6 Secale

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6.1 Introduction

6.1.1 Morphology

The genus *Secale* includes annual and perennial taxa, which generally contain two hermaphroditic florets in each spikelet. The lower leaf sheaths and blades are generally somewhat hairy, and a thin layer of wax is often seen, especially around the nodes. Most taxa are allogamous with rather long anthers (5 to 14 mm long). Only *S. sylvestre* is morphologically distinct from the other taxa, with the glume awn being several times longer in *S. sylvestre* than in any other taxa of *Secale* (Frederiksen and Petersen 1997). In addition, pollen of *S. sylvestre* is nearly spherical, whereas all other taxa have ovoid pollen (Frederiksen and Petersen 1997).

6.1.2 Cytology

The genus *Secale* is composed of diploid species with $2n = 2x = 14$ (Jain 1960; Petersen 1991). The genome has been designated R (Wang et al. 1996). However, some tetraploid strains have been created in cultivated rye (*S. cereale* L.). Giemsa banding or C-banding of chromosomes has shown the genus *Secale* to be characterized by large telomeric heterochromatic bands and a number of weak interstitial bands. The chromosomes of *S. sylvestre* differ from those of other taxa in the genus by their low number of interstitial bands and the small size of the telomeric bands (Singh and Röbbelen 1977). *Secale cereale* exhibits great variability in giemsa banding pattern, which is consistent with observations of such patterns in other allogamous species (Linde-Laursen et al. 1980). The distribution of some highly repeated DNA sequences detected by *in situ* hybridization (ISH) has shown that the self-pollinated annuals *S. sylvestre* and *S. vavilovii* contain considerably fewer repeated DNA sequences than the remaining taxa (Cuadrado and Jouve 1997). Karyotypes of some taxa are also known to differ from each other by a number of translocations (Riley 1955; Khush and Stebbins 1961; Khush 1962).

6.1.3 Origin of Cultivated Rye

There is general agreement that the weedy ryes of Central and Southeast Asia are direct progenitors of cultivated rye (Vavilov 1917, 1926). The weedy ryes taxonomically belong to *S. cereale*, occur only in connection with agriculture, and are much younger than wild species of the genus. However, there has been extensive disagreement about the ancestry of *S. cereale*. Vavilov (1926) and Roshevitz (1947) considered *S. vavilovii* to be the ancestor of *S. cereale*, and *S. vavilovii* to have evolved from *S. montanum*. Zhukhovsky (1933) and Schiemann (1948) showed that *S. cereale* descended from *S. ancestrale,* and that *S. ancestrale* and *S. montanum* diverged from a common ancestor. Riley (1955) concluded that *S. cereale* originated from *S. montanum* due to the chance fixation of two translocations. Stutz (1957) regarded *S. cereale* as a product of a hybridization involving *S. montanum* and *S. sylvestre* because *S. sylvestre* and *S. cereale* have the same chromosome arrangement. After considering ecological preferences, breeding habits, geographical distribution, and morphological and cytological affinities of wild species and cultivated rye, Khush and Stebbins (1961) concluded that *S. cereale* evolved from *S. montanum* as a result of progressive cytological and morphological differentiation and that this differentiation was probably facilitated by adaptive superiority of translocation heterozygotes and rearrangement ho-

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mozygotes. Furthermore, Khush (1962) showed that on the basis of geographical distribution, breeding system, growth habit, morphology, crossability, cytological, and genetic affinities, *S. sylvestre* differed from all of the other *Secale* species rather strikingly, while the other species seemed to be more closely related. It was suggested that *S. sylvestre* differentiated from *S. montanum* much earlier than the other species. In addition, Khush (1962) demonstrated *S. cereale* ssp. *segetale* was the immediate ancestor of cultivated ryes.

Based on extensive cytological, ecological, and morphological studies, Stutz (1972) concluded that cultivated rye originated from weedy products, which were derived from the introgression of *S. montanum* into *S. vavilovii*. *Secale vavilovii* appears to have been derived from *S. sylvestre* as a consequence of chromosomal translocations. *Secale sylvestre* was derived from *S. montanum* or a common ancestor. *Secale africanum, S. dalmaticum, S. ciliatoglume*, and *S. kuprijanovii* appeared to be only slightly modified, isolated populations of *S. montanum* (Fig. 1).

