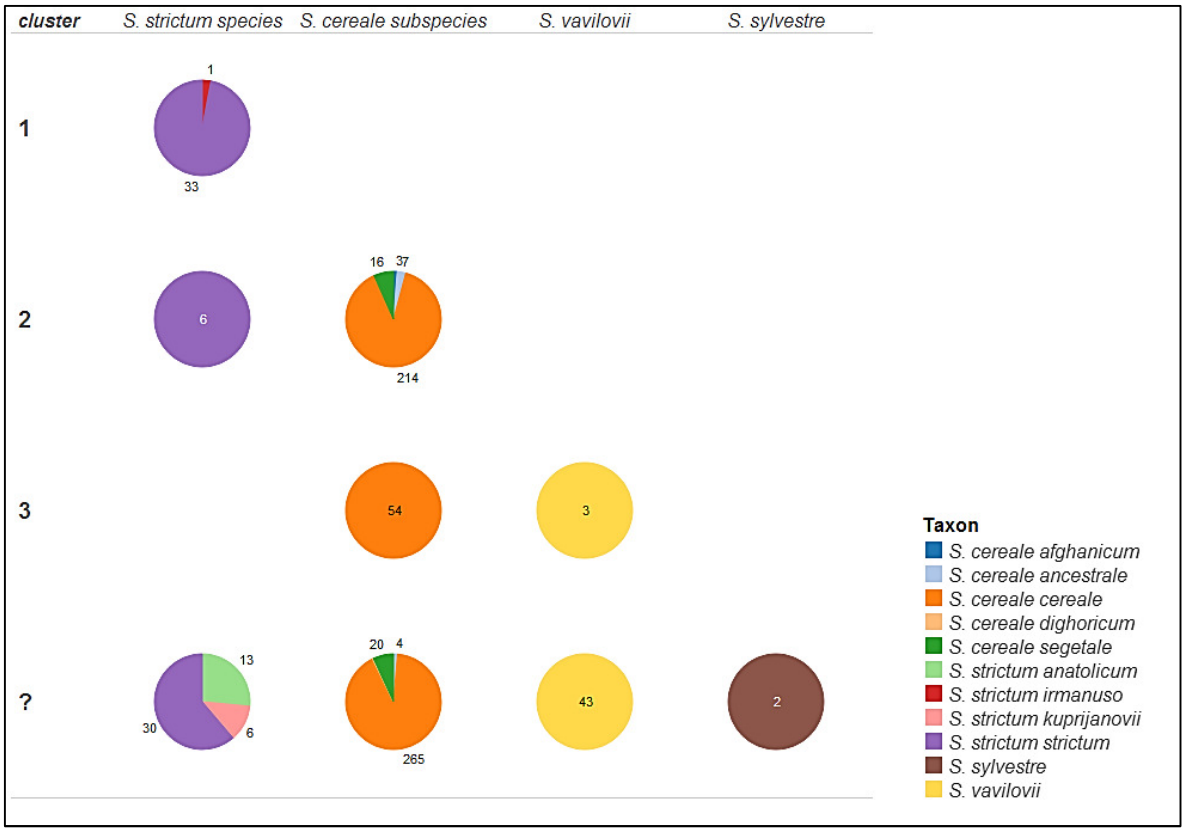

Figure 5.21. Partition of *Secale* species among three clusters. The question mark designates unassigned individuals.

To help resolve genetic relationships among different taxa, pairwise F_{ST} values were also compared (Table 5.10). The highest and lowest differentiation was observed between *S. cereale afghanicum* and *S. sylvestre* (F_{ST}=0.181) and *S. cereale* and *S. vavilovii* (F_{ST}=0.007), respectively. *S. cereale ssp. afghanicum* and *S. sylvestre* were found to be the most divergent from rest of the taxa.

Table 5.10. Pairwise F_{ST} values based on microsatellite data among *Secale* samples.

S. c. afghanicum	***S. c. ancestrale***	***S. cereale***	***S. c. segetale***	***S. vavilovii***	***S. strictum***	***S. s. anatolicum***	***S. s. kuprijanovii***	***S. sylvestre***	
0.000									***S. c. afghanicum***
0.075	0.000								***S. c. ancestrale***
0.057	0.032	0.000							***S. cereale***
0.059	0.035	0.009	0.000						***S. c. segetale***
0.066	0.039	0.007	0.013	0.000					***S. vavilovii***
0.063	0.037	0.016	0.015	0.015	0.000				***S. strictum***
0.081	**0.057**	0.021	0.030	0.021	0.030	0.000			***S. s. anatolicum***
0.089	0.049	0.023	0.035	0.028	0.031	0.038	0.000		***S. s. kuprijanovii***
0.181	**0.123**	**0.098**	**0.105**	**0.098**	**0.090**	**0.095**	**0.096**	0.000	***S. sylvestre***

Furthermore, in order to estimate relationships among different taxonomic groups of *Secale* genus more directly, Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendograms were constructed using microsatellite data. The first dendogram was constructed using *S. cereale* subspecies*, S. strictum* subspecies, *S. vavilovii* and *S. sylvestre* (Figure 5.22). The dendogram revaled a clear separation between *S. sylvestre* and the rest of genus. *S. cereale* ssp. *afghanicum* was also separated *S. cereale* subspecies and *S. strictum* subspecies. *S. strictum* ssp. *kuprijanovii* separated from remaining subspecies. *S. cereale ssp. cereale*, *S. cereale* ssp. segetale*, S. vavilovii, S. strictum ssp. anatolicum* and *S. strtictum* ssp. *strictum* constitute a group and *S. cereale* ssp. *ancestrale* remained outside off this group. *S. vavilovii* found to be closer to *S. cereale* ssp. *cereale* than *S. cereale* ssp. *afghanicum* and *S. cereale* ssp. *ancestrale*.

As the STRUCTURE analysis revealed a clear separation between *S. strictum ssp. strictum* samples originating from Iran, as a next step I separated these two populations (*S. strictum ssp. strictum* clade 2) and group the other *S. strictum ssp. strictum* samples together (*S. strictum ssp. strictum* clade 1) and rebuilt the dendogram (Figure 5.23). Branching of *S. strictum ssp. strictum* clade 2 with 99 bootstrap values revealed significantly divergent microsatellite composition. Except for this difference, both trees reflect nearly the same phylogenetic patterns.

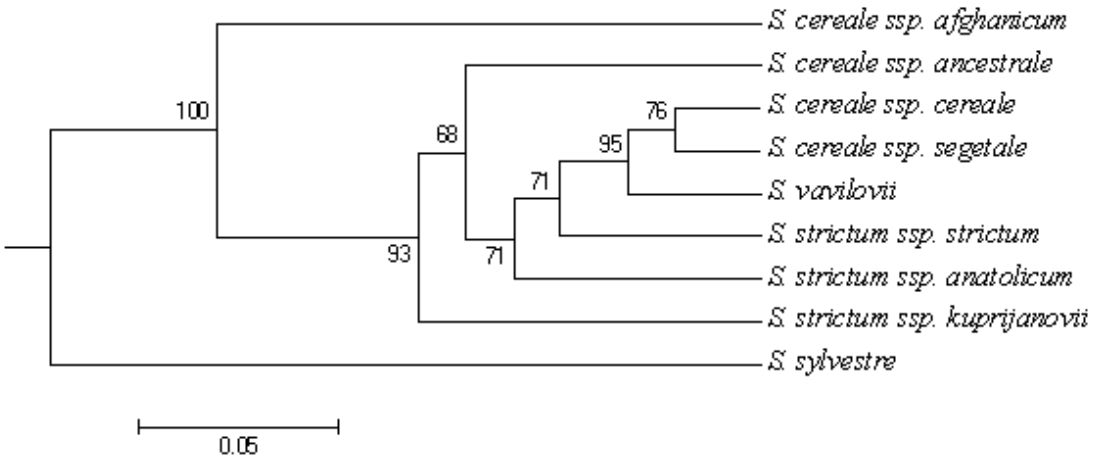

Figure 5.22. UPGMA dendogram I showing the phylogenetic relationship of *Secale* species based on pairwise D_A. Bootstrap values are provided on the nodes.

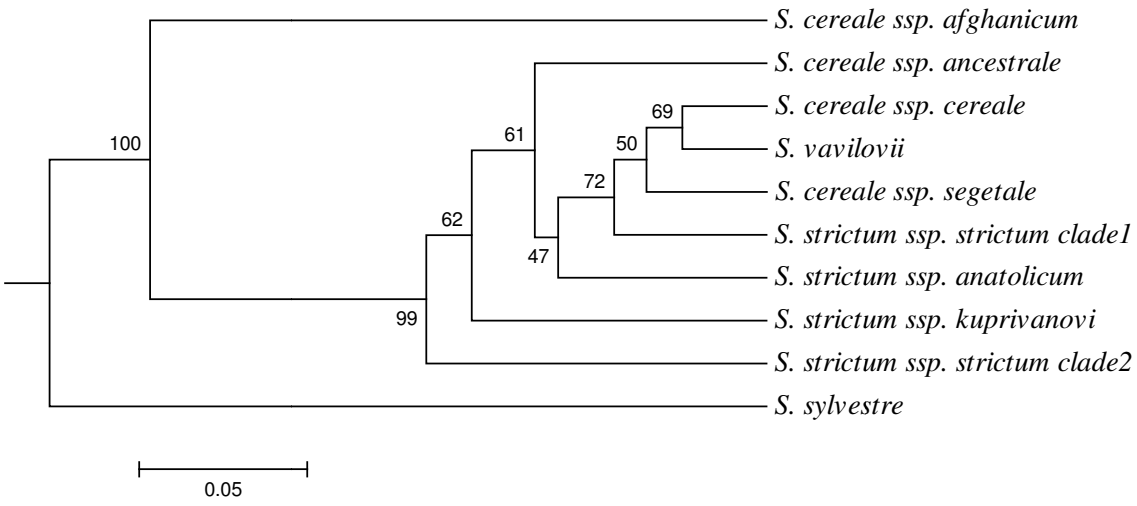

Figure 5.23. UPGMA dendogram II showing the phylogenetic relationship of *Secale* species based on pairwise D_A. Bootstrap values are provided on the nodes.

5.4.2. Chloroplast DNA Diversity of Genus *Secale*

To understand chloroplast phylogeny of all genus, a comprehensive SNP analysis of chloroplast *ndhF-rpl32* intergenic region was conducted using all *S. cereale*, *S. strictum*, *S. vavilovii* samples. Furthermore two samples of *S. sylvestre* and six hybrids were also

included in the study. The *ndhF-rpl32* intergenic region of chloroplast DNA was amplified in a total of 178 samples. Fifteen nucleotide substitutions and 89 insertions and deletions were observed in 103 sites. Haplotype diversity and average number of nucleotide differences were found to be 0.811 and 4.314, respectively.

The haplotype network of entire genus *Secale* was constructed considering the insertions and deletions as fifth character, as shown in Figure 5.24 (The detailed information about identified haplotypes was shown previously). Total number of haplotypes was 39. Among these 23 haplotypes were not shared among individuals. The remaining 16 haplotypes were shared among not only the subspecies of a particular species, but also among different species. No clear geographical structure was obtained from the haplotype network. However differentiation of certain haplotypes in *S. cereale* spp. *cereale* (e.g. H22) and *S. sylvestre* (H38 and H39) should be noted.

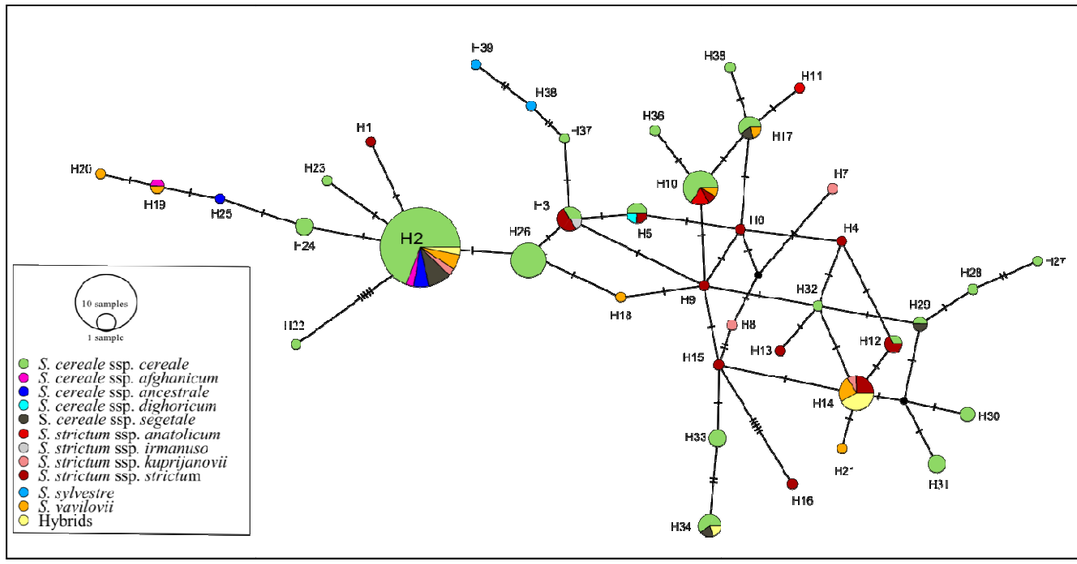

Figure 5.24. The haplotype network based on *ndhF-rpl32* intergenic region from *Secale* species. The pie charts represent distinctive haplotypes and their frequencies in each subspecies. The sizes of the circles are proportional to the frequency of each haplotype. Each line represents a single mutational change. Black circles represent hypothetical haplotypes.

Pairwise F_{ST} values of four species based on *ndhF-rpl32* intergenic region were also compared (Table 5.11).The results affirmed that *S. sylvestre* was genetically different from the rest of genus. Furthermore genetic differentiation between *S. cereale* and *S. strictum* was also confirmed. The difference between *S. cereale* and *S. vavilovii* was not significant (0.01).

Table 5.11. Pairwise F_{ST} values based on *ndhF-rpl32* intergenic region from *Secale* species. Significant *P* valuesare indicated in bold.

S. cereale	*S. vavilovii*	*S. strictum*	*S. sylvestre*	
0				*S. cereale*
0.01	0			*S. vavilovii*
0.14	0.04	0		*S. strictum*
0.41	**0.43**	**0.44**	0	*S. sylvestre*

5.4.3. Nuclear SNP Diversity of Genus *Secale*

A 667 bp nuclear expressed sequence tag sequence was amplified using GBS0551 primers (Varshney et al., 2007a) in 66 samples representing four species in the genus *Secale*. The obtained sequences were used to construct a Maximum-likelihood (ML) tree (Figure 5.25). The general topology showed that there were two main lineages. However, these groups did not correspond to neither taxonomic nor spatial delimitations. *S. vavilovii* accessions were dispersed within *S. cereale* subspecies in both groups. The two *S. sylvestre* samples clustered together in a subgroup (pink box) rather than forming a separate linage. *S. strictum* subspecies clustered together forming two (yellow and green boxes) and one subgroups (purple box) within first and second lineage, respectively. Furthermore a separated subgroup recovered in first linage containing *S. cereale* and *S. vavilovii* samples from South and Central Asia, except for a *S. cereale* ssp. *cereale* sample originating from Argentina (blue box). In the second group no clear relationships were recognized between the genealogy of *Secale* species and their geographic origin.

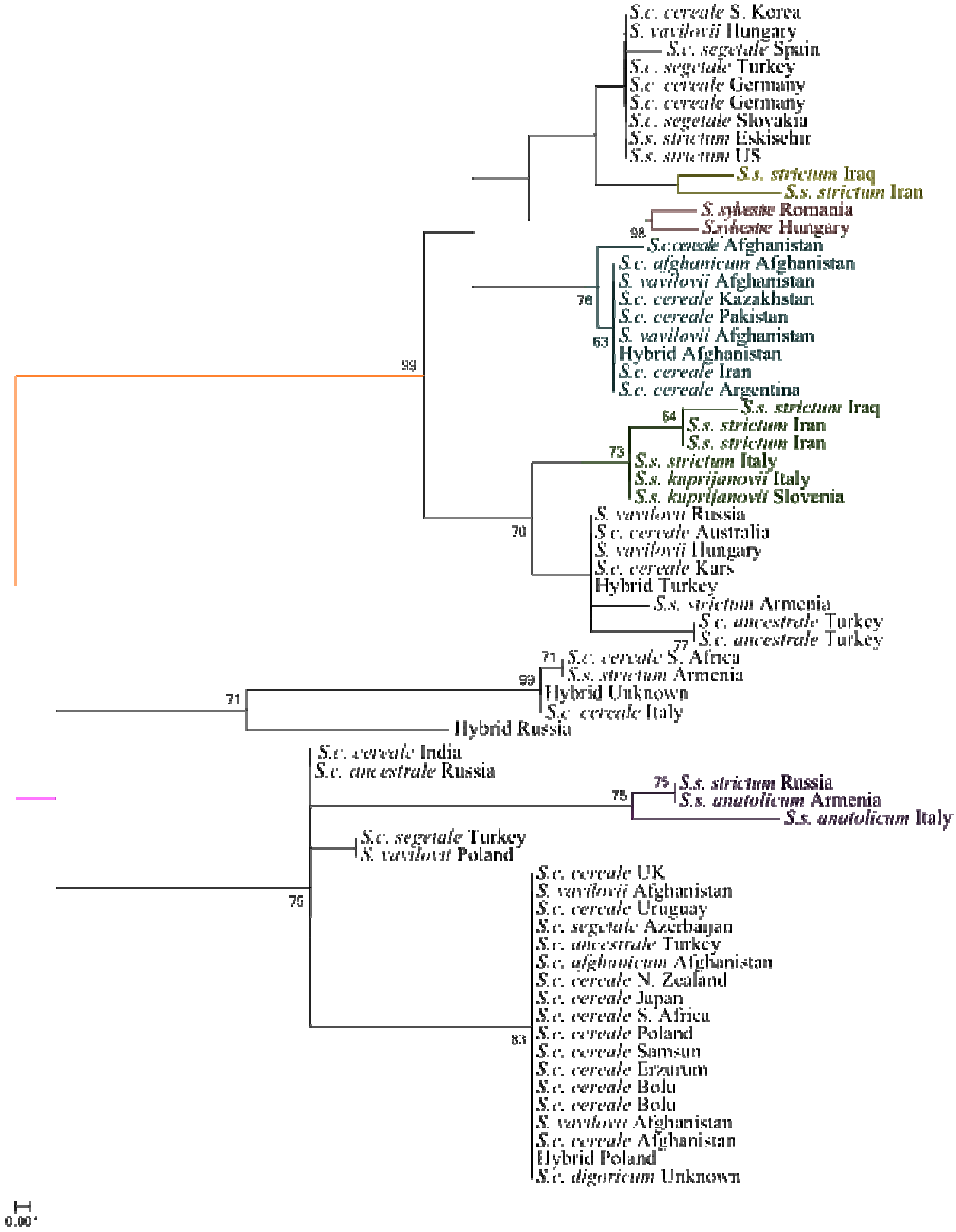

Figure 5.25. Maximum likelihood phylogenetic tree based on the nuclear GBS0551 region.

6. DISCUSSION

6.1. Taxonomy and Phylogeny of Genus *Secale*

In 1917 Vavilov published a paper titled “On the origin of cultivated rye”. About 50 years later, Stutz (1972) published another paper under the same title to point out that the phylogeny of the genus *Secale* is still contradictory. Today despite the availability of sophisticated molecular methods, the classification of taxa in the genus *Secale* and phylogenetic relationship of different species remains, obscure and nearly all of the taxa in the genus have been proposed as the ancestor of cultivated rye at some point (Hammer et al., 1987; Hammer, 1990; Frederiksen and Petersen, 1998). In this study, in order to better understand the phylogenetic relationships between different species and subspecies of *Secale*, 142 different accessions were investigated using nuclear SSRs, nuclear SNP and a chloroplast SNP.

The clear separation of *S. strictum* ssp. *strictum* accessions from northwest and west of Iran, from the rest of the *S. strictum* ssp. *strictum* accessions and other *S. strictum* subspecies in the STRUCTURE analysis indicated that this subspecies is genetically different. Considering that subspecies of *S. strictum* have a relatively wide geographical distribution, it is expected that different accessions may have adapted to various environmental conditions. Furthermore restriction of gene flow between *S. strictum* ssp. populations from and outside of Iran may have contributed to the observed diversification, due to geographical separation of the accessions. *S. strictum* ssp. *strictum* is significantly different from its subspecies (Shang et al., 2006; Hammer, 1990; Cuadrado and Jouve, 2002), indicating that *S. strictum* ssp. *strictum* has been evolving independently of other *S. strictum* subspecies (Acherem et al., 2014). The divergence of *S. strictum* ssp. *strictum* samples from Iran and rest of subspecies partly supports this idea. In the UPGMA dendogram constructed, the ancestral position of this group compared to the rest of *S. strictum* ssp. *strictum* samples was observed with high bootstrap support. The same dendogram also revealed that rest of the *S. strictum* ssp. *strictum* samples (*i.e.* other than the Iranian clade) originating from diverse areas, are closer to *S. cereale* accessions. This is

compatible with the hypothesis that cultivated rye evolved from *S. strictum* (Vavilov, 1917; 1922; Rilley, 1955; Khush and Stebbins, 1961). The STRUCTURE analysis also grouped *S. strictum* ssp. *strictum* samples out of Iran, *S. strictum* ssp. *kuprijanovii* and *S. strictum* ssp. *anatolicum* samples together, indicating there is gene flow between these taxa. This is also supported by F_{ST} comparisons based on microsatellite data and positioning of *S. strictum* subspecies accessions within *S. strictum* ssp. *strictum* accessions in maximum likelihood tree constructed using nuclear SNPs. Furthermore in the dendogram constructed based on microsatellite data *S. strictum* ssp. *anatolicum* and *S. strictum* ssp. *strictum* accessions originating from out of Iran were found to be closer to S. *cereale* subspecies compared to *S. strictum* ssp. *kuprijanovii* samples and *S. strictum* ssp. *strictum* accessions originating from Iran. This suggests existence of gene flow between *S. cereale* subspecies and *S. strictum* subspecies originating from outside of Iran.