Isozyme data showed that *S. cereale* and *S. montanum* were closely related and genetically similar. These data supported the opinion that *S. vavilovii* and *S. sylvestre* originated from *S. montanum*, the oldest species of the genus (Vences et al. 1987). In contrast, most molecular data showed that *S. sylvestre* was the most ancient species (Reddy et al. 1990; Petersen and Doebley 1993; Pozo et al. 1995). A recent study by Cuadrado and Jouve (1997) was in accordance with prior molecular data and showed that the lack of a 480-bp repeated sequence in all telomeres of *S. sylvestre* supported the early separation and clear distinction of this species from the rest of *Secale* species. *S. vavilovii*, which possesses the 480-bp repeat family in the telomere, was considered to be more evolved than *S. sylvestre*. *S. cereale, S. montanum*, and *S. kuprijanovii*, which showed amplification and complex organization of repeated sequence families in the telomeres, interstitial formation, and a tendency toward the doubling of loci for the 120- and 480-bp sequences, were considered the most evolved species. The appearance of a new locus or 5S rRNA in *S. cereale* and *S. ancestrale* suggested that cultivated ryes evolved from this weedy species.

Phylogenetic relationships among the *Secale* species based on the presence and distribution of two simple sequence repeats (SSRs), three highly repeated sequences from rye, and 5S rDNA supported the notion that *S. sylvestre* had split off from *S. strictum* in the Miocene Period (Cuadrado and Jouve 2002). The second stage in the evolution of the genus occurred in the Pleistocene Period, after the geographical separation of the perennial species *S. africanum*. A similar pattern of distribution of the clusters (AAC)n, (AAG)n, and the wild rye species demonstrated that *S. sylvestre* was the species that showed the greatest number of comparative sequence differences and therefore was the most distant of all the taxonomic units analyzed. *S. strictum* (Presh) ssp. *strictum* was most closely related to *S. strictum* ssp. *africanum* (Stapf) and *S. strictum* ssp. The presence of the 5S rDNA locus in chromosome arm 3RS of *S. cereale* and *S. vavilovii* supported the close relationship and common origin of both species. After an indefinite time, they became disjoined and evolved separately.

The internal transcribed spacer sequences of the 18S-5.8S rDNA (ITS-1) region of cultivated rye and *prijanovii* (Grossh) were compared to *S. strictum* ssp. *anatolicum* (Boiss.) Hammer. No significant differences were found between the weedy forms of *S. cereale* and cultivated rye (de Bustos and Jouve 2002).

6.1.4 Distribution of the Genus *Secale*

The genus *Secale* is a typical representative of Mediterranean flora and has a wide distribution from central Europe and the western Mediterranean through the Balkans, Anatolia, Israel, and the Caucasus to Central Asia (Fig. 2). An isolated population also appears in South Africa (Sencer and Hawkes 1980).

Perennial wild rye taxa grow mainly in primary habitats (meadows, rangeland, among bushes and rocks on calcareous slope, and forests) in the subalpine and alpine regions but may be found in segetal habitats (roadside, field borders, and cultivated land) in the Mediterranean basin, Southwest Asia, Transcaucasia, and South Africa (Roshevitz 1947). Among the annual wild species, *S. sylvestre* has been reported living in sandy pastures, sand dunes in river deltas, and seashores. Its range of distribution embraces an area that includes Eastern Europe, the Caucasus, and Central Asia. *S. vavilovii* has been reported from sandy ground by the Aras river, to mountain ranges, cultivated fields, and field borders in eastern Turkey. Annual weedy rye is always found as a weed in cultivated fields and field borders in Anatolia, the Caucasus, and Central Asia (Roshevitz 1947). Cultivated rye is commonly found in fields and open spaces from

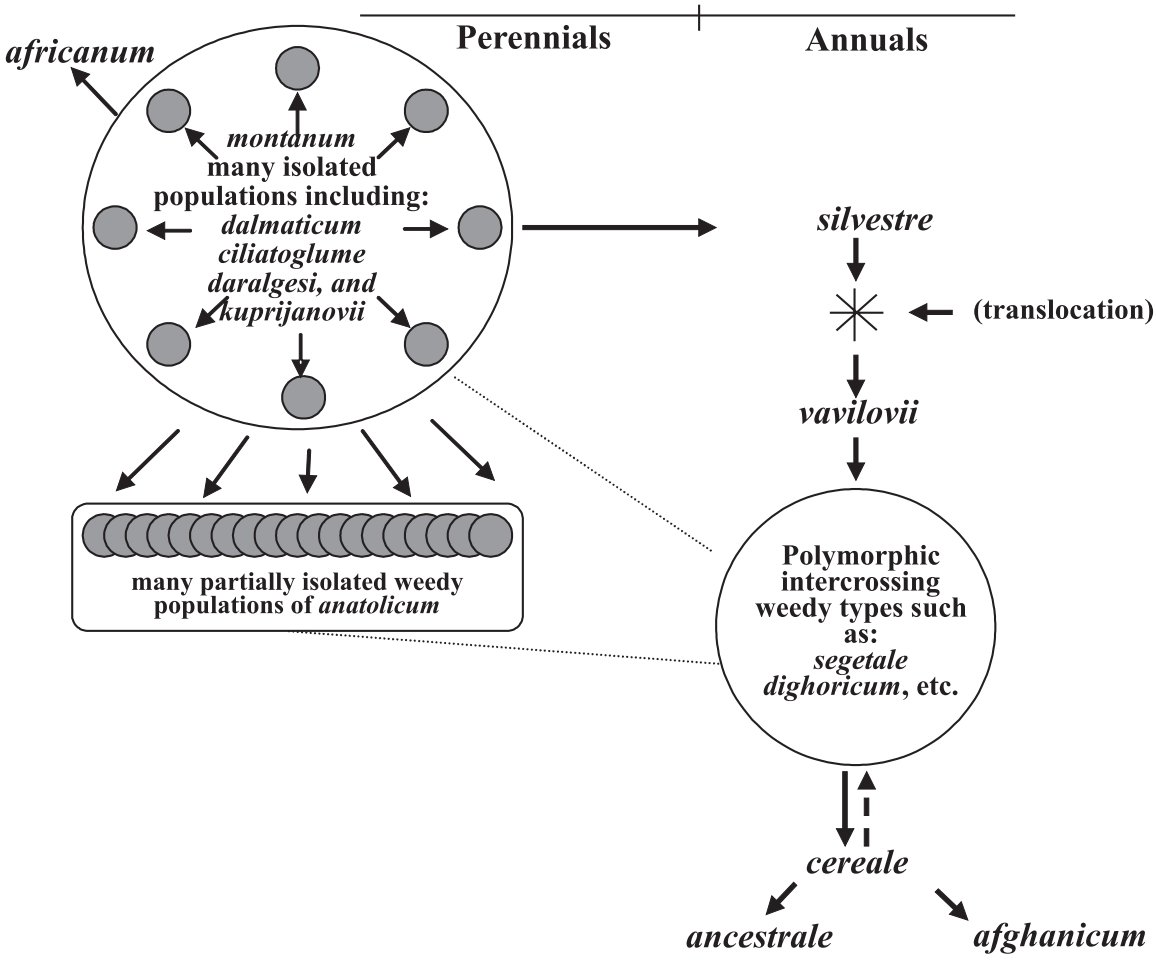

Fig. 1. Phylogenetic relationship in the genus *Secale* by Stutz (1972)

Europe to Southwest Asia. The geographical origin of cultivated rye was defined by de Candolle (1886) as the area between the Austrian Alps and the Caspian Sea.