Although the STRUCTURE analysis conducted using *S. cereale* samples only exhibited two separate clusters, these clusters did not correspond to subspecies delimitations, despite their clear separation. This is indicative of gene flow between the four *S. cereale* subspecies investigated in this study. This was also supported by the observation that several haplotypes in the haplotype network based on *ndhF-rpl32* intergenic region sequences were shared between *S. cereale* subspecies. The same pattern was apparent in maximum likelihood tree constructed using nuclear SNPs, where all of the *S. cereale* subspecies were grouped together. On the other hand the pairwise F_{ST} comparisons revealed that *S. cereale* ssp. *afghanicum* was genetically different from rest of the *S. cereale* subspecies. This differentiation was reinforced by UPGMA dendogram, in which *S. cereale* ssp. *afghanicum* was separated from rest of *S. cereale* subspecies with %100 support, and basal to the other clades containing various *S. cereale* and *S. strictum* subspecies. Based on the same dendogram *S. ancestrale* was seen to be basal to the clade that included *S. cereale* ssp. *cereale, S. vavilovii*, *S. cereale* ssp. *segetale*, *S. strictum* ssp. *anatolicum* and *S. strictum* ssp. *strictum* accessions originating from outside of Iran. The taxonomic position of *S. cereale* ssp. *ancestrale* had been disputed in the past. It was classified as separate species (Roshevitz, 1947; Pozo et al., 1995), whereas some other authors classified it as subspecies of *S. cereale* (Nürnberg-Krüger, 1960, Vences et al., 1987; Frederiksen and Petersen, 1998). Although the results revealed that *S. cereale afghanicum* and *S. cereale* ssp. *ancestrale* are genetically divergent from and basal to other

S. cereale subspecies, more conclusive taxonomic recommendations should be made using additional genetic markers.

From a taxonomical point of view, the classification of *S. vavilovii* as a species or a subspecies has also been contradictory. In some studies *S. vavilovii* was considered to be a distinct species close to *S. cereale* (Khush, 1962; Cuadrado and Jouve, 1997; Hammer et al., 1987; Hammer, 1990; Del Pozo et al., 1995; Chikmawatiet al., 2005; De Bustos and Jouve, 2002). On the other hand, some researchers postulated that *S. vavilovii* should be classified as a subspecies of *S. cereale* (Frederiksen and Petersen, 1998; Shang et al., 2006; Cuadrado and Jouve, 2002; Achrem et al., 2014; Ren et al., 2011). In the STRUCTURE analysis conducted for this taxon only, no structuring was observed indicating *S. vavilovii* accessions are similar to each other, despite their relatively large geographic separation, from Italy to Afghanistan. Furthermore the STRUCTURE analysis conducted for the whole *Secale* genus revealed no separation between *S. cereale* subspecies and *S. vavilovii*, indicating that there is no clear genetic separation between these two species. The UPGMA tree constructed using microsatellite data, in which *S. vavilovii* was found to be closer to *S. cereale* ssp. *cereale* than to *S. cereale* ssp. *afghanicum* and *S. cereale* ssp. *ancestrale* was also consistent with STRUCTURE results. This finding was also supported by the placement of *S. vavilovii* accessions within *S. cereale* subspecies in the maximum likelihood tree constructed using nuclear SNPs. Finally, pairwise comparisons of F_{ST} values based on *ndhF-rpl32* intergenic region and microsatellite data did not reveal any significant differentiation between *S. cereale* and *S. vavilovii*. Therefore based on obtained results of this study, *S. vavilovii* should be considered as a subspecies of *S. cereale*.

Pairwise comparisons of F_{ST} values based on *ndhF-rpl32* intergenic region and microsatellite data affirmed that *S. sylvestre* is genetically the most divergent species. These finding are consistent with the general agreement that *S. sylvestre* is the first species that diverged from *S. strictum* during the Pliocene (Stutz, 1972; Skuza et al., 2007; Achrem et al., 2013). These results are consistent with *S. sylvestre* being the morphologically and genetically the most distinct species, compared to the other annual species, observed in previous studies (Stutz, 1972; Khush and Stebbinz, 1961; Vences et al., 1987; Murai et al., 1989; Reddy et al., 1990; Petersen and Doebley, 1993; Skuza et al., 2007; Cuadrado and Jouve, 1997; Shang et al., 2006).

In the STRUCTURE analyses of whole genus, three distinct clusters were observed, indicating presence of three different gene pools. The first cluster was composed of only perennial taxa, *S. strictum* ssp. *strictum* subspecies indicating a clear separation between annual forms and perennial *S. strictum* ssp. *strictum*. Divergence between perennial *S. strictum* species from annual taxa had been shown previously based on rDNA spacer-length variation (Reddy et al., 1990), rDNA ITS sequences (De Bustos and Jouve, 2002), and AFLP approaches (Chikmawati et al., 2005). It was noteworthy that on the UPGMA dendogram *S. strictum* ssp. *strictum* samples that originated from Iran were seen to be basal to the clade that included the rest of *S. strictum* subspecies and all of the annual taxa, except for *S. cereale* ssp *afghanicum* and *S. sylvestre*. This is consistent with previous studies that show *S. strictum* being the ancestral species and rest of the taxa having originated from it (Khush and Stebbins, 1961; Khush, 1962; Zohary, 1971; Vences et al., 1987; Hammer et al., 1987; Hammer, 1990; Jones and Flavell, 1982). This finding also underpins the hypothesis that Northeastern Turkey and the adjacent area including Armenia and northwestern Iran could be the center of origin for the genus (Sencer and Hawkes, 1980; Zohary and Hopf, 2000; Willcox, 2005). In the same analysis, further separation of annual taxa belonging to different species or subspecies into two groups based on eco-geographical, rather than taxonomic origin, was observed, indicating the structuring in the annual taxa does not correspond to taxonomic delimitations.

Previous studies have shown high morphological (Frederiksen and Petersen 1997) and molecular similarity (De Bustos and Jouve, 2002; Shang et al., 2006; Hammer, 1990; Ren et al., 2011) between annual wild and weedy forms (*eg, S. vavilovii, S. cereale* ssp. *ancestrale, S. cereale* ssp. *afghanicum,* and *S. cereale* ssp. *segetale*) and *S. cereale* ssp. *cereale*. The obtained results also supported that *S. cereale* subspecies (including *S. vavilovii*) are so similar to each. This can be explained by hypothesis that *S. cereale* is considered to have a relatively recent origin, dating back to only a few centuries ago (Vavilov, 1917; Schiemann, 1948; Schwanitz, 1966). Evolution by natural selection is known to be a gradual process that can require long time frames to show its effects (Fisher, 1930). On the other hand, compared to their wild relatives, crop plants are believed to have evolved much more rapidly. Theoretically, the time required for domestication is estimated as a few hundred generations (Eyre-Walker et al., 1998; Rieseberg et al., 2002). However, Harlan (1992) reported domestication, especially the early stages of the process to be very

slow and transition from wild to domesticated forms could take thousands of years. Moreover, based on archeological data, Tanno and Willcox (2006) also supported Harlan's hypothesis. As a result, it is likely that there was insufficient time for the evolution of isolation mechanisms or barriers between cultivated rye, and its wild and weedy relatives. Consequently, the lack of structuring among annual taxa can be explained by introgression between cultivated rye and its weedy relatives. Interbreeding between different taxa, except for *S. sylvestre* and subsequent formation of hybrids with high pollen and seed fertility is very common in *Secale* genus (Khush and Stebbins; 1961; Khush, 1962, 1963a, b). This indicates that crossing barriers are not strong enough and genetic material can be exchanged between sympatric populations of cultivated rye and wild and weedy forms. Therefore high degree of gene flow even between species due to the lack of isolating mechanisms may have led to the lack of divergence.

6.2. Genetic Diversity and Genetic Differentiation of Cultivated Rye

6.2.1. Genetic Diversity

6.2.1.1. Genetic Diversity Levels in *Secale cereale*. The assessment of genetic diversity is very important for plant breeding and marker assisted selection of desirable traits, such as resistance to biotic and abiotic stress, water use efficiency, and evolutionary studies (Gonzalez-Astorga and Castillo-Campos, 2004). Furthermore considering global environmental change, determination of genetically diverse populations is crucial for defining management and conservation strategies to support future global human food security (Altieri, 1999). In plant species, in a similar manner to animals, genetic diversity can be maintained and genetic differentiation between populations of can be affected by specific characteristics like mating systems, gene flow, introgression and drift. Mating system is one of the major determinants of population structure in plant populations (Allard et al., 1975; Schoen and Brown, 1991), and existence of high gene flow between different populations of allogomous plants is a very well known phenomenon (Hamrick and Godt, 1989, Delye et al., 2010). Furthermore gene flow by introgression between crops and their wild and weedy relatives (Ellstrand, 1999; Simard, 2010) have substantial evolutionary impact on genetic diversity.

The analysis, conducted in order to understand the extent and partitioning of diversity of cereal rye at a global scale, revealed that the genetic diversity of cultivated rye as measured by different genetic diversity parameters at species and population levels were high, compared to other allogamous crops like sorghum (Cuevas and Prom, 2013) and maize (Liu et al., 2003). Previous studies investigating genetic diversity of cultivated rye by AFLP markers (Chikmawati, 2003), isoenzyme assays (Ramirez and Pisabarro, 1986; Persson and Bothmer, 2000; 2002; Matos et al., 2001) and RADP markers (Matos et al., 2001; Persson et al., 2001; Ma et al., 2004) also reported comparably high levels of genetic diversity. Cultivated rye is a wind pollinated allogamous species with a highly developed self-incompatibility system. Considering that gene flow is one of the most important evolutionary mechanisms increasing genetic diversity (Anderson et al., 2011), the observed genetic diversity rates in this study can be attributable to out-crossing nature of the *Secale*

species. Furthermore introgression between cultivated rye, and weedy and wild forms in the genus *Secale* is very common in the nature (Khush and Stebbins, 1961; Khush, 1962, 1963a, b), also increasing genetic diversity. Moreover, due to its high tolerance of different environmental conditions, rye has a broad geographic distribution. Considering genetic diversity is a result of environmental heterogeneity, the wide distribution may also contribute to high levels of genetic diversity.

6.2.1.2. Geographical Distribution of Genetic Diversity. In the scope of the present study, genetic diversity levels of different gene pools were compared. Genetic diversity of cultivated rye was found to be remarkably higher in the Middle East region (Turkey, Iran and Israel) compared to other regions. Although sample size of the region is larger than the others, to avoid any bias due to sample size, corrected genetic diversity measures (independent of sample size) were also used. The degree of genetic diversity was found to be highest in the Middle East for the corrected parameters, as well. Therefore it was concluded that obtained results reflect the real genetic diversity patterns of the region, rather than being a sampling artifact. Taking into account that many wild and weedy forms of the genus *Secale* inhabits in the area between Northeastern Turkey and northwestern Iran, gene flow between wild forms and cultivated forms by introgression is quite possible, resulting in an increase in genetic diversity. Furthermore, the topographic and geomorphologic features and climatic conditions exhibit significant variety in the region. High genetic diversity in the region can also be explained by environmental heterogeneity in the region. Another explanation for high genetic diversity observed in the region is that most of the populations in this region are landraces. Landraces are cultivated by traditional agricultural practices through many generations of selection, and they have become locally adapted to various environments by accumulating new alleles (Lanteri and Barcaccia, 2006). Therefore compared to modern cultivars, the genetic diversity of landraces of *Secale cereale* ssp. *cereale* are high.

Genetic diversity in South-Central Asia and East Asia regions was considerably low compared to Middle East. Besides, genetic diversity was lower in North America that contains populations from Mexico, USA and Canada. This probably stems from extensive use of genetically uniform cultivars in these regions. On the other hand, genetic diversity of Balkan group (that contained samples from European part of Turkey (Thrace),

Montenegro, Serbia, Macedonia, Yugoslavia, and Bosnia and Herzegovina) and Caucasus gene pool (that consists of two accessions from Georgia and East Azerbaijan) was high. Finally, the European gene pool contains samples from a wide geographical range containing Germany, Switzerland, UK, Poland and Sweden. Although agricultural systems of many countries in Europe favor the genetically uniform cultivars (Hammer et al., 2003), genetic diversity levels were found to be moderately high. This phenomenon can be explained by diverse origins of the European populations.

6.2.2. Genetic Differentiation

Genetic differentiation among populations of each geographical region was evaluated by G_{ST} values, which can be regarded as an indirect measure of gene flow between populations. Gene flow among populations homogenizes genetic structure by decreasing genetic differentiation (Anderson et al., 2011). Therefore higher rate of genetic differentiation is expected as gene flow is restricted. The genetic differentiation among populations was high within Balkans and North America, compared to other regions, indicating lower rates of gene flow.

Furthermore, genetic differentiation among geographical regions measured by pairwise F_{ST} values revealed a significant differentiation between African gene pool and remaining gene pools. Considering climatic conditions of the region being discrete and that the region is physically separated from remaining gene pools by geographical barriers, it can be concluded that rye became locally adapted to the continent and remained separated. This is consistent with the idea that *S. cereale* ssp. *cereale* evolved as an isolated population in Africa (Sencer and Hawkes, 1980). Similarly based on AFLP data, Chikmawati (2003) previously reported that African populations of cultivated rye were genetically more distant when compared to other populations.

6.3. Phylogeography of Cultivated Rye (*S. cereale* ssp. *cereale)*

The results obtained from the microsatellite analysis showed that the structuring in *S. cereale* corresponds to spatial distribution of samples, and prove the presence of two genetically distinct lineages. The first cluster (A), was mainly composed of samples from the Middle East and South-Central Asia. The second cluster (B) contains samples that originated from outside of Asia, mainly Europe. The samples having mixed ancestry had a worldwide distribution (this can be considered as a third cluster). Based on the results obtained from the study, it can be speculated that each of the two clusters obtained in the study originated from two distinct gene pools. Subsequently, the two main distinctive lineages retrieved in this study were initially separated probably due to restriction of gene flow because of geographical separation. Considering that in crop plants geographical distribution patterns reflect prevailing human mediated selection pressures in a particular environment (Hawtinet al., 1997), another explanation for this separation could be due to cultivated rye having been introduced into new geographical ranges in which climatic and environmental conditions are quite different compared to center of origin. This was possibly followed by anthropogenic selection of adaptable phenotypes to the conditions in those regions, leading to adaptive divergence.

The Middle Eastern samples were observed in all three clusters, indicating their potential ancestral position. Furthermore in the haplotype network, based on the *ndhF-rpl32* intergenic region, Middle East and South-Central Asia haplotypes are the most common. Based on these data, it can be speculated that the Middle Eastern populations are progenitors of cultivated rye and they recently expanded globally due to human mediated distribution and long-distance gene flow.

In the context of the study, the origin of the samples collected from outside of Asia was also investigated. Samples from South America clustered together with European samples into Cluster B. This is consistent with the idea that many crop plants dispersed to South America from Europe, after the voyages of Colombus (Ceccarelli, 2009). On the other hand, samples from Australia grouped into cluster A, indicating that cultivated rye was possibly introduced into Australia from the Middle East or South-Central Asia. Furthermore North American samples, originated from both clusters.

6.3.1. Geographical Origins of Cultivated Rye (*S. cereale* ssp. *cereale)*

Different theories exist on geographical origin of genus *Secale*. It was proposed that cultivated rye may have emerged independently from the two gene centers: it was first cultivated in Transcaucasia, Afghanistan and Turkestan (Vavilov, 1926). This was followed by cultivation of the varieties in northeastern Kazakhstan, Uzbekistan and northern Transcaucasia (Vavilov, 1926; Khush, 1963b). Therefore, many authors have postulated that all *Secale* taxa have originated somewhere in the Middle East or South-Central Asia (Sencer and Hawkes, 1980; Zohary and Hopf, 2000; Willcox, 2005) that also covers the geographical area known as "Fertile Crescent", the center of origin for many crop species like wheat, barley, pulses, pea and flaxes (Gopher et al., 2002; Zohary and Hopf, 2000; Brown et al., 2008).

Genetic diversity of crop species on interspecific and intraspecific level is not evenly distributed: the genetic diversity in the center of origin is higher (Vavilov, 1926). Comparing genetic diversity levels of Middle East and South-central Asia, our results indicate that most likely origin of cultivated rye was the Middle East and humans have recently distributed cultivated rye to other parts of the world leading to long-distance gene flow.

This was probably followed by dispersal to the rest of the world from this regional center by humans migrating to out of Middle East. Two migration routes were proposed: first was from Middle East to Europe across the Balkan Peninsula (Kornicke, 1885; Schultz, 1911; Regel, 1922; Popov, 1939; Deodikar, 1963). The second hypothesis suggests that rye was carried from the Middle East to Europe through Transcaucasia (Vavilov 1917 and Schiemann, 1932). In our study, genetic diversity of Balkan group was high. Furthermore, the genetic differentiation between Balkan populations vs. the Middle East and European populations was low. This might indicate that Balkan populations originated from the Middle East. Crop plants disperse from their center of origin by movement of people, which is known as demic diffusion (Ammerman and Cavalli-Sforza, 1984). During and after Bronze Age several human migrations have occurred from the Middle East to Europe via the Balkan Peninsula. These migrating communities may have brought cultivated rye from different origins. This might also explain the relatively high

total genetic diversity observed in the region, and this is consistent with the hypothesis that rye dispersed from Anatolia across the Balkan Peninsula into north/central Europe (Kornicke, 1885; Schultz, 1911; Regel, 1922**;** Popov, 1939; Deodikar 1963). Genetic diversity of the cultivated rye in Caucasus, the other proposed migration route for dispersal of rye from Middle East to Europe (Vavilov, 1917; Schiemann, 1932) was lower compared to Balkans. Although genetic similarity between this region and Europe and Middle East was high, based on recent excavation reports from Neolithic sites in Slovakia (Tempir, 1966; 1969; Behre, 1962), it is more probable that cultivated rye arrived in Europe via Balkans, not through the Caucasus.

7. CONCLUSION

In this study, a large scale analysis of genetic diversity and phylogenetic relationships of *Secale* genus was undertaken. A total of 727 genotypes from 142 different accessions of *Secale* genus representing the *S. cereale*, *S. vavilovii*, *S. strictum* and *S. sylvestre* from different eco-geographical areas of the world with a concentrated focus on Turkey and Middle East were investigated using nuclear SSR and SNP markers, and a chloroplast SNP marker.

The SSR markers separated perennial *S. strictum* subspecies from annual taxa. Further separation of annual taxa belonging to different species or subspecies into two groups based on eco-geographical origin was also observed, suggesting existence of geographical structuring. Separation of Asian accessions from remaining samples was also observed. The lack of any structuring within different species or subspecies belonging to annual taxa can be explained by recent separation of the species or subspecies, insufficient time for the evolution of isolation mechanisms and consequent gene flow even between species. Furthermore the lack of a clear genetic separation between *S. cereale* and *S. vavilovii* led us to conclude that *S. vavilovii*, rather than a distinct species, should be classified as a subspecies of *S. cereale*. The phylogenetic relationship of different species in the genus should be investigated in greater detail using high resolution molecular markers such as RAD sequencing.

The evaluation of genetic diversity of cultivated rye populations from different geographical origins by microsatellite markers led us to conclude that a significant amount of genetic variation exists in cultivated rye. The highest allelic variation and genetic diversity was found in Middle Eastern landrace populations. This finding supports the idea that the area could be the center of origin for the genus. Nearly all of the populations examined in Near East are locally adapted landraces that have not been exposed to intense artificial selection pressure. Therefore in contrast to modern crop varieties that have undergone a genetic bottleneck associated with the process of domestication, resulting in a decrease in genetic diversity, landraces constitute a large pool of genetic variation and

contain many interesting traits like strong tolerance to abiotic and biotic stresses (Zeven, 1998). Considering high genetic diversity in crop plant populations is directly related to adaptive potential of those populations to changing environmental conditions, landraces should be regarded as genetic resources reservoirs for new niches and future breeding programs. From a conservation point of view, the results obtained from the study suggest that an immediate action plan is required for in-situ conservation of Middle Eastern landrace populations.

8. REFERENCES

Achrem M., Kalinka, A., Rogalska, S.M., 2014. Assessment of genetic relationships among *Secale* taxa by using ISSR and IRAP markers and the chromosomal distribution of the AAC microsatellite sequence. Turkish Journal of Botany 38, 221-225.

Agarwal, M.K., Jaiswal J.P., Bhatt U., 2013. Molecular analysis of wheat genome by the use of random amplified polymorphic DNA (RAPD) markers. Indian Journal of Agricultural Research, 47,

Akkaya, M.S., Bhagwat, A.A., Cregan, P.B., 1992. Length polymorphisms of simple sequence repeat DNA in soybean. Genetics, 132, 1131-1139.

Alix, K., Heslop-Harrison, J. S., 2004. The diversity of retroelements in diploid and allotetraploid *Brassica* species. Plant Molecular Biology, 54, 895–909

Allard, R.W., Kahler. A.L., Clegg, M.T., 1975. Isozymes in plant population genetics. Ninth eds., Academic Press, New York., 47-58.