6.1.5 Classification of the Genus *Secale*

Systematic classifications have recognized from three to 14 species within the genus *Secale* depending on the identification criteria used. Early studies involved the systematic classifications of the genus *Secale* based on morphological characteristics, life cycle, and geographical distribution. Vavilov (1917, 1926) accepted four species in the genus *Secale*: *S. africanum* Stapf., *S. cereale* L., *S. fragile* Marsch, and *S. montanum* Guss. Zhukovsky (1928) proposed three subspecies, *S. cereale* subsp. *cereale* Zhuk. for cultivated rye, *S. cereale* subsp. *ancestrale* Zhuk., and S. *cereale* subsp. *segetale* for weedy rye. However, Zhukovsky (1933) subsequently raised subsp. *ancestrale* to species status. Roshevitz (1947) distinguished as many as 14 species based on crossability, which were grouped into three major series. The first series was composed of *S. montanum* and all perennial forms (*S. kuprijanovii, S. dalmaticum, S. ciliatoglume, S. daralagesi, S. anatolicum,* and *S. africanum*) constituting the series Kuprijanovia Rashev. The second group consists of *S. cereale* and all weedy annual relatives (*S. vavilovii, S. dighoricum, S. afghanicum, S. ancestrale,* and *S. segetale*), which constituted the series Cerealia Roshev. in which all members contained three translocated chromosomes (with respect to the *S. montanum* group chromosomes). Finally, the third group consisted of *S. sylvestre*, which stood alone as an annual species with the same chromosomal arrangement as *S. montanum* and constituted the series silvestria Ro-