Almgard, G., Landegren, V., 1974. lsozymatic variation used for the identification of barley cultivars. Z. Pflanzenztichttmg 72, 63-73.

Althoff, D. M., Gitzendanner, M. A., Segraves, K. A., 2007. The utility of amplified fragment length polymorphisms in phylogenetics: a comparison of homology within and between genomes. Systematic Biology, 56, 477–484.

Altieri, M.A., 1999. The ecological role of biodiversity in agroecosystems Agriculture, Ecosystems and Environment, 74, 19-31.

Altıntas, S., Toklu, F., Kafkas, S., Kilian, B., Brandolini, A., Özkan, H., 2008. Estimating genetic diversity in durum and bread wheat cultivars from Turkey using AFLP and SAMPL markers. Plant Breeding, 127, 9–14.

Ammerman, A., J., Cavalli-Sforza, L., L., 1984. The neolithic transition and the genetics of populations in Europe. Princeton University Press, Princeton

Amos, W., Hoffman, J.I., Frodsham, A., Zhang, L., Best, S., Hill, A.V.S., 2007. Automated binning of microsatellite alleles: problems and solutions. Molecular Ecology Notes, 7, 10–14.

Andeden, E.E., Baloch, F.S., Derya, M., Kilian, B., Özkan H., 2012. iPBS-Retrotransposons-based genetic diversity and relationship among wild annual Cicer species. Journal of Plant Biochemistry and Biotechnology, 22, 453-466.

Anderson, J.A., Churchill, G.A., Autrique, J.E., Tanksley, S.D., Sorrells, M.E., 1993. Optimizing parental selection for genetic linkage maps. Genome, 36. 181-186.

Anderson, J.T., Willis, J.H., Mitchell-Olds, T., 2011 Evolutionary genetics of plant adaptation. Trends in Genetics, 27, 258–266.

Avise, J.C., 1994. Molecular markers, natural history and evolution. New York Chapman and Hall.

Badr, A., Muller, K., Schafer-Pregl R., El Rabey, H., Effgen, S., Ibrahim, H.H., Pozzi C., Rohde, W., Salamino, F., 2000. On the origin and domestication of barley (*Hordeum vulgare*). Molecular Biology and Evolution, 17, 499–510.

Bar-Yosef, O., 1998. The Natufian culture in the Levant, threshold of the origin of agriculture. Evolutionary Anthropology, 6, 159–177.

Barrett, B.A., Kidwell, K.K., 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Science, 38, 1261–1271.

Barnes, P., Putnam, A. R., 1983. Rye residues contribute weed suppression in no-tillage cropping systems. Journal of Chemical Ecology, 9, 1045-1057.

Battisti, D.S., Naylor R. L., 2009. Historical warnings of future food insecurity with unprecedented seasonal heat. Science 323, 240–244.

Becker, J., Heun, M., 1995. Barley microsatellites: Allele variation and mapping. Plant Molecular Biology, 27, 835–845.

Behre, K.E., 1992. The history of rye cultivation in Europe. Vegetation History and Archeobotany, 1, 141-156.

Bellon, M. R., Adato, M., Becerril, J., Mindek D., 2006. Poor farmers' perceived benefits from different types of maize germplasm: the case of creolization in lowland tropical Mexico. World Development 34, 113–129.

Benito, C., Perez de la Vega, M., Salinas, J., 1980. The inheritance of wheat kernel peroxidases. Journal of Heredity, 71, 416-418.

Benito, C., Silva-Navas, J., Fontecha, G., Hernández-Riquer, M. V., Eguren, M., Salvador N., Gallego, F. J., 2009. From the rye Alt3 and Alt4 aluminum tolerance loci to orthologous genes in other cereals. Plant and Soil, 327, 107-120.

Bento, M., Pereira, H.S., Rocheta, M., Gustafson, P., Viegas, W., Silva, M., 2008. Polyploidization as a retraction force in plant genome evolution: sequence rearrangements in triticale. Plos One, 3, e1402.

Benz, B., 2012. The Conservation of Cultivated Plants. Nature Education Knowledge 3, 4-10.

Bolibok, H., Rakoczy-trojanowska, M., Wyrzykowska, M., Radecka, M., Orczyk, W., 2006. Identification of microsatellite markers in the rye genome. Cellular and Molecular Biology Letters, 11, 291-298.

Bot, A.J., Nachtergaele, F.O., Young A., 2000. Land Resource Potential and Constraints at Regional and Country Levels. Food and Agricultural Organization of the United Nations, Rome.

Botstein, D., White, R. L., Skolnick, M., Davis, R. W., 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American. Journal of Human Genetics, 32, 314–331.

Boyko, E., Kalendar, R., Korzun, V., Gill, B., Schulman, A.H., 2002. Combined mapping of *Aegilops tauschii* by retrotransposon, microsatellite, and gene markers. Plant Molecular Biology, 48, 767–790.

Branco, C.J.S., Vieira, E.A., Malone, G., Kopp, M. M., Malone, E., Bernardes, A., Mistura, C.C., Carvalho, F.I.F., Oliveira, C.A., 2007. IRAP and REMAP assessments of genetic similarity in rice. Journal of Applied Genetics 48, 107-113.

Brink, M., 2006. *Secale cereale* L. Record from Protabase. In: Brink M, Belay G (eds) PROTA (Plant Resources of Tropical Africa/Ressources ve´ge´tales de l'Afrique Tropicale). Wageningen, Netherlands.

Brown, T.A., Jones, M.K., Powell, W., Allaby, R.G., 2008. The complex origins of domesticated crops in the Fertile Crescent. Trends in Ecology and Evolution, 24, 103-109.

Brush, S.B., 1993. Indigenous Knowledge of Biological Resources and Intellectual Property Rights: The Role of Anthropology. American Anthropologist, 653-671.

Bryan, G.J., Collins, A.J., Stephenson, P., Orry, A., Smith, J.B., Gale, M.D., 1997. Isolation and characterisation of microsatellites from hexaploid bread wheat. Theoretical and Applied Genetics, 94, 557-563.

Burger, J.C., Lee, S., Ellstrand, N.C., 2006. Origin and genetic structure of feral rye in the Western United States. Molecular Ecology, 15, 2527–2539.

Bushuk W., 2001. Rye production and uses worldwide. In Bushuk W (Eds) Rye: production, chemistry, and technology, Second edition, 1–11. American Association of Cereal Chemists, USA.

Ceccarelli S., 1989. Wide Adaptation. How Wide? Euphytica, 40, 197-205.

Ceccarelli S., 2009. Evolution, plant breeding and biodiversity. Journal of Agriculture and Environment for International Development, 103, 131-145.

Chao, S., Sharp, P.J., Worland, A.J., Warham, E.J., Koebner, R.M.D., Gale, M.D., 1989. RFLP-based genetic maps of wheat homeologous group 7 chromosomes. Theorotical Applied Genetics, 78, 495-904.

Charles, M., Belcram, H., Just, J., Huneau, C., Viollet, A., Couloux, A., Segurens, B., Carter, M., Huteau, V., Coriton, O., Appels, R., Samain, S., Chalhoub, B., 2008. Dynamics and differential proliferation of transposable elements during the evolution of the B and A genomes of wheat. Genetics 180, 1071-1086.

Chikaiza, O., Khan, I.A. Zhang, X., Brevis, J.C., Jackson, L., Chen, X., Dubcovsky, J.D., 2006. Registration of five wheat isogenic lines for leaf rust and stripe rust resistance genes. Crop Science, 46, 485-487.

Chikmawati, T., 2003. Phylogenetic relationships in *Secale* and pcr-based est mapping in wheat. PhD Thesis, University of Missouri.

Chikmawati, T., Skovmand, B., Gustafson J.P. 2005. Phylogenetic relationships among *Secale* species revealed by amplified fragment length polymorphisms. Genome, 48, 729-801.

Ching, A., Caldwell, K.S., Jung, M., Dolan, M., Smith, O.S., Tingey, S., Morgante, M., Rafalski, A.J., 2002. SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbredlines. BMC Genetics, 3, 19-32.

Cho, R.J., Mindrinos, M., Richards, D.R., Sapolsky, R.J., Anderson, M., et al., 1999. Genome-wide mapping with biallelic markers in *Arabidopsis thaliana*. Nature Genetics 23, 203–207.

Clegg, M.T., 1990. Dating the monocot–dicot divergence. Trends in Ecology and Evolution, 5, 1-2.

Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology, 9, 1657-1659.

Cohen, J.I., Williams, J.T., Plucknett, D.L. and Shands, H., 1991. Ex situ conservation of plant genetic resources: global development and environmental concerns. Science 253, 866-72.

Collins, N.C., Shirley, N.J., Saeed, M., Pallotta, M., Gustafson, J.P., 2008. An ALMT1 Gene Cluster Controlling Aluminum Tolerance at the Alt4 Locus of Rye (*Secale cereale* L.). Genetics, 179, 669–682.

Condit, R., Hubbell, S.P., 1991. Abundance and DNA sequence of two-base repeat regions in tropical tree genomes. Genome, 34, 66-71.

Corriveau, J.L., Coleman, W., 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. American Journal of Botany, 75, 1443–1458.

Coulon, A., Fitzpatrick, J.W., Bowman, R., Stith, B.M., Makarewich, C.A., et al., 2008.

Congruent population structure inferred from dispersal behaviour and intensive genetic surveys of the threatened Florida scrub-jay (*Aphelocoma coerulescens*). Molecular Ecology, 17, 1685-1701.

Cox, P.M., Betts, R.A., Jones, C.D., Spall, S.A., Totterdell, J.I, 2000. Acceleration of global warming due to carbon-cycle feedbacks in a coupled climate model. Nature, 408, 184-187.

Craig, N.L., Craigie, R., Gellert, M., Lambowitz, A.M., 2002. Mobile DNA II. ASM Press, Washington, USA.

Cuadrado, A., Jouve, N. 1997. Distribution of highly repeated DNA sequences in species of the genus *Secale. Genome*, 40, 309-317.

Cuadrado, A., Jouve, N**.,** 2002. Evolutionary trends of different repetitive DNA sequences during speciation in the genus *Secale*. The American Genetic Association, 93, 339–345.

Cuevas, H.E., Prom, L.K., 2013. Assessment of molecular diversity and population structure of the Ethiopian sorghum [*Sorghum bicolor* (L.) Moench] germplasm collection maintained by the USDA–ARS National Plant Germplasm System using SSR markers. Genetic Resources and Crop Evolution, 60, 1817-1830.

Culvenor, R.A., Oram, R.N., Groth, C., 1985. Variation of tolerance in *Phalarisaquatica* L. and related species to aluminium in nutrient solution and soil. Australian Journal of Agricultural Research, 36, 383–395.

De Bustos, A., Jouve N., 2002. Phylogenetic relationships of the renus *Secale* based on the characterization of rDNA ITS sequences. Plant Systematics and Evolution, 235, 147-154.

De Candolle, A., 1882. Origine des Plantes Cultivees. Germer Bailliere, Paris.

De Candolle, A., 1885. Origins of cultivated plants. Second edition, D. Appleton, New York.

Delhaize, E., Ryan, P. R., 1995. Aluminium toxicity and tolerance in plants. Plant Physiology, 107, 315–321.

Delhaize E., Ryan, P. R., Randall, P. J., 1993. Aluminum tolerance in wheat (*Triticum aestivum L.*): Aluminum-stimulated excretion of malic-acid from root apices. Plant Physiology, 103, 695–702.

Délye, C., Clément, J.A.C., Pernin, F., Chauvel, B., Le Corre, V.,2010. High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level. Basic and Applied Ecology, 11, 504-512.

Deodikar, G.B., 1963. Rye: *Secale cereale* Linn. Indian Council of Agricultural Research, New Delhi.

Doebley, J.F., Gaut, B.S., Smith, B.D., 2006. The Molecular Genetics of Crop Domestication. Cell, 127, 1309–1321.

Doyle J.J., Doyle J.L., 1990. Isolation of plant DNA from fresh tissue. Focus, 12:13-15.

Doyle, J.J., 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Systematic Botanny, 17, 144-163.

Duke, J.A., 1978, The quest for tolerant germplasm, ASA Special Symposium, 32, Crop tolerance to suboptimal land conditions. Madison, 1–61.

Earl, D.A., Von Holdt, B.M., 2012. Structure harvester: a website and program for visualizing structure output and implementing the Evanno method. Conservation Genetic Resources, 4, 359-361.

Ellegren, H., 2004. Microsatellites: simple sequences with complex evolution. Nature Reviews Genetics, 5, 435-445.

Ellis, R.P., McNicol, J.W., Baird, E., Booth, A., Lawrence, P., 1997. The use of AFLPs to examine genetic relatedness in barley. Molecular Breeding, 3, 359-369.

Ellis, T.H.N., Poyser, S.J., Knox, M.R., Vershinin, A.V., Ambrose, M.J., 1998. Ty1-copia class retrotransposon insertion site polymorphism for linkage and diversity analysis in pea. Molecular Genomics and Genetics, 260, 9-19.

Ellstrand, N.C., Prentice, H.C., Hancock, J.F., 1999. Gene flow and introgression from domesticated plants into their wild relatives. Annual Review of Ecoogy, Evolution and Systematics, 30, 539-563.

Ennos, R.A. et al., 1999. Using organelle markers to elucidate the history, ecology and evolution of plant populations. In Hollingsworth, P.M. (Ed.), Molecular Systematics and Plant Evolution, 1–19, Taylor and Francis, London.

Etterson, J. R., 2004a. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. Clinal patterns of selection along an environmental gradient in the Great Plains. Evolution, 58, 1446-1458.

Etterson, J.R. 2004b. Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change II. Genetic architecture of three populations reciprocally planted along an enivronmental gradient in the Great Plains. Evolution, 58:1459-1471.

Evanno, G., Regnaut S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE : a simulation study. Molecular Ecology, 14, 2611-2620.

Evans, L.E., Scoles, G.J. 1976. Cytogenetics, plant breeding, and agronomy. In: Rye: production, chemistry and technology, 13-26, American Association of Cereal Chemists, St Paul.

Eyre-Walker, A., Gaut, R.L., Hilton, H., Feldman, D.L., Gaut, B.S., 1998 . Investigation of the bottleneck leading to the domestication of maize. Proceedings of the National Academy of Sciences, USA 95, 4441- 4446.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetic analyses under Linux and Windows. Molecular Ecology Resources, 10, 564-567.

Feltus, F.A., Wan, J., Schulze, S.R., Estill, J.C., Jiang, N., Paterson, A.H., 2004. An SNP resource for rice genetics and breeding based on subspecies *indica* and *japonica* genome alignments. Genome Resources, 14, 1812-1819.

Fisher, R.A., 1930. The genetical theory of natural selection. Oxford University Press, Oxford.

Foy, C., 1983. The physiology of plant adaptation to mineral stress. Iowa State Journal of Research, 57, 355-92.

Frederiksen, S., Petersen, G., 1997. Morphometrical analyses of *Secale* (Triticeae, Poaceae). Nordic Journal of Botany, 17, 185–197.

Frederiksen, S., Petersen, G., 1998. A taxonomic revision of *Secale.* Nordic Journal of Botany, 18, 399–420.

Fu, Y.X., 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics, 147, 915-925.

Fuhrer, J. 2003. Agroecosystem responses to combinations of elevated CO_2, ozone

and global climate change. Agriculture Ecosystems and Environment, 97, 1-20.

Gallego, F.J., Benito, C., 1997. Genetic control of aluminium tolerance in rye (*Secale cereale* L.). Theoretical and Applied Genetics, 95, 393–399.

Gallego, F.J., Calles, B., Benito, C., 1998. Molecular markers linked to the aluminium tolerance gene Alt1 in rye (*Secale cereale L.*), Theoretical and Applied Genetics, 97, 1104-1109.

Gallego, F.J., Lopez-Solanilla, E., Figueiras, A. M., Benito, C., 1998. Chromosomal location of PCR fragments as a source of DNA markers linked to aluminium tolerance genes in rye. Theoretical and Applied Genetics, 96, 426–434.

Gawronska, H., Nalborczyk, E., 1989. Photosynthetic productivity of winter rye *Secale cereale*: photosynthetic area of six cultivars of winter rye *Secale cereale*. Acta Physiologiae Plantarum 11, 251-264.

Gielly, L., Taberlet, P., 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus *rbc*L sequences. Molecular Biology and Evolution, 11, 769–777.

Gonzalez-Astorga, J., Castillo-Campos, G., 2004. Genetic variability of the narrow endemic tree Antirhea aromatica Castillo-Campos & Lorence, (Rubiaceae, Guettardeae) in a tropical forest of Mexico. Annals of Botanny, 93, 521–528.

Goodwin, S., Hu, B.X., Shaner, G., 1998. An AFLP marker linked to a gene for resistance to Septoria tritici blotch in wheat. In Slinkard, A.E., (Ed), Eighth International Wheat Genetics Symposium, 108-110, Saskatoon.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Journal of Heredity, 86, 485-186.

Gopher, A., Abbo, S., Lev-Yadun, S., 2002. The 'when', the 'where' and the 'why' of the Neolithic revolution in the Levant. Documenta Praehistorica, 28, 49-62.

Graner, A., Jahoor, A., Schondelmaier, J., Siedler, H., Pillen, K., Fischbeck, G., Wenzel, G., Hedges, D.J., Batzer, M.A., 2005. From the margins of the genome: mobile elements shape primate evolution. BioEssays, 27, 785-794.

Groben, R., Wricke, G., 1998. Occurrence of microsatellites in spinach sequences from computer databases and development of polymorphic SSR markers. Plant Breeding, 117, 271-274.

Guo, S.W., Thompson, S.W., 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics, 48, 361-372.

Gupta, H.S., Balyan, P., Sharma C., Ramesh, B., 1996. Microsatellites in plants: A new class of molecular markers. Current Science, 70, 45-54.

Gupta, A.K., 2004. Origin of agriculture and domestication of plants and animals linked to early Holocene climate amelioration. Current Science, 87, 54-59.

Hackauf B., Wehling P., 2002. Identification of microsatellite polymorphisms in an expressed portion of the rye genome. Plant Breeding, 121, 17-25.

Hammer, K., Skolimowska, E., Knupffer, H., 1987. Vorarbeiten zur monographischen Darstellung von Wildpflanzensortimenten: *Secale* L. Kulturpflanze, 35, 135–177.

Hammer K., 1990. Breeding system and phylogenetic relationships in *Secale L.* Zentralbl fur Biologie, 109, 45-50.

Hammer, K., Khoshbakht, K., 2005. Towards a 'red list' for crop plant species. Genetic Resources and Crop Evolution, 52, 249-265.

Hamrick, J.L., Godt, M.J.W., 1989. Allozyme diversity in plant species, in: A.H.D. Brown, M.T. Clegg, A.L. Kahler, B.S. Weir (Eds.), Plant Population Genetics, Breeding and Genetic Resources, Sunderland: Sinauer Associates Inc., Massachusetts USA, 1989, pp. 43–63.

Harlan, H.R., Martini, M.L., 1936. Problems and results of barley breeding. USDA Yearabook of Agriculture. Washington DC: US, 303-346.

Harlan, J.R., 1970. Evolution of cultivated plants. In: Frankel, O.H. and Bennett, E. (Eds) Genetic Resources in Plants – IBP Handbook No 11. London: International Biological Programme, 19-32.

Harlan, J.R., 1975. Our vanishing genetic resources. Science 188, 618-621.

Harlan, J.R. 1992 . Crops and man. American Society of Agronomy, Madison, Wisconsin, USA.

Harris, S.A., 1999. RAPDs in systematics: a useful methodology? In: Hollingsworth PM, Bateman RM, Gornall RJ (Eds). Molecular systematics and plant evolution, 211-228, Taylor and Francis, London:.

Hawtin, G., Iwanaga, M., Hodgkin, T., 1996. Genetic resources in breeding for adaptation. Euphytica, 92, 255-266.

Herrmann, R.G., 1991. Construction of an RFLP map of barley. Theoretical Applied Genetics, 83, 250-256

Hazen, P.S., Leroy, P., Ward. R., 2002. AFLP in *Triticum aestivum* L. Patterns of diversity and genome distribution. Euphytica, 125, 89-102

Hedrick, PW. 2005. A Standardized Genetic Differentiation Measure. Evolution, 59, 1633-1638.

Heemert, C.V., Sybenga, J., 1972. Identification of the three chromosomes involved in the translocation which structurally differentiates the genome of *Secale cereale* L., from those of *Secale montanum* and *Secale vavilovii* Grossh. Genetica, 43, 387-393.

Helentjaris, T., 1987. A genetic linkage map for maize based on RFLPs. Trends in Genetics, 3, 217-221.

Hillman, G.C., 1978. On the origins of domestic rye - *Secale cereale*: the finds from Aceramic Can Hasan III in Turkey. Anatolian Studies, 28, 157-174.

Hillman, G.C., 2000. Village on the Euphrates, from Foraging to Farming at Abu Hureyra, Oxford Univ. Press, Oxford, 327–398.

Hirosawa, S., Takumi, S., Ishii, T., Kawahara, T., Nakamura, C., Mori, N., 2004. Chloroplast and nuclear DNA variation in common wheat: insight into the origin and evolution of common wheat. Genes and Genetic Systems, 79, 271-282.

Hoekenga, O.A., Maron, L.G., Pineros, M.A., Cancado, M.A.G., Shaff J., et al., 2006. *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis*. Proceedings of the National Academy of Sciences of the United States of America, 103, 9738-9743.