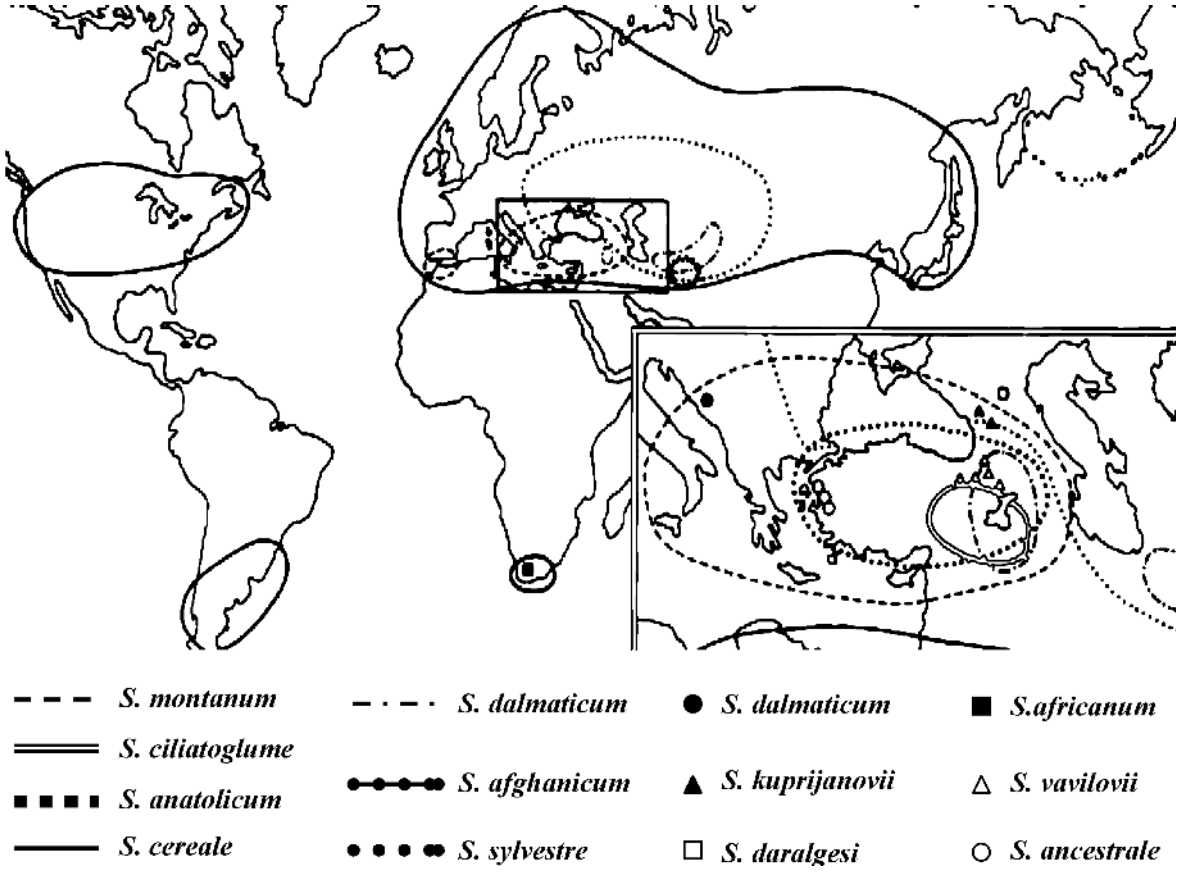

Fig. 2. Geographic distribution of the genus *Secale* (Vence 1987)

shev. Series Cerealia, which included cultivated rye, was the youngest series derived from the series Kuprijanovia. *S. cereale* arose under cultivation relatively recently, having been selected by humans for a nonbrittle rachis.

Khush and Stebbins (1961) conducted a series of studies using cytogenetic characteristics as criteria, which showed *S. sylvestre* and *S. cereale* to differ in three reciprocal translocations, of which two were the same as those found between *S. cereale* and *S. montanum*, with the third involving a short chromosome segment. *S. sylvestre* and *S. montanum* were found to differ by a small, single translocation. Khush (1962) did not find cytogenetic support to classify perennial ryes (*S. montanum*, *S. africanum*, *S. kuprijanovii* Grossh.) as separate species and proposed that they should be regarded as subspecies of *S. montanum*, while the weedy ryes (*S. ancestrale*, *S. afghanicum* Vav., *S. dighoricum* Vav., and *S. segetale* Zhuk.) should be considered as subspecies of *S. cereale*. Based on morphological characters, as well as cytogenetic and other studies, Sencer (1975) proposed three biological species within the genus *Secale* L.; first, *S. montanum* including all the wild perennial taxa with high morphological resemblance and cytogenetic affinity to each other; second, *S. sylvestre*, a wild, annual species, which is isolated from *S. montanum* geographically, ecologically, and reproductively; and third, *S. cereale* containing the annual wild, weedy, and cultivated ryes.

On the basis of numerical taxonomy of phenolic compounds, Dedio et al. (1969) showed that *S. sylvestre* had a distinct chromatogram of its own, whereas *S. cereale*, *S. dighoricum*, and *S. segetale* were grouped together, and *S. montanum*, *S. africanum*, *S. dalmaticum*, and *S. kuprijanovii* appeared more closely related to each other than to the rest of the taxa. Using isozymes, Vences et al. (1987) supported Vavilov (1926) by accepting four species in the genus *Secale*. *S. cereale* and *S. montanum* appeared closely related and genetically similar and were almost equally related to *S. vavilovii* and *S. sylvestre* on the basis of genetic distance. *S. sylvestre* was easily distinguished

from *S. vavilovii*, and both were distinguished from the two open-pollinated species. However, there was no clear differentiation between *S. cereale* and *S. montanum*.

Recently, molecular data have been used to analyze phylogenetic relationships among species within *Secale*. Reddy et al. (1990), using rDNA spacer length variation, and Petersen and Doebley (1993), using RFLPs from the plastid genome, showed that DNA provided a useful character to supplement the conventional methods used for studying relationships between *Secale* species. They showed that only the annual species *S. sylvestre* was really distinct from the rest of the taxa, and that cultivated rye together with both the wild annual and perennial accessions were mixed. Using a polymerase chain reaction (PCR) technique, Pozo et al. (1995) agreed with the *Secale* group phylogeny as proposed by Khush (1962). However, *S. cereale* subsp. *ancestrale* was not included in *S. cereale*. *S. cereale* subsp. *anatolicum* was closer to *S. cereale* than to *S. montanum*, while *S. montanum* subsp. *kuprijanovii* was closer to *S. sylvestre*.