Hubisz, M., Falush, D., Stephens, M., Pritchard, J., 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources, 9, 1322-1332.

Hulbert, S.H., 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology, 52, 577-586.

Ishii, T., Mori, N., Ogihara, Y., 2001. Evaluation of allelic diversity at chloroplast microsatellite loci among common wheat and its ancestral species. Theoretical and Applied Genetics, 103, 896-904.

Ishii, T., Chisa, T., Nobuyuki, T., Osamu, K., Naoki, M., 2006. Mitochondrial microsatellite variability in common wheat and its ancestral species. Genes and Genetic Systems, 81, 211-214.

Isik, Z., Parmaksiz, I., Coruh, C., Geylan-Su, Y.S., Cebeci, O., Beecher, B., Budak, H., 2007. Organellar genome analysis of rye (*Secale cereale*) representing diverse geographic regions. Genome, 50, 724-734.

Jaaska, V. 1975. Evolutionary variation of enzymes and phylogenetic relationships in the genus *Secale* L. Proceedings of Academia and Sciences of.Estonian Soviet Socialist Republic, Biology, 24, 179-198.

Jaaska, V., 1999. On the origin and in statu nascendi domestication of rye and barley: a review. In: Damania, A.B., Valkoun, J., Willcox, G., Qualset, C.O., (Editors). The origins of agriculture and crop domestication, Part 4: Domestication of crop plants. Proceedings of Harlan symposium, 10–14 May 1997, IPGRI, Aleppo, Syria

Jain, S. K., 1960. Cytogenetics of rye *(Secale* spp.). Bibliographia Genetica, 19, 1-86.

Jones, J.D.G., Flavell, R.B., 1982. The mapping of highly-repeated DNA families and their relationship to C-bands in chromosomes of *Secale cereale*. Chromosoma, 86, 595-612.

Kalendar, R., Grob, T., Regina, M., Suoniemi. A., Schulman, A.H., 1999. IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theoretical and Applied Genetics, 98, 704-711.

Kalendar, R., Schulman, A.H., 2006. IRAP and REMAP for retrotransposon-based genotyping and fingerprinting. Nature Protocols, 1, 2478–2484.

Kalendar, R., Tanskanen, J., Immonen, S., Nevo, E., Schulman, A. H., 2000. Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proceedings of the National Academy of Sciences of the United States of America, 97, 6603–6607.

Kalendar, R., Antonius, K., Smykal, P., Schulman, A.H., 2010. iPBS: a universal method for DNA fingerprinting and retrotransposon isolation. Theoretical and Applied Genetics, 121, 1419-1430.

Kalinowski, S.T., 2005. HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. Molecular Ecology Notes, 5, 187-189.

Kantety, R.V., La Rota, M., Matthews, D.E., Sorrells, M.E., 2002. Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. Plant Molecular Biology, 48, 501-510.

Khlestkina, E.K., Ma, H., Myint, T., Pestsova, E.G., Röder, M.S., Malyshev, S.V., Korzun, V., Börner, A., 2004. Mapping of 99 new microsatellite-derived loci in rye (*Secale cereale L.*) including 39 expressed sequence tags. Theoretical and Applied Genetics, 109, 725-732.

Khush, G.S., Stebbins, G.L., 1961. Cytogenetic and evolutionary studies in *Secale.* I. Some new data on the ancestry of *S. cereale.* American Journal of Botany, 48, 723-730.

Khush, G.S.. 1962. Cytogenetic and evolutionary studies in *Secale*: Interrelationships of the wild species. Evolution, 16, 484-496.

Khush, G.S., 1963a. Cytogenetic and evolutionary studies in *Secale*: Cytogenetics of weedy ryes and origin of cultivated rye. Economical Botany, 17, 60-71.

Khush, G.S., 1963b. Cytogenetic and evolutionary studies in *Secale.* IV. *Secale vavilovii* and its biosystematic status. Zeitschnfi fur Pjantmzfichtung, 50, 34-43.

Kim, B.Y., Baier, A.C., Somers, D.J., Gustafson, J.P., 2001. Aluminum tolerance in triticale, wheat, and rye. Euphytica, 120, 329-337.

Kochian, L.V., Hoekenga, O.A., Pineros M.A., 2004. How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. Annual Review of Plant Biology, 55, 459-493.

Kochian, L.V., Pineros, M.A., Hoekenga, O.A., 2005. The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. Plant Soil, 274, 175-195.

Koebner, R.M.D., Miller, T.E., Snape, J.W., Law, N.V., 1998. Wheat endopeptidase genetic control of polymorphism intra-chromosomal gene location and alien variation. Genome 30, 186-192.

Kornicke, F.A., 1885. *Secale cereale L.* Der Roggen. Handbuchd er GetreidebauesI. Berlin, Parey.

Korzun, V.S., Malyshev, A.V., Voylokov, A., Börner, A., 2001. Genetic map of rye (*Secale cereale L.*) combining RFLP, isozyme, protein, microsatellite and gene loci, Theoretical and Applied Genetics, 102, 709-717.

Kota, R., Varshney, R.K., Thiel, T., Dehmer, K.J., Graner, A., 2001. Generation and comparison of EST-derived SSR and SNP markers in barley (*Hordeum vulgare* L.). Hereditas, 135, 141-151.

Kranz, A. R., 1957. Populationsgenetische Untersuchungen am Iranischen Primitivroggen. Zeitschni zur PfEanzenzuchtung, 38, 101-146.

Kranz, A. R., I961, Cytologische Untersuchungen und genetische Beobachtungen an der Bastarden zwischen *Secale cereale* L. und *Secale vavilvuii* Grossh. *Zuchter,* 31, 19-225.

Kuckuck, J., 1937. Zur Entstehung und Abstammung des Roggens. Z. Gesamte Getreidewes, 24, 131-132.

Kuckuck, H., 1974. Bedeutung der Nutzung, Erhaltung und Weiterentwicklung der natu¨rlichen genetischen Formenmannigfaltigkeit fur die Pflanzenzu chtungein Beitrag zur grunen Revolution'. Naturwiss. Rundschau 27, 267-272.

Lanteri, S., Barcaccia, G., Molecular marker based analysis for crop germplasm preservation. In: RUANE, J.; SONNINO, A. (Ed.). The role of biotechnology in exploring and protecting agricultural genetic resources. Rome: FAO, 2006. p.55-66.

Leigh, F., Kalendar, R., Lea, V., Lee, D., Donini, P., Schulman, A.H., 2003. Comparison of the utility of barley retrotransposon families for genetic analysis by molecular marker techniques. Molecular Genetic and Genomics, 269, 464-474.

Librado, P., Rozas, J., 2009. DNASP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451-1452.

Linde-Laursen, L,R., von Bothmer, and N. Jacobson. 1980. Giemsa C-banding in Asiatic taxa of *Hordeum* sect Stenostachys with notes on chromosome morphology. Hereditas, 93, 235-254.

Liu, Z.W., Biyashev, R.M., Saghai Maroof, M.A., 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theoretical and Applied Genetics, 93, 869-876.

Liu, K.J., Goodman, M., Muse, S., Smith, J.S., Buckler, E., et al., 2003. Genetic structure and diversity among maize inbred lines as inferred from DNA microsatellites. Genetics, 165, 2117-2128

Lorenz K.J., 1991. Rye. In: Lorenz KJ, Kulp K (Eds) Handbook of cereal science and technology,331–371, Marcel Dekker, New York, USA.

Ma, J.F., 2000. Role of organic acids on detoxification of aluminium in higher plants. Plant Cell Physiology, 41, 383-390.

Ma, J.F., Furukawa, J., 2003. Recent progress in the research of external Al detoxification in higher plants: a minireview. Journal of Inorganic Biochemistry, 97, 46-51.

Mackill, D.J., Zhang, Z., Redona, E.D., Colowit, P.M., 1996. Level of polymorphism and genetic mapping of AFLP markers in rice. Genome, 39, 969-977.

Manninen, O., Kalendar, R., Robinson. J., Schulman, A.H., 2000. Application of BARE -1 retrotransposon markers to map a major resistance gene for net blotch in barley. Molecular Genomics and Genetics, 264, 325-334.

Marshall, D.R., Allard, R.W., 1970. Isozyme polymorphism in natural populations of *Avena fatua* and *Avena barbata*. Heredity, 25, 373-382.

Matos, M., Pinto-Carnide, O., Benito, C., 2001. Phylogenetic relationships among Portuguese rye based on isozyme, RAPD and ISSR markers. Hereditas, 134, 229-236.

Matos, M., Camacho, M.V., Pérez-Flores, V., Pernaute, B., Pinto-Carnide, O. et al, 2005. A new aluminium tolerance gene located on rye chromosome arm 7RS. Theoretical and Applied Genetics, 111, 360–369.

McClintock, B., 1950. The origin and behavior of mutable loci in maize. Proceedings of National Academy of Sciences of the USA, 36, 344-55.

McClintock, B., 1965. Components of action of the regulators Spm and Ac. Carnegie Institution of Washington Year Book.

McCouch, S.R., Kochert, G., Yu, Z.H., Wang, Z.Y., Khush, G.S., Coffman, W.R., Tanksley, S.D., 1988. Molecular mapping of rice chromosomes. Theoretical and Applied Genetics, 76, 815-829.

McLeod, E., 1982. Feed the Soil Organic Agriculture Research Institute, Graton, CA.

Meimberg, H., Rice, K.J., Milan N.F., Njoku C.C., Mckay J.K., 2009. Multiple origins promote the ecologıcal amplitude of allopolyploid *aegilops* (poaceae). American Journal of Botany, 96, 1262-1273.

Meyers, B.C., Tingey, S.V., Morgante, M., 2001. Abundance, distribution, and transcriptional activity of repetitive elements in the maize genome. Genome Resources, 11, 1660-1676.

Miftahudin, G.J.S., Gustafson, J.P., 2002. AFLP markers tightly linked to the aluminium-tolerance gene Alt3 in rye (*Secale cereale L.*). Theoretical and Applied Genetics, 104, 626-631.

Miftahudin, G.J.S., Gustafson, J.P., 2004. Development of PCR-based codominant markers flanking the *Alt3* gene in rye. Genome, 47, 231-238.

Mogensen, H.L., 1996. The hows and whys of cytoplasmic inheritance in seed plants. American Journal of Botany, 83, 383-404.

Moore, A.M.T., Hillman, G.C., Legge, A.J., 2000. Village on the Euphrates, from Foraging to Farming at Abu Hureyra, Oxford Univ. Press, Oxford.

Moöhring, S., Salamini, F., Schneider, K., 2004. Multiplexed, linkage group-specific marker sets for rapid genetic mapping and fingerprinting of sugar beet (*Beta vulgaris* L.). Molecular Breeding, 14, 475-488.

Mörchen, M., Cuguen, J., Michaelis, G., Hänni, C., Saumitou-Laprade, P., 1996. Abundance and length polymorphism of microsatellite repeats in *Beta vulgaris* L. Theoretical and Applied Genetics, 92, 326-333.

Murai, K., Naiyu, U., Tsunewaki K., 1989. Studies on the origin of crop species by restriction endonuclease analysis of organellar DNA. III. Chloroplast DNA variation and interspecific relationships in the genus *Secale*. Japanese Journal of Genetics, 64, 35-47.

Myers, N., 1994. Protected Areas -protected from a Greater What?; Biodiversity and Conservation, 3, 411-418.

Nasu, S., Suzuki, J., Ohta, R., Hasegawa, K., Yui, R., Kitazawa, N., Monna, L., Minobe, Y., 2002. Search for and analysis of single nucleotide polymorphisms (SNPs) in rice (Oryza sativa,Oryza rufipogon) and establishment of SNP markers. DNA Resources, 9, 163-171.

Nei, M. 1972. Genetic distance between populations. The American Naturalist, 106, 283-292.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 76, 379-390.

Nei, M., Chesser, R.K., 1983. Estimation of fixation indices and gene diversities. Annals of Human Genetics, 47, 253-259.

Nei, M., 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nesbitt, K.A., Potts, B.M., Vaillancourt, R.E., West A.K., Reid, J.B., 1995. Partioning and distribution of RAPD variation in a forest tree species, *Eucalyptus globulus, Myrtaceae*. Heredity, 74, 628-637.

Nuijten, E., Van Treuren, R., Struik, P.C., Mokuwa, A., Okry, F., Teeken, B., Richards, P., 2009. Evidence for the emergence of new rice types of interspecific hybrid origin in West African farmers' fields. Plos One, 4, 1-9.

Nürnberg-Krüger, U. 1960. Cytogenetioche Untersuchungen der Gattung *Secale*, L. Zeit für Pflanzenzüchtung, 44, 63-72.

Olmstead, R.G., 1994. Palmer Chloroplast DNA systematics: a review of methods and data analysis. American Journal of Botanny, 81, 1205-1224.

Oosterhout, C.V., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes, 4, 535–538.

Pachauri, R.K, Reisinger, A, (Eds), 2007. Climate Change 2007: Synthesis Report. Fourth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, Geneva, Switzerland, 104 pp.

Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6, 288-295.

Perez de la Vega, M., Allard, R.W., 1984. Mating system and genetic polymorphism in populations of *Secale cereale* and *S. vavilovii*. Canadian Journal of Genetics and Cytology, 26, 308-317.

Persson, K., Bothmer, R. V., 2000. Assessing the allozyme variation in cultivars and Swedish landraces of rye (*Secale cereale L.*) Hereditas, 132, 7-17.

Persson, K., Diaz, O., Bothmer, R.V., 2001. Extent and patterns of RAPD variation in landraces and cultivars of rye *(Secale cereale* L.) from Northern Europe. Hereditas, 134, 237-243.

Persson, K., Bothmer R. V., 2002. Genetic diversity amongst landraces of rye (*Secale cereale* L.) from Northern Europe. Hereditas, 136, 29-38.

Petersen, G., Doebley, J.F., 1993. Chloroplast DNA variation in the genus *Secale* (*Poaceae*). Plant Systematics and Evolution, 187, 115-125.

Pinto-Carnide, O., Guedes-Pinto, H., Garcia, J., 1989. Aluminum tolerance behaviour of Portuguese rye populations. Vortrage für Pflanzenzuchtung, 15, 6–5.

Pinto-Carnide, O., Guedes-Pinto, H., 1999. Aluminum tolerance variability in rye and wheat Portuguese germplasm. Genetic Resources and Crop Evolution, 46, 81-85.

Plucknett, D.L., Smith, N.J.H., Williams, J.T., Anishetty, N.M., 1987. Gene Banks and the World's Food: Princeton University Press, Princeton.

Popov, A., 1939. Investigations on the botanical diversity and gapping in our native rye. Annuals of University of Sofia, 17, 523.

Pozo, J.C., Figueiras, A.M., Benito, C., De la Pena, A., 1995. PCR derived molecular markers and phylogenetic relationships in the *Secale* genus. Plant Biology, 37, 481-489.

Pritchard, J. K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics, 155, 945-959.

Qualset, CO.,. Damania, A.B., Zanatta, A.C.A., Brush, S.B., 1997. Locally based crop plant conservation. In Plant Genetic Conservation: The in situ Approach, Maxted, N., Ford-Lloyd, B.Y., Hawkes, J.G., (Eds.). Chapman and Hall, London.

Queen, R.A., Gribbon, B.M., James, C., Jack, P., Flavell, A.J., 2004. Retrotransposon-based molecular markers for linkage and genetic diversity analysis in wheat. Molecular Genetics and Genomics, 271, 91-97.

Rafalski, J.A., 2002. Application of single nucleotide polymorphisms in crop genetics. Current Opinion in Plant Biology, 5, 94-100.

Ragupathy, R., Banks, T., Cloutier, S., 2010. Molecular characterization of the Sasanda LTR copia retrotransposon family uncovers their recent amplification in *Triticum aestivum* (L.) genome. Molecular Genetics and Genomics, 283, 255-271.

Ramirez, L., Pisabarro, G., 1986. Studies on enzymatic polymorphism in rye (*Secale cereale L.*) cultivars. Genetica, 69, 209-212.

Raymond, M., Rousset, F., 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, 86, 248-249.

Reddy, P., Appel, R., Baum, B.R., 1990. Ribosomal DNA spacerlength variation in *Secale* spp. (Poaceae). Plant Systematics and Evolution, 171, 205-220.

Regel, C., 1922. "Uber den Ursprung der Getreidearten", Naturwissenschaftliche Wochen-schrift, XXI, 328-330.

Reif, J.C., Xia, X.C., Melchinger, A.E., Warburton, M.L., Hoisington, D.A., Beck, D., Bohn, M. et al. 2004. Genetic diversity determined within and among CIMMYT maize populations of tropical, subtropical and temperate germplasm by SSR markers. Crop Science, 44, 326-334.

Reiter, R.S., Williams, J.G.K., Feldmann, K.A., Rafalski, J.A., Tingey, S.V., and Scolnik, P.A., 1992. Global and local genome mapping in Arabidopsis thaliana by using recombinant inbred lines and random amplifed polymorphic DNAs. Proceedings of National Academy of Sciences, USA, 89, 1477-1481.

Ren, T.H., Chen, F., Zou, Y.T., Jia, Y.H., Zhang, H.Q., Yan, B.J., Ren, Z.L., 2011. Evolutionary trends of microsatellites during the speciation process and phylogenetic relationships within the genus *Secale*. Genome, 54, 316-326.

Rieseberg, L.H., Widmer, A., Arntz, A.M., Burke, J.M., 2002. Directional selection is the primary cause of phenotypic diversification. Proceedings of the National Academy of Sciences, USA, 99, 12242-12245 .

Riley, R., 1955. The cytogenetics of the differences between some *Secale* species. Journal of Agricultural Science, 46, 377-383.

Roshevits, R.I., 1947, Monographie of the Genus *Secale L.* Acta Inst Bot. Acad. Sci. USSR, I6, 105-163.

Rostoks, N., Mudie, S., Cardle, L., Russell, J., Ramsay, L., Booth, A., Svensson, J.T., Wanamaker, S.I., Walia, H., Rodriguez, E.M., Hedley, P.E., Liu, H., Morris, J., Close, T.J., Marshall, D.F., Waugh, R., 2005. Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. Molecular Genetics and Genomics, 274, 515-527.

Rousset, F., 2008. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. Molecular Ecology Resources, 8, 103-106.

Roy, J.K., Balyan, H.S., Prasad, M., Gupta, P.K., 2002. Use of SAMPL for a study of polymorphism, genetic diversity and possible gene tagging in bread wheat. Theoretical and Applied Genetics, 104, 465-472.

Russell, J., Booth, A., Fuller, J., Harrower, B., Hedley, P., Machray, G., Powell, W., 2004. A comparison of sequence-based polymorphism and haplotype content in transcribed and anonymous regions of the barley genome. Genome, 47, 389-398.

Ryan, P.R., Ditomaso, J.M., Kochian, L.V., 1993. Aluminium toxicity in roots - an investigation of spatial sensitivity and the role of the root cap. Journal of Experimental Botany, 44, 437-446.

Saal, B., Wricke, G., 1999. Development of simple sequence repeat markers in rye (*Secale cereale L.*). Genome, 42, 964-972.

Salamini, F., Özkan H., Brandolini, A., Schäfer-Pregl, R., Martin, W., 2002. Genetics and geography of wild cereal domestication in the Near East. Nature Reviews, 3, 429-441.

SanMiguel, P., Bennetzen, J.L., 1998. Evidence that a recent increase in maize genome size was caused by the massive amplification of intergene retrotransposons. Annals of Botanny, 81, 37–44.

Scheibe, A., 1935. *Secale africanum* und seine Bastarde mit *Secale montanum* und *Secale cereale.* Naturwissenschaflen, 39, 136-137.

Schnable, P.S., Ware, D., Fulton, R.S. et al, 2009. The B73 maize genome: complexity, diversity, and dynamics. Science, 326, 1112–1115.

Schlegel, R.H.J., 2013. Rye: Genetics, Breeding, and Cultivation. CRC Press.

Schiemann, E ., 1932. Entstehungd er Kulturpflanzen, Berlin, Borntraeger.1 74-184.

Schiemann, E., 1948. Weiten, Roggen, Gerste. Systematih, Geschichte und Verwendung. Jena: Fischer.

Schiemann, E., Nornberg-Kruger, U**.,** 1952. Neue Untersuchungen an *Secale aricanum* Stapf. 11.

Schoen, D.J., Brown, A.H.D., 1991. Intraspecific variation in population gene diversity and effective population size correlates with the mating system in plants. Proceedings of the National Academy of Sciences U.S.A, 88, 4494 -4497.

Schulman, A.H., Flavell, A.J., Ellis, T.H.N., 2004. The application of LTR retrotransposons as molecular markers in plants. Methods in Molecular Biology, 260, 145-173.

Schut, J.W., Qi, X., Stam, P., 1997. Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. Theoretical and Applied Genetics, 95, 1161-1168.

Schultz, A., 1911. "Die Geschichte des Roggens", Jahresberichte des Westfalischen Vereins f. Wissenschaftu Kunst XXXIX, 153-163.

Schwanitz, F., 1966. The origin of cultivated plants. Harvard Univ. Press, Cambridge.

Sederoff, R.R., Levings, C.S., Timothy, D.H., Hu, W.W.L., 1981. Evolution of DNA sequence organization in mitochondrial genomes of Zea. Procedings of National Academy of Sciences of the USA, 78, 5953-5957.

Semagn, K., Bjørnstad, Å., Ndjiondjop, M.N., 2006. An overview of molecular marker methods for plants. African Journal of Biotechnology, 5, 2540-2568.

Sencer, H.A., 1975. Study of variation in the genus *Secale L.* and on the origin of the cultivated rye, Ph.D. thesis, The University of Birmingham.

Sencer, H.A., Hawkes, J.G., 1980. On the origin of cultivated rye. Biological Journal of the Linnean Society, 13, 299-313.

Shang, H.Y., Baum, B.R., Wei, Y., Zheng, Y., 2007. The 5S rRNA gene diversity in the genus Secale and determination of its closest haplomes. Genetic Resources and Crop Evolution, 54, 793-806.

Shang, H.Y., Wei, Y., Wang, X., Zheng, Y., 2006. Genetic diversity and phylogenetic relationships in the rye genus *Secale* L. (rye) based on *Secale cereale* microsatellite markers. Genetics and Molecular Biology, 29, 685-691.

Shedlock, A.M., Okada, N., 2000. SINE insertions: powerful tools for molecular systematics. Bioassays, 22, 148-160.

Simard, M.J., 2010. Gene flow between crops and their wild relatives. Evolutionary Applications, 3, 402-403.

Skuza, L., Rogalska, S., Bocianowski, J., 2007. RFLP Analysis of Mitochondrial DNA in the Genus *Secale*. Acta Biologica Cracoviensia Series Botanica, 49, 77-87.

Simon, C.J., Muehlbauer, F.J., 1997. Construction of a chickpea linkage map and its comparison with maps of pea and lentil. Journal of Heredity, 88, 115-119.

Somers, D.J., Kirkpatrick, R., Moniwa, M., Walsh, A., 2003. Mining single-nucleotide polymorphisms from hexaploid wheat ESTs. Genome, 46, 431-437.

Stebbins, G.L., 1957. Self-fertilization and population variability m the higher plants. The American Naturalist, 91, 337-354.

Stephens, M., Smith, N.J., Donnelly, P., 2001. A new statistical method for haplotype reconstruction from population data. American Journal of Human Genetics, 68, 978-989.

Stojalowski, A., Lapinski, M., Masojc, P., 2004. RAPD markers linked with restorer genes for the C-source of cytoplasmic male sterility in rye (*Secale cereale* L.). Plant Breeding, 123, 428-33.

Stutz, H.C., 1957. A cytogenetic analysis of the hybrid *Secale cereale L* x *Secale montanum guss* and its progeny. Genetics, 42, 199-222.

Stutz, H C., 1972. On the origin of cultivated rye. American Journal of Botany, 59, 59-70.

Takezaki, N., Nei, M., Tamura, K., 2010. POPTREE2: software for constructing population trees from allele frequency data and computing other population statistics with Windows interface. Molecular Biology and Evolution, 27, 747-752.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution, 28, 2731-2739.

Tang, S., Knapp, S.J., 2003. Microsatellites uncover extraordinary diversity in native American landraces and wild populations of cultivated sunflower. Theoretical and Applied Genetics, 106, 990-1003.

Tanksley, S.D., Young, N.D., Paterson, A.H., Bonierbale, M.W., 1989. RFLP mapping in plant breeding: new tools for an old science. Nature Biotechnology, 7, 257-264.

Tanno, K.S., Taketa, Takeda, K., Komatsuda, T., 2002. A DNA marker closely linked to the *vrs1* locus (row-type gene) indicates multiple origins of six-rowed cultivated barley (*Hordeum vulgare* L.). Theoretical and Applied Genetics, 104, 54 -60.

Taramino, G., Tingey, S., 1996. Simple sequence repeats for germplasm analysis and mapping in maize. Genome, 39, 277-287.

Tautz, D., Renz, M., 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Resources, 12, 4127-4138.

Tempir, Z., 1968. Archologische Funde yon landwirtschafllichen Pflanzen und Unkrutern in Bohmen und Mahren. Ved Pr Cesk Zeroed Muz 1968, pp 15-88.

Tempir Z (1969) Archaeological finds of food plants and weeds in Slovakia. Agricultfira 8, 7-66.

Tenaillon, M.I., Sawkins, M.C., Long, A.D., Gaut, B., Doebley, J.F., Brandon, S., 2001. Patterns of DNA sequence polymorphism along chromosome 1 of maize *(Zea mays* ssp. *mays* L.) Procedings of National Academy of Sciences of USA, 98, 9161-9166.

Tzvelev, N., 1973. Conspectus specierum tribus Triticeae Dum. familiae Poaceae in flora URSS. Novosti Sistematiki Vysshikh Rastenii, 10, 19-59.

Van, K., Hwang, E.Y, Young Kim, M., Kim, Y.H., Cho, Y.I., Cregan, P.B., Lee, S.H., 2004. Discovery of single nucleotide polymorphisms in soybean using primers designed from ESTs. Euphytica, 139, 147-157

Van Zeist, W., Casparie, W.A., 1968. Wild einkorn wheat in northern Syria. Acta Botanica Neerlandica, 17, 44-53.

Varshney, R.K., Graner, A., Sorrells, M.E., 2005. Genic microsatellite markers in plants: features and applications. Trends in Biotechnology, 23, 48,55.

Varshney, R.K, Beier, U., Khlestkina, E., Kota, R., Korzun, V., Graner, A., Börner, A., 2007a. Single nucleotide polymorphisms in rye (*Secale cereale* L.): discovery, frequency, and applications for genome mapping and diversity studies. Theoretical and Applied Genetics, 114, 1105-1116.

Varshney, R.J., Mahendar, T., Aggarwal, R.K., Börner, A., 2007b. Genic Molecular Markers in Plants: Development and Applications Genomics-Assisted Crop Improvement 13-29.

Vavilov, N.I., 1917. On the origin of cultivated rye. Bulletin of Applied Botany, Genetics and Plant Breeding, 10, 561-590.

Vavilov, N.I., 1928. Geographische Genzentrenunserer Kulturpflanzen, Zeitschriftf induktierte Abstammungu. Vererbungslehre, 1, 342-369.

Vavilov, N.I., Dorofeev, V.F., 1992. Origin and geography of the cultivated plants, Cambridge University Press, Cambridge.

Vavilov, N.I., 1926. Studies on the origin of cultivated plants. Bulletin Applied Botany, 16, 1-248.

Vavilov, N.I., 1951. The Origin, Variation, Immunity and Breeding of Cultivated Plants. The Chronica Botanica, 13,1-6

Vences, F.J., Vaquero, F., Garcla, P., Perez De La Vega, M., 1987. Further Studies on Phylogenetic Relationships in *Secale:* On the Origin of its Species. Plant Breeding, 98, 281-291.

Vicient, C.M., Suoniemi, A., Anamthawat-Jónsson, K., Tanskanen, J., Beharav, A., Nevo, E., Schulman, A.H., 1999. Retrotransposon BARE-1 and its role in genome evolution in the genus *Hordeum*. Plant Cell, 11, 1769-1784.

Vicient, C.M., Kalendar, R., Schulman, A.H., 2001. Envelope-class retrovirus-like elements are widespread, transcribed and spliced, and insertionally polymorphic in plants. Genome Research, 11, 2041-2049.

Vos, P., Hogers, R., Blecker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Peleman, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23, 4407-4414.

Warburton, M.L., Reif, J.C., Frisch, M., Bohn, M., Bedoya, C., Xia, X.C., Crossa, J., et al., 2008. Genetic diversity in CIMMYT nontemperate maize germplasm: landraces, open pollinated varieties, and inbred lines. Crop Science 48:617-624.

Watkins, W.S., Rogers, A.R., Ostler, C.T., Wooding, S., Bamshad, M.J., Brassington, A.M., et al., 2003. Genetic variation among world populations: inferences from 100 Alu insertion polymorphisms. Genome Research, 13, 1607-1618.

Waugh, R., McLean, K., Flavell, A.J., Pearce, S.R., Kumar, A., Thomas, B.B., Powell, W., 1997. Genetic distribution of BARE-1-like retrotransposable elements in the barley genome revealed by sequence-specific ampliWcation polymorphisms (S-SAP). Molecular Genetics and Genomics, 253, 687-694.

Welsh, J., McClelland, M., 1990. Fingerprinting Genomes using PCR with arbitrary primers. Nucleic Acids Research, 18, 1358-1370.

Wicker, T., Krattinger, S.G., Lagudah, E.S., Komatsuda, T., Pourkheirandish, M., Matsumoto, T., Cloutier, S., Reiser, L., Kanamori, H., Sato, K., Perovic, D., Stein, N., Keller, B., 2009). Analysis of intraspecies diversity in wheat and barley genomes identifies breakpoints of ancient haplotypes and provides insight into the structure of diploid and hexaploid triticeae gene pools. Plant Physiology, 149, 258-270.

Willcox, G., 2005. The distribution, natural habitats and availability of wild cereals in relation to their domestication in the Near East: Multiple events, multiple centres. Vegetation History and Archaeobotany, 14, 534-541.

Wright, S., 1931. Evolution in Mendelian populations. Genetics, 16, 97-159.

Wouw, M., Kik, C., Hintum, T., Treuren, R., Visser, B., 2009. Genetic erosion in crops: concept, research results and challenges. Plant Genetic Resources: Characterization and Utilization, 8, 1-15.

Yamane, K., Kawahara, T., 2005. Intra- and interspecific phylogenetic relationships among diploid triticum-aegilops species (*poaceae)* based on base-pair substitutions, indels, and microsatellites in chloroplast noncoding sequences. American Journal of Botany, 92, 1887-1898.

Yamasaki, M., Tenaillon, M.I., Bi, I.V., Schroeder, S.G., Sanchez-Villeda, H.J., Doebley, F., Gaut, B.S., et al., 2005. A large-scale screen for artificial selection inmaize identifies candidate agronomic loci for domestication and crop improvement. The Plant Cell, 17, 2859-2872.

Yu, G.X., Wise, R.P., 2000. An anchored AFLP- and retrotransposon-based map of diploid *Avena*. Genome, 43, 736-749.

Zeder, M.A., Emshwiller, E., Smith, B.D., Bradley, D.G., 2006. Documenting domestication: the intersection of genetics and archaeology. Trends in Genetics, 22, 139-155.

Zeven, A.C., 1998. Landraces: A review of definitions and classifications. Euphytica, 104, 127-139.

Zhou, J.P., Yang, Z.J., Li, G.R., Liu, C., Tang, Z.X., Zhang, Y., Ren, Z.L. 2010. Diversified chromosomal distribution of tandemly repeated sequences revealed evolutionary trends in *Secale* (Poaceae). Plant Systematics and Evolution, 287, 49-56.

Zhu, J., Gale, M.D., Quarrie, S., Jackson, M.T., Bryan, G.J., 1998. AFLP markers for the study of rice biodiversity. Theoretical and Applied Genetics, 96, 602-611.

Zhu, Y.L., Song, Q.J., Hyten, D.L., van Tassell, C., Matukumalli, L.K., Grimm, D.R., Hyatt, S.M., Fickus, E.W., Young, N.D., Cregan, P.B., 2003. Single-nucleotide polymorphisms in soybean. Genetics, 163, 1123-1134.

Zhukovsky, P.M., 1928. A new wild growing form of rye in Anatolia. Bulletin of Applied Botany, 19, 49-52.S

Zhukovsky, P.M., 1933. La Turguie Agracole: 274-275. Leningrad.

Zohary, D., 1971. Origin of south-west Asiatic cereals: wheats, barley, oats and rye. In Davis, P**.**H. (Eds), Plant Lye of South-west Asia, 235-263. The Botanical Society of Edinburgh, Edinburgh.

Zohary, D., 1996. The mode of domestication of the founder crops of Southwest Asian agriculture. In The Origins and Spread of Agriculture and Pastoralism in Eurasia (Harris, D.R., ed.), pp. 142–158, UCL Press

Zohary, D., Hopf, M., 2000. Domestication of Plants in the Old World, Third ed., Oxford Univ. Press, Oxford.

APPENDIX A: INFORMATION ON ACCESSIONS USED IN THE STUDY

Table A1. Accessions used in the study

Taxon	N	Source	Accession	Origin	Type	Lat.	Lon.
S. cereale ancestrale	7	USDA	PI 618666	Turkey	Wild	39	35
S. cereale ancestrale	1	IPK	R0029	Russian Federation	Wild	62	102
S. cereale ancestrale	1	IPK	R0062	Turkey	Wild	40	36
S. cereale ancestrale	1	IPK	R0767	Turkey	Wild	38	36
S. cereale ancestrale	1	IPK	R1054	Turkmenistan	Wild	40	60
S. cereale cereale	12	USDA	CIse 108	Japan	Cultivar	36	138
S. cereale cereale	15	USDA	CIse 110	South Korea	Cultivar	37	128
S. cereale cereale	9	USDA	CIse 12	South Dakota, US	Cultivar	45	-100
S. cereale cereale	10	USDA	CIse 37	Afghanistan	Landrace	34	66
S. cereale cereale	9	USDA	CIse 79	Australia	Cultivar	-25	135
S. cereale cereale	5	USDA	PI 168130	Antalya, Turkey	Landrace	37	31
S. cereale cereale	6	USDA	PI 168136	Izmir, Turkey	Landrace	38	27
S. cereale cereale	7	USDA	PI 168168	Bursa, Turkey	Landrace	40	29
S. cereale cereale	8	USDA	PI 168176	Konya, Turkey	Landrace	38	32
S. cereale cereale	3	USDA	PI 168181	Samsun, Turkey	Landrace	41	36
S. cereale cereale	1	USDA	PI 168194	Yozgat, Turkey	Landrace	40	35
S. cereale cereale	7	USDA	PI 168195	Kirklareli, Turkey	Landrace	42	28
S. cereale cereale	4	USDA	PI 168196	Edirne, Turkey	Landrace	41	27
S. cereale cereale	8	USDA	PI 168199	Isparta, Turkey	Landrace	38	31
S. cereale cereale	3	USDA	PI 168205	Corum, Turkey	Landrace	40	34
S. cereale cereale	6	USDA	PI 168209	Kutahya, Turkey	Landrace	39	30
S. cereale cereale	3	USDA	PI 168211	Afyon, Turkey	Landrace	39	31
S. cereale cereale	4	USDA	PI 168213	Kirsehir, Turkey	Landrace	39	34
S. cereale cereale	9	USDA	PI 168218	Kayseri, Turkey	Landrace	39	36
S. cereale cereale	14	USDA	PI 168220	Nigde, Turkey	Landrace	38	34
S. cereale cereale	1	USDA	PI 173587	Amasya, Turkey	Landrace	41	36
S. cereale cereale	11	USDA	PI 173589	Ordu, Turkey	Landrace	41	37
S. cereale cereale	1	USDA	PI 205221	Ankara, Turkey	Landrace	40	33
S. cereale cereale	4	USDA	PI 218110	Pakistan	Landrace	35	73
S. cereale cereale	3	USDA	PI 220118	Afghanistan	Landrace	32	66
S. cereale cereale	4	USDA	PI 227870	Iran	Landrace	32	51
S. cereale cereale	1	USDA	PI 228360	Iran	Landrace	35	47
S. cereale cereale	9	USDA	PI 234655	Kazakhstan	Cultivar	48	68
S. cereale cereale	17	USDA	PI 240675	Colonia, Uruguay	Cultivar	-34	-58

Table A1. Accessions used in the study (Cont.)

Taxon	N	Source	Accession	Origin	Type	Lat.	Lon.
S. cereale cereale	1	USDA	PI 243741	West Azerbaijan, Iran	Landrace	38	45
S. cereale cereale	2	USDA	PI 250745	East Azerbaijan, Iran	Landrace	38	47
S. cereale cereale	8	USDA	PI 252002	Eskisehir, Turkey	Landrace	39	32
S. cereale cereale	3	USDA	PI 261400	Ontario, Canada	Cultivar	50	-86
S. cereale cereale	10	USDA	PI 268281	Bakhtaran, Iran	Landrace	34	48
S. cereale cereale	11	USDA	PI 289814	Khorasan, Iran	Landrace	36	60
S. cereale cereale	7	USDA	PI 314964	Sao Paulo, Brazil	Cultivar	-22	-49
S. cereale cereale	5	USDA	PI 323450	Lublin, Poland	Cultivar	51	19
S. cereale cereale	6	USDA	PI 330407	South Africa	Cultivar	-25	30
S. cereale cereale	13	USDA	PI 330526	England/UK	Cultivar	53	-2
S. cereale cereale	12	USDA	PI 344970	Bosnia and Herzegovina	Landrace	43	19
S. cereale cereale	17	USDA	PI 344975	Montenegro	Landrace	43	19
S. cereale cereale	11	USDA	PI 344990	Serbia	Landrace	43	21
S. cereale cereale	8	USDA	PI 345739	Australia	Cultivar	-35	149
S. cereale cereale	5	USDA	PI 405812	Macedonia	Landrace	42	22
S. cereale cereale	12	USDA	PI 410534	Pakistan	Landrace	34	73
S. cereale cereale	6	USDA	PI 429371	Semnan, Iran	Landrace	36	53
S. cereale cereale	9	USDA	PI 429373	Zanjan, Iran	Landrace	36	48
S. cereale cereale	9	USDA	PI 429377	Hamadan, Iran	Landrace	35	49
S. cereale cereale	4	USDA	PI 430003	India	Landrace	33	77
S. cereale cereale	6	USDA	PI 436190	Los Lagos, Chile	Landrace	-40	-73
S. cereale cereale	11	USDA	PI 445977	Israel	Cultivar	32	35
S. cereale cereale	9	USDA	PI 445996	Malmohus, Sweden	Cultivar	56	14
S. cereale cereale	9	USDA	PI 446023	Mexico	Cultivar	23	-102
S. cereale cereale	13	USDA	PI 446027	New Zealand	Cultivar	-42	174
S. cereale cereale	4	USDA	PI 447337	China	Cultivar	44	87
S. cereale cereale	6	USDA	PI 535147	Yugoslavia	Landrace	43	20
S. cereale cereale	13	USDA	PI 543398	Argentina	Cultivar	-35	-64
S. cereale cereale	11	USDA	PI 561793	Manisa, Turkey	Landrace	39	28
S. cereale cereale	5	USDA	PI 561796	Bolu, Turkey	Landrace	41	31
S. cereale cereale	4	USDA	PI 561797	Bolu, Turkey	Landrace	41	32
S. cereale cereale	9	USDA	PI 561798	Sinop, Turkey	Landrace	42	35
S. cereale cereale	8	USDA	PI 561799	Sinop, Turkey	Landrace	42	35
S. cereale cereale	5	USDA	PI 561802	Bitlis, Turkey	Landrace	39	42
S. cereale cereale	6	USDA	PI 561804	Van, Turkey	Landrace	38	43
S. cereale cereale	10	USDA	PI 561806	Erzurum, Turkey	Landrace	40	41

Table A1. Accessions used in the study (Cont.)

Taxon	N	Source	Accession	Origin	Type	Lat.	Lon.
S. cereale cereale	10	USDA	PI 561806	Erzurum, Turkey	Landrace	40	41
S. cereale cereale	5	USDA	PI 561809	Pakistan	Landrace	36	74
S. cereale cereale	1	USDA	PI 568118	Bilecik, Turkey	Landrace	40	30
S. cereale cereale	11	USDA	PI 584782	Georgia	Landrace	42	46
S. cereale cereale	1	IPK	R0191	Germany	Cultivar	50	10
S. cereale cereale	1	IPK	R1092	Austria	Cultivar	48	13
S. cereale cereale	1	IPK	R1133	Portugal	Uncertain	40	-8
S. cereale cereale	1	IPK	R1148	Turkey	Landrace	41	36
S. cereale cereale	1	IPK	R1151	Switzerland	Cultivar	47	8
S. cereale cereale	1	IPK	R1480	Germany	Cultivar	52	9
S. cereale cereale	1	IPK	R1489	Germany	Cultivar	51	9
S. cereale cereale	1	IPK	R1490	Germany	Cultivar	51	10
S. cereale cereale	1	IPK	R1653	Germany	Cultivar	52	11
S. cereale cereale	1	IPK	R1658	USA	Cultivar	40	-99
S. cereale cereale	1	IPK	R1756	Austria	Cultivar	47	13
S. cereale cereale	1	IPK	R2022	Finland	Cultivar	64	26
S. cereale cereale	1	IPK	R2199	Italy	Cultivar	43	13
S. cereale cereale	12	IPK	TR1	Gökova/Turkey	Landrace	37	29
S. cereale cereale	10	IPK	TR2	Kars/Turkey	Landrace	41	43
S. cereale dighoricum	1	IPK	R0250	Unknown	Wild		
S. cereale segetale	12	USDA	PI 326284	Azerbaijan	Wild	42	47
S. cereale segetale	12	USDA	PI 326286	Kazakhstan	Wild	48	68
S. cereale segetale	2	USDA	PI 618671	Turkey	Wild	39	35
S. cereale segetale	6	USDA	PI 618673	Turkey	Wild	38	33
S. cereale segetale	1	IPK	R0061	Russian Federation	Wild	60	100
S. cereale segetale	1	IPK	R0279	Turkey	Wild	40	36
S. cereale segetale	1	IPK	R0607	Slovakia	Wild	49	20
S. cereale segetale	1	IPK	R0788	Spain	Wild	40	-4
S. cereale ssp. *afghanicum*	1	IPK	R0567	Afghanistan	Wild	35	65
S. cereale ssp. *afghanicum*	1	IPK	R0569	Afghanistan	Wild	36	65
S. cereale ssp. *afghanicum*	1	IPK	R1038	Afghanistan	Wild	36	65
S. strictum anatolicum	1	IPK	R1055	Armenia	Wild	41	46
S. strictum anatolicum	12	USDA	PI 445973	United States	Wild	38	-98
S. strictum irmanuso	1	IPK	R0858	Italy	Wild	44	12
S. strictum kuprijanovii	1	IPK	R0590	Russian Federation	Wild	64	104

Table A1. Accessions used in the study (Cont.).

Taxon	N	Source	Accession	Origin	Type	Lat.	Lon.
S. strictum kuprijanovii	1	IPK	R1053	Slovenia	Wild	46	15
S. strictum kuprijanovii	1	IPK	R1056	Azerbaijan	Wild	41	48
S. strictum kuprijanovii	1	IPK	R1151	Kazakhistan	Wild	48	68
S. strictum kuprijanovii	1	IPK	R1154	Russian Federation	Wild	57	101
S. strictum kuprijanovii	1	IPK	R579	Azerbaijan	Wild	41	48
S. strictum strictum	10	USDA	PI 205222	Eskisehir, Turkey	Wild	40	31
S. strictum strictum	5	USDA	PI 253956	Dahuk, Iraq	Wild	37	43
S. strictum strictum	13	USDA	PI 401402	Lorestan, Iran	Wild	33	49
S. strictum strictum	7	USDA	PI 401404	East Azerbaijan	Wild	38	46
S. strictum strictum	10	USDA	PI 531829	Armenia	Wild	41	45
S. strictum strictum	13	USDA	PI 568257	Russian Federation	Cultivar	60	47
S. strictum strictum	1	IPK	R0914	Italy	Wild	42	15
S. strictum strictum	1	IPK	R0920	Italy	Wild	42	14
S. strictum strictum	1	IPK	R0939	Italy	Wild	42	13
S. strictum strictum	1	IPK	R1000	Italy	Wild	43	12
S. strictum strictum	1	IPK	R1047	Armenia	Wild	40	45
S. strictum strictum	5	USDA	PI 383757	Erzurum, Turkey	Wild	40	41
S. strictum x cereale	1	IPK	R0278	Turkey	Hybrid	41	37
S. sylvestre	1	IPK	R1045	Hungary	Wild	47	19
S. sylvestre	1	IPK	R1046	Romania	Wild	46	25
S. vavilovii	13	USDA	PI 253957	Afghanistan	Wild	34	68
S. vavilovii	11	USDA	PI 284842	Hungary	Wild	47	20
S. vavilovii	9	USDA	PI 573648	Russian Federation	Wild	43	44
S. vavilovii	8	USDA	PI 573649	Afghanistan	Wild	35	66
S. vavilovii	1	IPK	R1027	Italy	Wild	44	13
S. vavilovii	1	IPK	R1063	Poland	Wild	52	21
S. vavilovii	1	IPK	R1125	Turkey	Wild	41	35
S. vavilovii	1	IPK	R1126	Turkey	Wild	41	34
S. vavilovii	1	IPK	R2433	Unknown	Wild		
S. vavilovii x cereale	1	IPK	R0227	Unknown	Hybrid		
S. vavilovii x cereale	1	IPK	R1064	Poland	Hybrid	52	20
S. vavilovii x cereale	1	IPK	R1127	Turkey	Hybrid	38	36
S. vavilovii x cereale	1	IPK	R1128	Russian Federation	Hybrid	58	98
S. vavilovii x cereale	1	IPK	R1156	Russian Federation	Hybrid	56	100
S. vavilovii x cereale	1	IPK	R2432	Afghanistan	Hybrid	37	65
S. vavilovii x cereale	1	IPK	R2434	Russian Federation	Hybrid	60	104

Table A2. Cultivated rye populations used in the genetic diversity analysis

Region	Origin	Cultivation	Lat.	Lon.	Accesion No
Middle East	Afyon, Turkey	Landrace	39	31	PI 168211
	Bursa, Turkey	Landrace	40	29	PI 168168
	Corum, Turkey	Landrace	40	34	PI 168205
	Eskisehir, Turkey	Landrace	39	32	PI 252002
	Isparta, Turkey	Landrace	38	31	PI 168199
	Izmir, Turkey	Landrace	38	27	PI 168136
	Kayseri, Turkey	Landrace	39	36	PI 168218
	Kirsehir, Turkey	Landrace	39	34	PI 168213
	Konya, Turkey	Landrace	38	32	PI 168176
	Kutahya, Turkey	Landrace	39	30	PI 168209
	Nigde, Turkey	Landrace	38	34	PI 168220
	Ordu, Turkey	Landrace	41	37	PI 173589
	Bakhtiari va Chahar, Iran	Landrace	32	51	PI 227870
	Bakhtaran, Iran	Landrace	34	48	PI 268281
	Khorasan, Iran	Landrace	36	60	PI 289814
	Semnan, Iran	Landrace	36	53	PI 429371
	Zanjan, Iran	Landrace	36	48	PI 429373
	Bitlis, Turkey	Landrace	39	42	PI 561802
	Bolu, Turkey	Landrace	41	32	PI 561796
	Erzurum, Turkey	Landrace	40	41	PI 561797
	Bitlis, Turkey	Landrace	39	42,3	PI 561802
	Bolu, Turkey	Landrace	41	32,2	PI 561797
	Manisa, Turkey	Landrace	39	28	PI 561793
	Sinop, Turkey	Landrace	42	34,7	PI 561798
	Van, Turkey	Landrace	38	43,4	PI 561804
	Hamadan, Iran	Landrace	35	49	PI 429377
	Israel	Cultivar	32	35	PI 445977
	Gökova/Turkey	Landrace	37	29	TR1
	Kars/Turkey	Landrace	41	43	TR2
Africa	Transvaal, South Africa	Cultivar	-25	30	PI 330407

Table A2. Cultivated rye populations used in the genetic diversity analysis (Cont.)

Region	Origin	Cultivation	Lat.	Lon.	Accesion No
Australia	Australia	Cultivar	-25	135	CIse 79
	Capital Terr., Australia	Cultivar	-35	149	PI 345739
	New Zealand	Cultivar	-42	174	PI 446027
Balkan	Edirne, Turkey	Landrace	41	27	PI 168196
	Kirklareli, Turkey	Landrace	42	28	PI 168195
	Bosnia and Herzegovina	Landrace	43	19	PI 344970
	Montenegro	Landrace	43	19	PI 344975
	Serbia	Landrace	43	21	PI 344990
	Macedonia	Landrace	42	22	PI 405812
	Yugoslavia	Landrace	43	20	PI 535147
Caucasus	Goergia	Landrace	42	46	PI 584782
	East Azerbaijan, Iran	Landrace	38	47	PI 250745
East Asia	Japan	Cultivar	36	138	CIse 108
	South Korea	Cultivar	37	128	CIse 110
	Xinjiang, China	Cultivar	44	87	PI 447337
Europe	Switzerland	Cultivar	47	8	TR4
	Lublin, Poland	Cultivar	51	19	PI 323450
	England/UK	Cultivar	53	-2	PI 330526
	Malmohus, Sweden	Cultivar	56	14	PI 445996
	Germany	Cultivar	51	10	TR3
North America	Mexico	Cultivar	19	-99	PI 446023
	USA	Cultivar	40	-99	CIse 12
	Ontario, Canada	Cultivar	50	-86	PI 261400
South America	Colonia, Uruguay	Cultivar	-34	-58	PI 240675
	Sao Paulo, Brazil	Cultivar	-22	-49	PI 314964
	Los Lagos, Chile	Landrace	-40	-73	PI 436190
	Argentina	Cultivar	-35	-64	PI 543398
South and Central Asia	Afghanistan	Landrace	34	66	CIse 37
	North-West Frontier, Pakistan	Landrace	35	73	PI 218110
	Kandahar, Afghanistan	Landrace	32	66	PI 220118
	Kazakhstan	Cultivar	48	68	PI 234655
	Azad Kashmir, Pakistan	Landrace	34	73	PI 410534
	Himachal Pradesh, India	Landrace	33	77	PI 430003

APPENDIX B: MORPHOLOGICAL ANALYSIS

Table B1. Morphological characters evaluated in the study.

Character	Explanation	Catagories
Growth Habit	Appearance of the young plant during tillering	Upright, intermediate, or postrate
Stem Color	Stem color during early development	White or purple
Leaf Width	Leaf width of each population	Thin, intermediate, or wide
Stem Rust Susceptibility	Susceptibility to stem rust	Not infected, mildly infected or infected
Days to Ear Emergence	Days from sowing to when the ears of approximately half of the plants had emerged	
Plant Height	The height of five typical mature plants f from ground to top	cm
Length of Spike	The lengths of five typical spikes	cm
Hairiness of Stem	The stem hairiness below ear	Hardly visible, weak, intermediate or strong
Length of Awns	Length of awns of five typical spikes from each growing accessions	Short, intermediate, conspicuous
Number of Spikelets	The number of spikelets per spike	
Shattering of Ear	Fragility of rachis in mature spike was evaluated for five individual spikes	If no part disintegrated non-fragile If the upper 1/3 weak shatteringand If 1/2 disintegrated medium shattering If the whole spike disintegrated, shattering
Ear Erectness	The erectness of the ear	Erect, semi-bent or bent
Seed Color	The color of the seeds at maturity	Dark, light
Seed size	The size of seeds at maturity	Big, intermediate or small
Seed Shape	The shape of seeds at maturity	Ovate, Ovate-oblangate, Compressed
Attachment of Glumes	The attachment status of glumes around the seeds	Tightly attached, attached, loosely attached, or free threshing

Table B2. Pre-harvesting morphological characters.

Accession No	Taxon	Growth habit	Stem color	Rust	Leaf width	Days to ear emergence
R567	*S. cereale afghanicum*	Postrate	White	Infected	Intermediate	146
R569	*S. cereale afghanicum*	Postrate	White	Infected	Intermediate	142
R1038	*S. cereale afghanicum*	Upright	White	Infected	Intermediate	146
PI 618666	*S. cereale ancestrale*	Intermediate	Purple	Mild	Intermediate	153
R62	*S. cereale ancestrale*	Postrate	Purple	Mild	Intermediate	157
R1054	*S. cereale ancestrale*	Intermediate	Purple	Mild	Thin	153
R767	*S. cereale ancestrale*	Postrate	Purple	Mild	Thin	156
R29	*S. cereale ancestrale*	Postrate	Purple	Mild	Thin	165
PI 568118	*S. cereale cereale*	Intermediate	Purple	none	Thin	132
PI 561802	*S. cereale cereale*	Upright	White	none	Thin	136
PI 561796	*S. cereale cereale*	Intermediate	Purple	none	Intermediate	133
PI 561797	*S. cereale cereale*	Intermediate	Purple	none	Intermediate	131
PI 561806	*S. cereale cereale*	Postrate	Purple	none	Thin	144
PI 561793	*S. cereale cereale*	Intermediate	White	Mild	Intermediate	135
PI 168181	*S. cereale cereale*	Postrate	Purple	none	Intermediate	133
PI 561799	*S. cereale cereale*	Intermediate	White	none	Wide	136
PI 561804	*S. cereale cereale*	Intermediate	Purple	none	Wide	131
PI 561809	*S. cereale cereale*	Upright	White	none	Wide	131
PI 584788	*S. cereale cereale*	Postrate	White	none	Thin	148
R1148	*S. cereale cereale*	Intermediate	White	none	Thin	134
PI 168211	*S. cereale cereale*	Intermediate	White	none	Intermediate	142
PI 173587	*S. cereale cereale*	Upright	Purple	Mild	Intermediate	147
PI 205221	*S. cereale cereale*	Upright	White	none	Intermediate	143
PI 168130	*S. cereale cereale*	Intermediate	White	none	Thin	142
PI 168168	*S. cereale cereale*	Upright	Purple	none	Wide	122
PI 168205	*S. cereale cereale*	Intermediate	Purple	none	Thin	138
PI 168196	*S. cereale cereale*	Postrate	Purple	Mild	Intermediate	133
PI 252002	*S. cereale cereale*	Intermediate	Purple	none	Intermediate	130
PI 168199	*S. cereale cereale*	ND.	ND.	ND.	ND.	ND.
PI 168136	*S. cereale cereale*	Intermediate	White	none	Wide	123
PI 168218	*S. cereale cereale*	Postrate	Purple	none	Intermediate	135
PI 168195	*S. cereale cereale*	Intermediate	White	Mild	Intermediate	135
PI 168213	*S. cereale cereale*	Intermediate	White	Mild	Wide	123
PI 168176	*S. cereale cereale*	Upright	White	none	Intermediate	126
PI 168209	*S. cereale cereale*	Postrate	Purple	Mild	Thin	136
PI 168220	*S. cereale cereale*	Intermediate	White	none	Intermediate	135
PI 173589	*S. cereale cereale*	Intermediate	Purple	none	Wide	131
PI 168181	*S. cereale cereale*	Postrate	White	none	Thin	139
PI 168194	*S. cereale cereale*	Intermediate	Purple	Mild	Intermediate	131
PI 227870	*S. cereale cereale*	Intermediate	Purple	none	Intermediate	141
PI 228360	*S. cereale cereale*	Upright	White	Mild	Intermediate	132
PI 243741	*S. cereale cereale*	Upright	White	Infected	Intermediate	132
PI 250745	*S. cereale cereale*	Postrate	White	Mild	Thin	139
PI 268281	*S. cereale cereale*	Intermediate	White	Infected	Intermediate	132
PI 289814	*S. cereale cereale*	Intermediate	White	none	Intermediate	133
PI 429371	*S. cereale cereale*	Postrate	White	none	Thin	138
PI 429373	*S. cereale cereale*	Intermediate	White	Mild	Intermediate	139
PI 429377	*S. cereale cereale*	Postrate	White	Infected	Intermediate	134

Table B2. Pre-harvesting morphological characters (Cont.).

Accession No	Taxon	Growth habit	Stem color	Rust	Leaf width	Days to ear emergence
PI 445977	*S. cereale cereale*	Upright	White	none	Wide	135
PI 323450	*S. cereale cereale*	Intermediate	Purple	Infected	Thin	136
PI 330526	*S. cereale cereale*	Intermediate	Purple	Infected	Intermediate	137
PI 344979	*S. cereale cereale*	Postrate	Purple	Infected	Intermediate	134
PI 344975	*S. cereale cereale*	Intermediate	White	Infected	Intermediate	133
PI 344990	*S. cereale cereale*	Postrate	White	none	Intermediate	132
PI 405812	*S. cereale cereale*	Postrate	Purple	none	Thin	132
PI 445996	*S. cereale cereale*	Postrate	Purple	Infected	Thin	142
PI 535147	*S. cereale cereale*	Postrate	Purple	Infected	Thin	154
PI 240675	*S. cereale cereale*	Upright	White	Mild	Wide	116
PI 314964	*S. cereale cereale*	Upright	White	none	Wide	116
PI 436190	*S. cereale cereale*	Postrate	Purple	none	Intermediate	137
PI 446023	*S. cereale cereale*	Intermediate	White	Infected	Thin	124
PI 543398	*S. cereale cereale*	Intermediate	White	Infected	Thin	127
CIse 12	*S. cereale cereale*	Postrate	Purple	none	Thin	139
PI 261400	*S. cereale cereale*	Postrate	Purple	Infected	Thin	149
CIse 37	*S. cereale cereale*	Postrate	Purple	none	Intermediate	131
CIse 108	*S. cereale cereale*	Intermediate	Purple	Mild	Intermediate	132
CIse 110	*S. cereale cereale*	Intermediate	Purple	Infected	Thin	127
PI 218110	*S. cereale cereale*	Intermediate	White	Infected	Thin	134
PI 220118	*S. cereale cereale*	Postrate	Purple	Infected	Thin	137
PI 234655	*S. cereale cereale*	Postrate	White	Infected	Intermediate	148
PI 410534	*S. cereale cereale*	Upright	White	Infected	Wide	131
PI 430003	*S. cereale cereale*	Intermediate	Purple	none	Intermediate	127
PI 447337	*S. cereale cereale*	Postrate	Purple	Infected	Thin	140
CIse 79	*S. cereale cereale*	Intermediate	White	Infected	Intermediate	134
PI 330407	*S. cereale cereale*	Intermediate	White	none	Intermediate	134
PI 345739	*S. cereale cereale*	Postrate	Purple	Mild	Intermediate	135
PI 446027	*S. cereale cereale*	Upright	White	Mild	Wide	123
TR1	*S. cereale cereale*	Upright	White	none	Wide	118
TR2	*S. cereale cereale*	Intermediate	White	none	Thin	133
R1133	*S. cereale cereale*	Intermediate	Purple	Mild	Intermediate	143
R1489	*S. cereale cereale*	Postrate	White	Mild	Intermediate	149
R2022	*S. cereale cereale*	Intermediate	Purple	Mild	Intermediate	ND.
R1756	*S. cereale cereale*	Intermediate	Purple	none	Thin	161
R1490	*S. cereale cereale*	Intermediate	White	none	Thin	145
R2199	*S. cereale cereale*	Intermediate	White	none	Thin	138
R1151	*S. cereale cereale*	Intermediate	Purple	Mild	Wide	131
R1480	*S. cereale cereale*	Postrate	White	Mild	Thin	156
R1092	*S. cereale cereale*	Upright	Purple	none	Wide	134
R1658	*S. cereale cereale*	Intermediate	Purple	Infected	Wide	135
R1653	*S. cereale cereale*	Postrate	Purple	Mild	Thin	154
R191	*S. cereale cereale*	Postrate	White	none	Thin	159
PI 326284	*S. cereale segetale*	Postrate	Purple	none	Wide	130
PI 326286	*S. cereale segetale*	Postrate	Purple	none	Thin	154
PI 618671	*S. cereale segetale*	Intermediate	Purple	Mild	Intermediate	142
PI 618673	*S. cereale segetale*	Intermediate	Purple	Infected	Intermediate	142
R279	*S. cereale segetale*	Intermediate	White	Mild	Thin	124

Table B2. Pre-harvesting morphological characters (Cont.).

Accession No	Taxon	Growth habit	Stem color	Rust	Leaf width	Days to ear emergence
R788	*S. cereale segetale*	Upright	Purple	none	Intermediate	131
R607	*S. cereale segetale*	Postrate	Purple	none	Thin	149
R61	*S. cereale segetale*	Intermediate	White	Infected	Intermediate	144
PI 205222	*S. strictum strictum*	Intermediate	Purple	none	Thin	ND.
PI 253956	*S. strictum strictum*	Intermediate	Purple	none	Thin	ND.
PI 253956	*S. strictum strictum*	Postrate	Purple	none	Thin	ND.
PI 383757	*S. strictum strictum*	Intermediate	Purple	none	Thin	ND.
PI 253956	*S. strictum strictum*	ND.	ND.	ND.	ND.	ND.
PI 401402	*S. strictum strictum*	ND.	ND.	ND.	ND.	ND.
PI 401404	*S. strictum strictum*	Intermediate	Purple	none	Thin	ND.
PI 531829	*S. strictum strictum*	Postrate	Purple	Infected	Thin	144
PI 568257	*S. strictum strictum*	Upright	Purple	Mild	Thin	ND.
R1000	*S. strictum strictum*	Postrate	Purple	none	Thin	ND.
PI 445973	*S. strictum anatolicum*	Intermediate	White	Mild	Intermediate	134
R1055	*S. strictum anatolicum*	Postrate	Purple	Mild	Thin	ND.
R579	*S. strictum kuprijanovii*	Upright	Purple	Mild	Thin	ND.
R1056	*S. strictum kuprijanovii*	Upright	Purple	Mild	Thin	ND.
R590	*S. strictum kuprijanovii*	Postrate	White	Mild	Intermediate	ND.
R1151	*S. strictum kuprijanovii*	ND.	ND.	ND.	ND.	ND.
R1154	*S. strictum kuprijanovii*	Intermediate	Purple	none	Thin	ND.
R1053	*S. strictum kuprijanovii*	Intermediate	Purple	none	Thin	ND.
R939	*S. strictum strictum*	ND.	ND.	none	Thin	ND.
R1047	*S. strictum strictum*	Intermediate	Purple	none	Thin	ND.
R920	*S. strictum strictum*	ND.	ND.	ND.	ND.	ND.
R914	*S. strictum strictum*	Intermediate	White	none	Thin	ND.
R1046	*S. sylvestre*	Postrate	Purple	Infected	Thin	ND.
R1045	*S. sylvestre*	Intermediate	White	Infected	Thin	ND.
PI 253957	*S. vavilovii*	Postrate	White	Infected	Thin	142
PI 284842	*S. vavilovii*	Intermediate	White	Mild	Intermediate	134
PI 573648	*S. vavilovii*	Intermediate	White	none	Thin	153
PI 573649	*S. vavilovii*	Intermediate	Purple	Mild	Wide	133
R1063	*S. vavilovii*	Postrate	Purple	Infected	Thin	152
R1125	*S. vavilovii*	Postrate	White	Mild	Thin	153
R1027	*S. vavilovii*	Upright	White	Mild	Thin	132
R1126	*S. vavilovii*	Postrate	Purple	Infected	Thin	142
R2433	*S. vavilovii*	Intermediate	Purple	Infected	Intermediate	138
R2434	*S. vavilovii x cereale*	Intermediate	White	Infected	Intermediate	142
R2432	*S. vavilovii x cereale*	Intermediate	White	Infected	Thin	145
R1127	*S. vavilovii x cereale*	Intermediate	White	none	Thin	149
R1064	*S. vavilovii x cereale*	Postrate	White	Infected	Thin	161
R1128	*S. vavilovii x cereale*	Postrate	White	Infected	Thin	149
R227	*S. vavilovii x cereale*	Postrate	Purple	Mild	Thin	149
R1156	*S. vavilovii x cereale*	Upright	Purple	Mild	Thin	166
R278	*S. strictum x cereale*	Postrate	White	Infected	Thin	140

Table B3. Post-harvesting numeric morphological characters.

		Plant Height					Spike lenght					Number of Spikelets				
Accession No	**Taxon**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
R567	*S. cereale afghanicum*	130	132	137	135	140	19,5	16,3	15,0	16,3	12,3	56	52	46	50	58
R569	*S. cereale afghanicum*	169	156	159	161	164	14,2	14,5	14,3	15,0	14,2	50	52	56	54	52
R1038	*S. cereale afghanicum*	129	132	127	129	130	13,1	12,4	12,1	12,8	16,3	46	46	50	48	58
PI 618666	*S. cereale ancestrale*	163	170	164	168	165	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
R62	*S. cereale ancestrale*	162	164	170	171	171	17,2	18,0	17,6	19,0	18,2	46	50	50	52	50
R1054	*S. cereale ancestrale*	185	189	194	192	194	19,0	21,3	11,0	14,5	14,0	52	66	40	50	40
R767	*S. cereale ancestrale*	172	174	177	178	175	12,0	11,9	15,0	11,5	16,1	46	46	48	44	44
R29	*S. cereale ancestrale*	134	143	144	149	134	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
PI 568118	*S. cereale cereale*	166	178	192	184	187	21,9	17,5	13,5	15,9	16,9	64	53	48	52	54
PI 561802	*S. cereale cereale*	166	178	192	184	187	17,0	18,5	19,0	16,0	16,5	50	54	54	48	50
PI 561796	*S. cereale cereale*	166	178	192	184	187	20,3	18,5	16,3	16,5	18,0	52	48	50	44	40
PI 561797	*S. cereale cereale*	174	180	176	171	181	15,3	17,5	18,2	18,4	17,7	50	52	50	52	52
PI 561806	*S. cereale cereale*	149	154	163	138	126	15,0	17,1	20,5	20,2	20,4	40	60	68	62	54
PI 561793	*S. cereale cereale*	162	164	174	175	171	16,0	17,2	18,0	18,0	16,5	50	48	46	48	44
PI 168181	*S. cereale cereale*	170	185	188	173	182	14,5	15,4	18,5	18,3	16,7	44	46	54	52	52
PI 561799	*S. cereale cereale*	176	187	185	184	185	17,6	21,3	17,1	14,9	20,0	56	60	60	53	58
PI 561804	*S. cereale cereale*	135	137	140	143	147	15,0	14,7	14,5	15,5	15,1	42	46	50	52	46
PI 561809	*S. cereale cereale*	162	164	164	159	157	18,5	18,2	18,3	16,6	16,5	52	54	52	48	50
PI 584788	*S. cereale cereale*	124	128	130	138	139	13,6	14,2	14,0	18,3	16,4	54	56	50	64	62
R1148	*S. cereale cereale*	135	143	140	139	137	17,5	17,3	16,5	14,3	17,1	50	46	48	42	46
PI 168211	*S. cereale cereale*	180	175	168	170	169	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
PI 173587	*S. cereale cereale*	113	122	143	138	133	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
PI 205221	*S. cereale cereale*	144	148	150	158	161	17,3	19,7	16,0	14,2	16,2	54	56	44	42	50
PI 168130	*S. cereale cereale*	163	162	165	166	168	17,1	18,0	15,2	13,5	15,1	50	56	50	40	40
PI 168168	*S. cereale cereale*	170	190	191	183	184	15,3	17,5	16,5	15,5	16,1	46	50	48	44	46
PI 168205	*S. cereale cereale*	159	163	159	165	150	14,9	17,5	17,1	18,0	15,1	48	52	52	50	48

Table B3. Post-harvesting numeric morphological characters (Cont.).

		Plant Height					Spike lenght					Number of Spikelets				
Accession No	**Taxon**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
PI 168196	*S. cereale cereale*	191	194	195	193	186	16,7	15,7	19,0	19,3	18,3	60	50	58	60	53
PI 252002	*S. cereale cereale*	154	162	168	163	158	14,8	20,0	17,5	15,3	14,8	44	54	54	46	44
PI 168199	*S. cereale cereale*	ND.	ND.	ND.	ND.	ND.	15,4	15,2	16,2	18,4	17,9	50	48	46	54	54
PI 168136	*S. cereale cereale*	176	178	180	197	193	18,3	15,5	16,2	16,6	16,0	54	50	50	52	50
PI 168218	*S. cereale cereale*	154	158	157	158	167	15,3	14,8	15,2	13,7	19,2	46	52	46	44	58
PI 168195	*S. cereale cereale*	169	176	179	178	185	22,4	15,8	18,6	17,0	18,2	60	53	54	52	52
PI 168213	*S. cereale cereale*	170	175	175	174	169	22,0	22,3	21,7	19,6	22,2	60	64	64	60	62
PI 168176	*S. cereale cereale*	195	188	191	189	192	17,0	17,3	20,0	22,1	16,0	48	48	54	56	42
PI 168209	*S. cereale cereale*	160	158	155	159	152	16,0	18,9	16,6	19,3	21,7	44	54	50	50	58
PI 168220	*S. cereale cereale*	160	162	161	169	161	12,0	12,8	15,8	16,8	14,7	44	42	52	52	48
PI 173589	*S. cereale cereale*	178	177	176	179	175	17,1	20,2	15,2	16,0	13,9	52	56	50	49	46
PI 168181	*S. cereale cereale*	173	180	184	188	162	15,7	20,5	18,5	19,4	16,9	50	56	58	58	52
PI 168194	*S. cereale cereale*	171	177	179	172	173	15,4	19,3	15,4	16,9	19,5	52	60	54	58	58
PI 227870	*S. cereale cereale*	183	188	186	187	188	18,4	17,5	16,3	19,6	17,0	58	50	52	52	54
PI 228360	*S. cereale cereale*	189	192	191	188	187	18,0	17,7	16,4	17,8	14,9	58	46	44	50	42
PI 243741	*S. cereale cereale*	147	152	153	146	151	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
PI 250745	*S. cereale cereale*	161	162	160	170	177	19,0	18,5	17,0	16,2	16,5	58	52	54	50	58
PI 268281	*S. cereale cereale*	168	173	170	173	178	15,2	20,3	17,5	15,8	15,9	46	62	52	50	50
PI 289814	*S. cereale cereale*	164	165	168	162	164	17,3	17,4	18,2	13,6	14,0	54	58	52	42	48
PI 429371	*S. cereale cereale*	173	176	174	175	176	18,3	18,9	16,0	17,0	18,3	46	58	52	52	54
PI 429373	*S. cereale cereale*	168	162	168	161	164	16,7	16,0	18,5	20,3	17,6	50	58	62	60	54
PI 429377	*S. cereale cereale*	172	174	173	168	170	15,7	18,6	16,5	18,3	19,4	44	54	48	52	52
PI 445977	*S. cereale cereale*	194	183	192	182	185	17,0	16,5	16,3	17,9	16,0	50	50	48	52	48
PI 323450	*S. cereale cereale*	180	188	173	178	167	15,0	13,5	14,5	17,5	14,4	50	52	56	56	50
PI 330526	*S. cereale cereale*	173	180	178	164	175	14,3	18,0	17,0	17,0	17,0	46	60	58	58	60
PI 344979	*S. cereale cereale*	182	196	190	194	181	19,1	17,5	17,2	13,0	17,6	50	56	58	36	58

Table B3. Post-harvesting numeric morphological characters (Cont.).

		Plant Height					Spike lenght					Number of Spikelets				
Accession No	**Taxon**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
PI 344975	*S. cereale cereale*	173	175	178	180	171	17,5	18,1	17,7	17,1	20,5	52	52	54	44	54
PI 344990	*S. cereale cereale*	178	187	189	192	197	15,5	14,4	15,4	15,8	19,1	48	44	46	48	48
PI 405812	*S. cereale cereale*	176	183	195	205	207	19,2	18,0	14,9	17,2	17,0	58	48	42	48	50
PI 445996	*S. cereale cereale*	190	197	183	194	178	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
PI 535147	*S. cereale cereale*	131	136	150	163	149	21,0	18,5	17,5	17,3	18,3	60	62	54	56	52
PI 240675	*S. cereale cereale*	173	176	177	179	175	14,9	16,9	17,6	17,0	15,6	42	45	53	48	44
PI 314964	*S. cereale cereale*	170	169	162	172	170	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
PI 436190	*S. cereale cereale*	172	187	170	171	180	18,0	16,3	16,0	15,0	19,0	58	48	54	56	66
PI 446023	*S. cereale cereale*	179	170	181	189	175	14,5	16,5	14,0	16,5	15,2	46	58	50	56	52
PI 543398	*S. cereale cereale*	168	181	177	178	176	15,5	17,0	15,0	16,1	14,0	52	50	56	56	50
CIse 12	*S. cereale cereale*	151	154	160	158	163	17,5	18,3	13,5	13,1	16,1	68	60	54	56	62
PI 261400	*S. cereale cereale*	151	165	166	150	155	22,0	21,0	16,5	15,2	17,3	64	60	54	50	54
CIse 37	*S. cereale cereale*	170	172	176	175	173	19,1	17,8	15,1	19,6	18,9	58	56	50	56	58
CIse 108	*S. cereale cereale*	174	172	165	169	174	18,5	13,4	19,2	17,9	15,6	60	44	60	58	50
CIse 110	*S. cereale cereale*	169	176	170	177	182	14,1	14,0	15,2	14,1	16,1	50	52	50	48	50
PI 218110	*S. cereale cereale*	164	161	181	178	180	16,4	14,7	16,6	17,1	16,1	40	40	42	44	40
PI 220118	*S. cereale cereale*	165	173	164	172	162	18,0	17,0	16,9	16,4	15,7	56	54	49	48	52
PI 234655	*S. cereale cereale*	162	160	171	164	161	21,0	18,6	22,3	19,2	20,6	58	54	62	60	60
PI 410534	*S. cereale cereale*	166	148	151	149	145	17,7	20,3	15,1	17,6	14,9	54	60	49	50	54
PI 430003	*S. cereale cereale*	175	177	173	178	188	20,3	16,5	20,1	16,6	18,1	56	44	44	44	52
PI 447337	*S. cereale cereale*	156	157	174	156	144	15,5	17,0	19,0	13,0	18,3	60	54	66	48	50
CIse 79	*S. cereale cereale*	197	199	200	199	194	17,1	18,8	19,4	14,6	17,9	56	57	60	49	56
PI 330407	*S. cereale cereale*	194	198	190	194	189	21,0	17,2	19,5	18,3	19,4	64	56	60	58	60
PI 345739	*S. cereale cereale*	171	170	172	178	177	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
PI 446027	*S. cereale cereale*	170	173	171	178	184	19,0	16,0	18,0	17,5	17,0	56	46	48	46	46
TR1	*S. cereale cereale*	197	198	205	205	202	12,6	16,7	15,8	15,4	16,9	42	46	46	45	46

Table B3. Post-harvesting numeric morphological characters (Cont.).

		Plant Height					Spike lenght					Number of Spikelets				
Accession No	**Taxon**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
TR2	*S. cereale cereale*	155	156	142	145	150	15,4	16,2	18,5	15,2	16,0	48	52	58	54	54
R1133	*S. cereale cereale*	163	156	150	168	161	13,0	19,0	15,9	14,6	14,5	52	48	52	44	44
R1489	*S. cereale cereale*	130	132	128	129	134	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
R1756	*S. cereale cereale*	141	148	150	152	142	19,5	20,6	19,6	19,9	17,7	66	64	64	66	56
R1490	*S. cereale cereale*	138	142	142	146	143	18,5	19,3	18,3	14,4	16,3	62	56	54	44	54
R2199	*S. cereale cereale*	171	174	176	162	166	15,8	16,1	16,5	17,5	15,5	56	50	54	46	46
R1151	*S. cereale cereale*	158	165	165	168	169	15,5	16,5	15,2	12,5	16,5	44	52	54	40	52
R1480	*S. cereale cereale*	132	131	159	125	128	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
R1092	*S. cereale cereale*	183	186	183	180	181	15,8	17,0	14,5	19,0	16,0	46	52	44	40	46
R1658	*S. cereale cereale*	188	189	182	188	183	15,4	16,3	15,8	15,0	13,8	48	54	48	50	48
R1653	*S. cereale cereale*	142	146	136	124	123	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
R191	*S. cereale cereale*	115	114	116	110	112	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.	ND.
PI 326284	*S. cereale segetale*	184	187	189	192	190	20,8	18,0	18,5	18,0	17,4	60	56	58	52	52
PI 326286	*S. cereale segetale*	158	142	143	147	150	19,3	19,1	20,0	18,5	20,0	58	60	62	62	58
PI 618671	*S. cereale segetale*	130	130	138	139	137	14,4	15,7	16,5	19,3	15,5	50	58	50	63	52
PI 618673	*S. cereale segetale*	142	150	150	148	151	18,1	19,9	14,8	14,6	12,6	56	68	46	48	50
R279	*S. cereale segetale*	150	157	155	158	153	13,3	20,0	12,5	15,5	11,2	52	56	48	48	42
R788	*S. cereale segetale*	145	143	149	153	156	16,0	15,8	15,7	17,9	18,3	58	54	58	56	54
R607	*S. cereale segetale*	140	141	150	147	148	14,3	18,0	14,0	10,6	18,5	54	54	48	46	62
R61	*S. cereale segetale*	168	176	172	170	171	16,5	16,3	16,0	17,0	17,0	54	58	52	52	48
PI 531829	*S. strictum strictum*	145	147	144	147	142	14	16,2	14,4	14,5	15,5	34	52	40	34	32
PI 445973	*S. strictum anatolicum*	164	169	166	163	176	17,5	20,8	14,7	15,1	15,7	56	56	46	48	50
PI 253957	*S. vavilovii*	114	121	119	116	117	13,9	14,3	13,4	18,9	18,9	48	44	44	54	56
PI 284842	*S. vavilovii*	194	197	193	193	194	15,0	19,5	19,7	16,3	18,0	50	60	58	50	56
PI 573648	*S. vavilovii*	140	145	150	142	139	18,0	19,1	15,5	13,2	17,0	52	54	46	50	56
PI 573649	*S. vavilovii*	127	121	123	128	132	20,1	18,7	24,9	20,3	21,2	58	56	52	54	64

Table B3. Post-harvesting numeric morphological characters (Cont.).

		Plant Height					Spike lenght					Number of Spikelets				
Accession No	**Taxon**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
R1063	*S. vavilovii*	127	128	130	131	134	16,5	16,0	22,4	17,9	21,2	48	50	62	56	62
R1125	*S. vavilovii*	125	128	132	132	132	18,0	15,4	18,9	16,1	17,2	52	40	56	48	52
R1027	*S. vavilovii*	142	144	142	143	143	14,2	15,0	14,2	17,7	13,2	42	44	44	56	42
R1126	*S. vavilovii*	142	144	141	143	144	18,5	13,5	18,6	15,5	12,5	50	46	60	54	42
R2433	*S. vavilovii*	162	163	181	184	184	16,5	12,5	15,5	14,0	15,0	42	38	44	44	40
R2434	*S. vavilovii x cereale*	136	138	133	138	138	18,5	21,5	15,0	21,0	12,1	50	56	46	58	40
R2432	*S. vavilovii x cereale*	126	127	138	122	136	13,2	11,4	12,3	16,0	11,4	46	38	38	52	46
R1127	*S. vavilovii x cereale*	125	128	129	128	124	17,0	14,2	13,5	12,5	17,5	56	52	58	46	56
R1064	*S. vavilovii x cereale*	102	110	107	11	106	15,3	10,1	14,2	11,9	15,0	54	44	50	44	50
R1128	*S. vavilovii x cereale*	142	144	146	153	143	13,0	20,0	13,0	16,0	13,0	38	54	44	48	34
R227	*S. vavilovii x cereale*	151	158	161	159	149	14,5	17,0	16,5	19,5	19,5	42	48	46	60	56
R278	*S. strictum x cereale*	120	122	127	119	120	13,1	20,2	19,3	17,5	14,5	48	58	50	54	52

Table B4. Post-harvesting categorical morphological characters.

Accession No	Taxon	Lenght of awn	Stem hairness	Shattering	Ear erectness	Treshing	Seed color
R567	*S. cereale afghanicum*	Intermediate	Strong	Weak shattering	Semi-bent	Attached	Light
R569	*S. cereale afghanicum*	Conspicuous	Strong	Fragile	Semi-bent	Tightly attached	Dark
R1038	*S. cereale afghanicum*	Conspicuous	Strong	Totally fragile	Semi-bent	Tightly attached	Dark
R62	*S. cereale ancestrale*	Conspicuous	Intermediate	Totally fragile	Erect	Tightly attached	Dark
R1054	*S. cereale ancestrale*	Conspicuous	Intermediate	Totally fragile	Semi-bent	Tightly attached	Dark
R767	*S. cereale ancestrale*	Conspicuous	Intermediate	Totally fragile	Erect	Tightly attached	Dark
PI 568118	*S. cereale cereale*	Short	Intermediate	Non-fragile	Bent	Looseyl attached	Dark
PI 561802	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Erect	Looseyl attached	Light
PI 561796	*S. cereale cereale*	Intermediate	Strong	Weak shattering	Semi-bent	Looseyl attached	Light
PI 561797	*S. cereale cereale*	Short	Weak	Non-fragile	Semi-bent	Looseyl attached	Dark
PI 561806	*S. cereale cereale*	Intermediate	Strong	Non-fragile	Semi-bent	Looseyl attached	Light
PI 561793	*S. cereale cereale*	Conspicuous	Intermediate	Weak shattering	Semi-bent	Looseyl attached	Dark
PI 168181	*S. cereale cereale*	Short	Intermediate	Non-fragile	Erect	Looseyl attached	Light
PI 561799	*S. cereale cereale*	Short	Weak	Non-fragile	Semi-bent	Looseyl attached	Dark
PI 561804	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Semi-bent	Looseyl attached	Dark
PI 561809	*S. cereale cereale*	Short	Hardly visible	Weak shattering	Erect	Looseyl attached	Dark
PI 584788	*S. cereale cereale*	Short	Intermediate	Non-fragile	Erect	Looseyl attached	Light
R1148	*S. cereale cereale*	Intermediate	Intermediate	Weak shattering	Semi-bent	Looseyl attached	Light
PI 205221	*S. cereale cereale*	Short	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
PI 168130	*S. cereale cereale*	Conspicuous	Hardly visible	Non-fragile	Semi-bent	Free trashing	Dark
PI 168168	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
PI 168205	*S. cereale cereale*	Short	Weak	Non-fragile	Semi-bent	Free trashing	Light
PI 168196	*S. cereale cereale*	Short	Strong	Non-fragile	Semi-bent	Free trashing	Light
PI 252002	*S. cereale cereale*	Short	Weak	Non-fragile	Erect	Free trashing	Light
PI 168199	*S. cereale cereale*	Short	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
PI 168136	*S. cereale cereale*	Short	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
PI 168218	*S. cereale cereale*	Conspicuous	Strong	Non-fragile	Semi-bent	Free trashing	Light
PI 168195	*S. cereale cereale*	Short	Strong	Weak shattering	Semi-bent	Free trashing	Dark

Table B4. Post-harvesting categorical morphological characters (Cont.).

Accession No	Taxon	Lenght of awn	Stem hairness	Shattering	Ear erectness	Treshing	Seed color
PI 168213	*S. cereale cereale*	Intermediate	Strong	Non-fragile	Bent	Looseyl attached	Light
PI 168176	*S. cereale cereale*	Intermediate	Intermediate	Weak shattering	Semi-bent	Looseyl attached	Dark
PI 168209	*S. cereale cereale*	Short	Weak	Non-fragile	Semi-bent	Free trashing	Dark
PI 168220	*S. cereale cereale*	Short	Strong	Non-fragile	Erect	Looseyl attached	Dark
PI 173589	*S. cereale cereale*	Short	Strong	Non-fragile	Erect	Free trashing	Dark
PI 168181	*S. cereale cereale*	Short	Strong	Non-fragile	Semi-bent	Free trashing	Light
PI 168194	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
PI 227870	*S. cereale cereale*	Conspicuous	Weak	Non-fragile	Semi-bent	Free trashing	Light
PI 228360	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
PI 250745	*S. cereale cereale*	Conspicuous	Strong	Non-fragile	Erect	Free trashing	Light
PI 268281	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Erect	Looseyl attached	Dark
PI 289814	*S. cereale cereale*	Conspicuous	Weak	Non-fragile	Semi-bent	Free trashing	Dark
PI 429371	*S. cereale cereale*	Intermediate	Strong	Weak shattering	Semi-bent	Tightly attached	Light
PI 429373	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
PI 429377	*S. cereale cereale*	Conspicuous	Strong	Non-fragile	Semi-bent	Looseyl attached	Light
PI 445977	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
PI 323450	*S. cereale cereale*	Short	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
PI 330526	*S. cereale cereale*	Intermediate	Strong	Weak shattering	Bent	Tightly attached	Dark
PI 344979	*S. cereale cereale*	Short	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
PI 344975	*S. cereale cereale*	Conspicuous	Strong	Non-fragile	Semi-bent	Free trashing	Light
PI 344990	*S. cereale cereale*	Short	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
PI 405812	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Erect	Free trashing	Dark
PI 535147	*S. cereale cereale*	Conspicuous	Strong	Non-fragile	Semi-bent	Free trashing	Light
PI 240675	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	ND.	Light
PI 436190	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
PI 446023	*S. cereale cereale*	Conspicuous	Strong	Non-fragile	Erect	Free trashing	Dark
PI 543398	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
CIse 12	*S. cereale cereale*	Conspicuous	Weak	Non-fragile	Semi-bent	Free trashing	Light
PI 261400	*S. cereale cereale*	Intermediate	Weak	Non-fragile	Bent	Free trashing	Dark

Table B4. Post-harvesting categorical morphological characters (Cont.).

Accession No	Taxon	Lenght of awn	Stem hairness	Shattering	Ear erectness	Treshing	Seed color
CIse 37	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
CIse 108	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
CIse 110	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
PI 218110	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Erect	Looseyl attached	Light
PI 220118	*S. cereale cereale*	Short	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
PI 234655	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Erect	Looseyl attached	Dark
PI 410534	*S. cereale cereale*	Short	Weak	Non-fragile	Semi-bent	Looseyl attached	Dark
PI 430003	*S. cereale cereale*	Intermediate	Strong	Non-fragile	Semi-bent	Free trashing	Light
PI 447337	*S. cereale cereale*	Intermediate	Hardly visible	Non-fragile	Erect	Looseyl attached	Light
CIse 79	*S. cereale cereale*	Short	Weak	Non-fragile	Erect	Looseyl attached	Dark
PI 330407	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
PI 446027	*S. cereale cereale*	Intermediate	Intermediate	Non-fragile	Bent	Free trashing	Light
TR1	*S. cereale cereale*	Short	Intermediate	Non-fragile	Semi-bent	Free trashing	Light
TR2	*S. cereale cereale*	Conspicuous	Strong	Non-fragile	Semi-bent	Free trashing	Dark
R1133	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
R1756	*S. cereale cereale*	Conspicuous	Strong	Non-fragile	Semi-bent	Looseyl attached	Dark
R1490	*S. cereale cereale*	Conspicuous	Hardly visible	Non-fragile	Semi-bent	Looseyl attached	Dark
R2199	*S. cereale cereale*	Conspicuous	Weak	Non-fragile	Semi-bent	Looseyl attached	Light
R1151	*S. cereale cereale*	Short	Hardly visible	Non-fragile	Semi-bent	Looseyl attached	Light
R1092	*S. cereale cereale*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Free trashing	Dark
R1658	*S. cereale cereale*	Intermediate	Weak	Non-fragile	Semi-bent	Free trashing	Light
PI 326284	*S. cereale segetale*	Conspicuous	Intermediate	Weak shattering	Semi-bent	Attached	Light
PI 326286	*S. cereale segetale*	Intermediate	Intermediate	Weak shattering	Semi-bent	Attached	Light
PI 618671	*S. cereale segetale*	Conspicuous	Strong	Weak shattering	Semi-bent	Attached	Light
PI 618673	*S. cereale segetale*	Short	Strong	Weak shattering	Semi-bent	Attached	Light
R279	*S. cereale segetale*	Conspicuous	Weak	Weak shattering	Semi-bent	Attached	Light
R788	*S. cereale segetale*	Intermediate	Strong	Weak shattering	Semi-bent	Attached	Light
R607	*S. cereale segetale*	Conspicuous	Strong	Weak shattering	Semi-bent	Tightly attached	Dark
R61	*S. cereale segetale*	Intermediate	Strong	Weak shattering	Semi-bent	Attached	Light

Table B4. Post-harvesting categorical morphological characters (Cont.).

Accession No	Taxon	Lenght of awn	Stem hairness	Shattering	Ear erectness	Treshing	Seed color
PI 531829	*S. strictum strictum*	Conspicuous	Weak	Fragile	Erect	Tightly attached	Light
PI 445973	*S. strictum anatolicum*	Conspicuous	Intermediate	Fragile	Semi-bent	Tightly attached	Dark
R1046	*S. sylvestre*	Conspicuous	ND.	Totally fragile	ND.	Tighly atatched	Dark
PI 253957	*S. vavilovii*	Short	Hardly visible	Weak shattering	Semi-bent	ND.	Dark
PI 284842	*S. vavilovii*	Conspicuous	Intermediate	Non-fragile	Semi-bent	Looseyl attached	Dark
PI 573648	*S. vavilovii*	Intermediate	Intermediate	Fragile	Erect	Looseyl attached	Dark
PI 573649	*S. vavilovii*	Short	Strong	Fragile	Semi-bent	Free trashing	Dark
R1063	*S. vavilovii*	Intermediate	Hardly visible	Fragile	Erect	Tightly attached	Dark
R1125	*S. vavilovii*	Conspicuous	Intermediate	Totally fragile	Erect	Tightly attached	Light
R1027	*S. vavilovii*	Conspicuous	Strong	Weak shattering	Semi-bent	Tightly attached	Light
R1126	*S. vavilovii*	Conspicuous	Intermediate	Fragile	Erect	Tightly attached	Light
R2433	*S. vavilovii*	Intermediate	Intermediate	Non-fragile	Semi-bent	Looseyl attached	Light
R2434	*S. vavilovii x cereale*	Conspicuous	Intermediate	Fragile	Semi-bent	Tightly attached	Dark
R2432	*S. vavilovii x cereale*	Short	Hardly visible	Fragile	Erect	Tightly attached	Dark
R1127	*S. vavilovii x cereale*	Short	Intermediate	Fragile	Erect	Tightly attached	Light
R1064	*S. vavilovii x cereale*	Short	Weak	Non-fragile	Erect	Attached	Dark
R1128	*S. vavilovii x cereale*	Intermediate	Intermediate	Fragile	Semi-bent	Tightly attached	Dark
R227	*S. vavilovii x cereale*	Intermediate	Intermediate	Fragile	Semi-bent	Looseyl attached	Dark
R278	*S. strictum x cereale*	Conspicuous	Weak	Weak shattering	Erect	Tightly attached	Dark

APPENDIX C: ALLELE FREQUENCIES WITH GRAPHS BY SUBSPECIES AND LOCUS

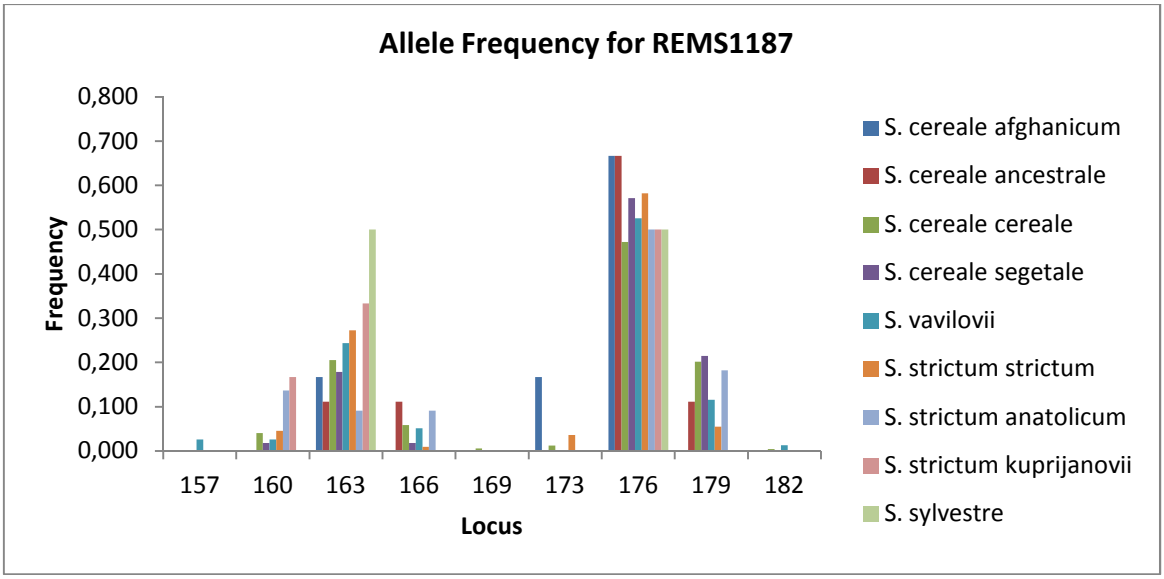

Figure C1. Allele frequencies for REMS1187.

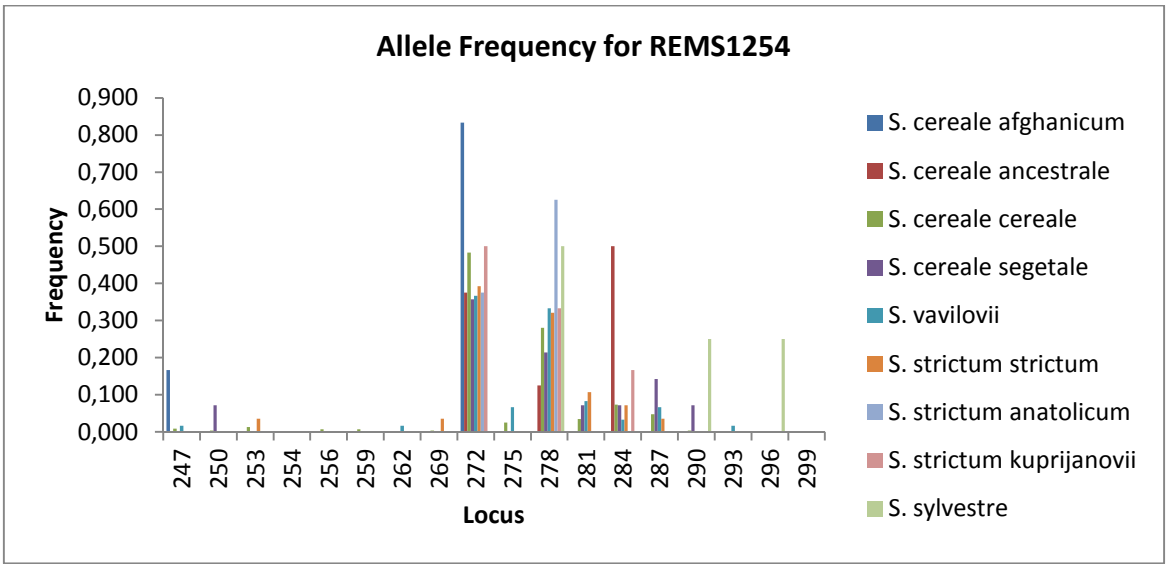

Figure C2. Allele frequencies for REMS1254.

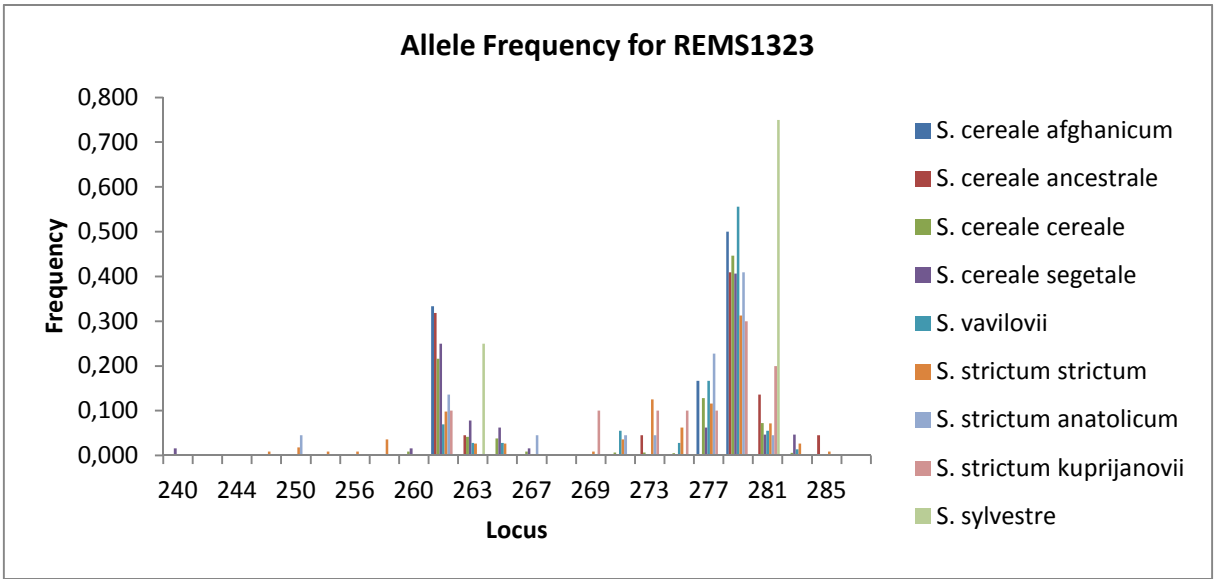

Figure C3. Allele frequencies for REMS1323.

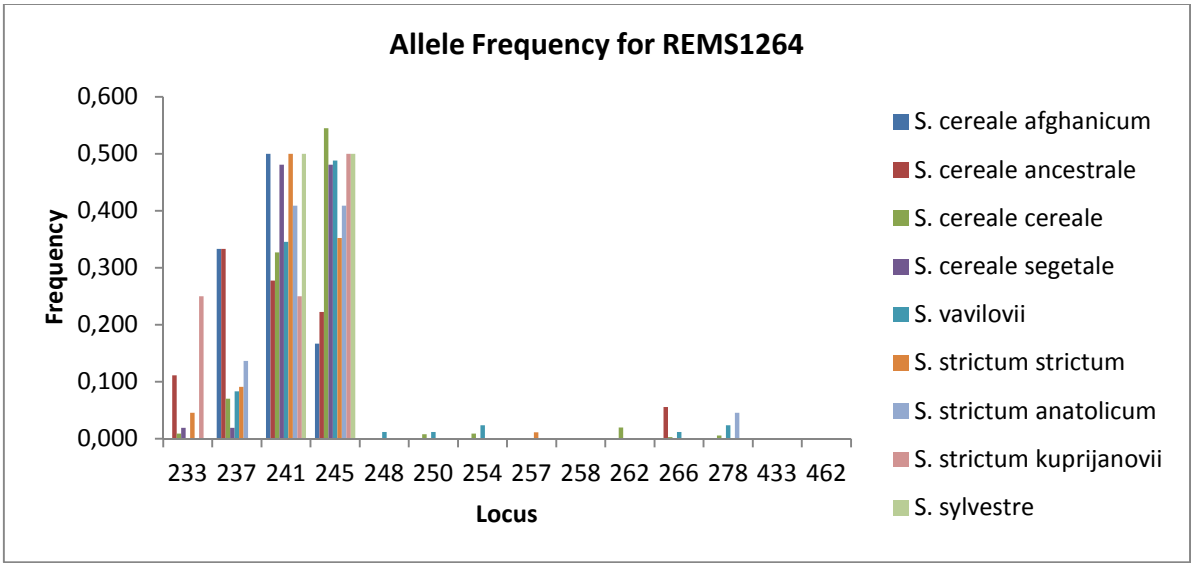

Figure C4. Allele frequencies for REMS1264.

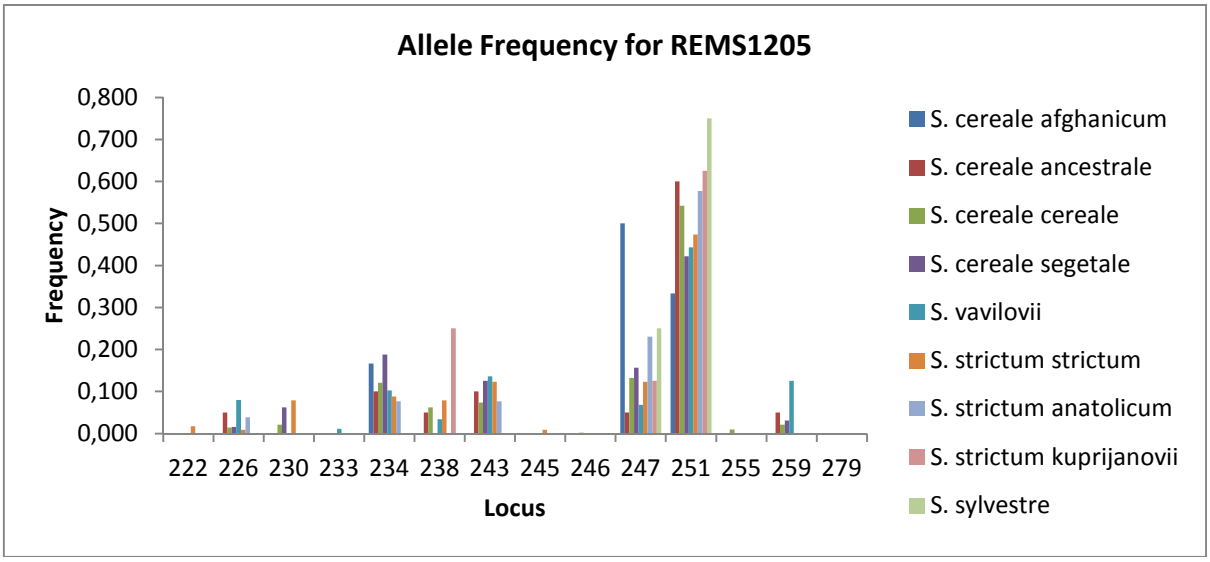

Figure C5. Allele frequencies for REMS1205.

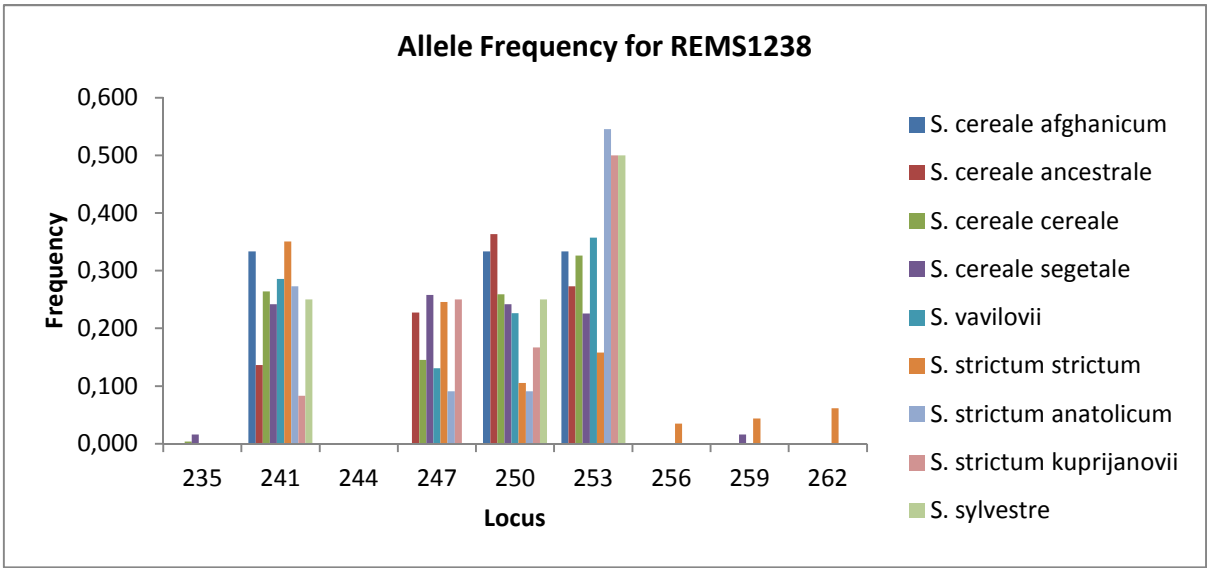

Figure C6. Allele frequencies for REMS1238.

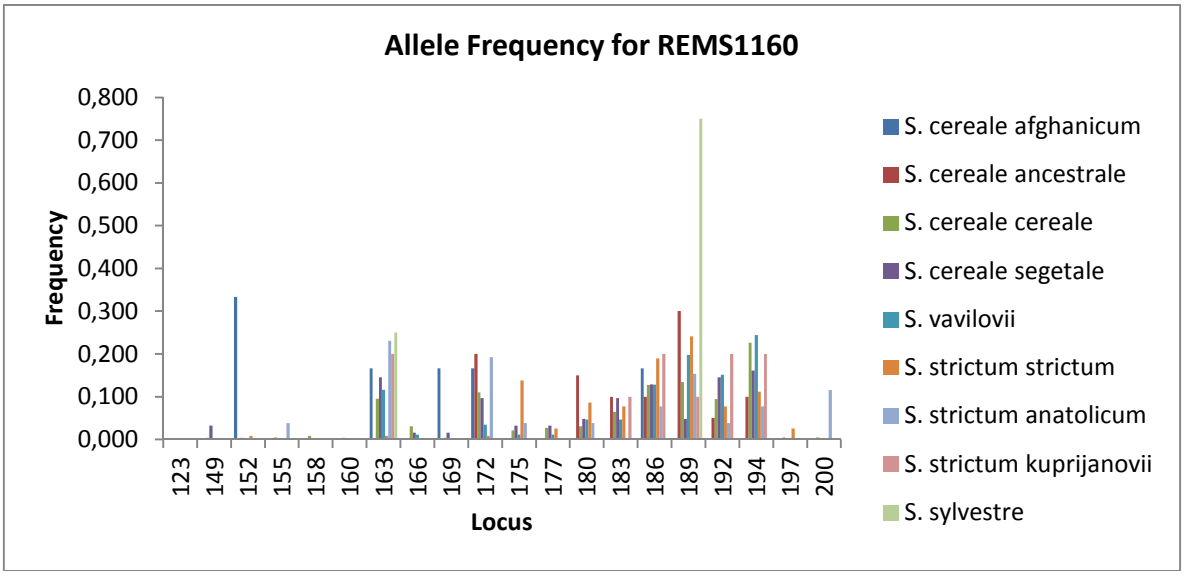

Figure C7. Allele frequencies for REMS1160.