Finally, Frederiksen and Petersen (1998) made a taxonomic revision of *Secale* based on an examination of material in several herbaria and recognized only three species: *S. sylvestre, S. strictum*, and *S. cereale*. *S. strictum* contained two subspecies, ssp. *strictum* and ssp. *africanum*, and two varieties within ssp. *strictum,* var. *strictum* and var. *ciliatoglume* comb. nov. *S. cereale* also contained two subspecies. The cultivated taxa, marked by their tough rachises, were placed in ssp. *cereale* and the wild or weedy taxa that have a more or less fragile rachis were placed in ssp. *ancestrale*.

6.2 Phylogenetic Relationships Among *Secale* Species Utilizing AFLP Analysis

Cluster analysis based on AFLP-based analyses grouped together the annual taxa except for *S. sylvestre* and grouped the perennial taxa close to each other (Chikmawati 2003). This result indicated that life cycle (perennial vs. annual) played an important role in determining the relationships among *Secale* species. Further analysis using Fisher's exact test showed that 24% of the AFLPs detected were associated with the life cycle character.

AFLP analysis clearly resolved all accessions into three major groups, group 1 consisting of perennial taxa, group 2 consisting of annual taxa, and group 3 consisting of *S. sylvestre* (Chikmawati 2003), strongly supporting the validity of the three major series within *Secale* recognized by Roshevitz (1947), series *Kuprijanovia* Roshev. (*S. montanum* and all perennial), series *Cerealia* Roshev. (*S. cereale* and all weedy annuals), and series *Silvestria* Roshev. (the annual, *S. sylvestre*).

AFLP analyses showed that among the annual taxa (*Cerealia* Roshev.), *S. cereale* was more closely related to *S. ancestral, S. afghanicum, S. dighoricum,* and *S. segetale* than to *S. vavilovii* (Chikmawati 2003). Although *S. cereale* and *S. turkestanicum* are both cultivated species, they exhibited the most distant relationship to each other. The differences in breeding systems between two taxa (*S. turkestanicum* is self-pollinated and *S. cereale* is cross-pollinated) may explain this observation.

Among perennial taxa (*Kuprijanovia* Roshev.), *S. ciliatoglume* showed the most distant relationship to others (Chikmawati 2003). *S. ciliatoglume* is an isolated weedy population with pubescent culms endemic to orchards and vineyards near Mardin, Turkey. It is possible that this taxon maintained its distinct identity from the others because of its very limited distribution. Among the perennials, *S. africanum* was most distantly related to *S. montanum*, while *S. anatolicum* and *S. kuprijanovii*, which are close to each other, showed the closest relationship to *S. montanum*.

Somewhat surprisingly, the annual species *S. sylvestre* (*Silvestria* Roshev.) was closer to the perennial taxa than to the annual taxa. Since this species had the closest relationship to the outgroups, it can be considered as the most ancient among all the *Secale* species. Cluster analysis showed that *S. sylvestre* was the oldest while *S. cereale* was the youngest of the *Secale* species.

Based on cytological, ecological, and morphological studies, Stutz (1972) demonstrated that cultivated rye (*S. cereale* L.) originated from a weedy progenitor, which in turn was derived from the introgression of *S. montanum* (syn. *S. strictum*) into *S. vavilovii*. *S. africanum, S. dalmaticum, S. ciliatoglume*, and *S. kuprijanovii* appeared to be only a slightly modified isolated population of *S. montanum*. Populations of *S. anatolicum* were thought to be weedy forms of *S. montanum*, genetically and chromosomally distinct from the weedy annual forms. The species relationships within genus *Secale* based on AFLP data were

consistent with Stutz (1972) (Chikmawati 2003). However, Stutz (1972) also suggested that *S. montanum* was the common ancestor of all the *Secale* species, which conflicts with the AFLP data of Chikmawati (2003), and demonstrated that *S. sylvestre* was the most ancient species and the first to diverge from the common ancestor, while *S. montanum* diverged later.

6.3 Molecular Taxonomy of *Secale*

6.3.1 Distinction Among Annual Species

S. sylvestre, a low growing plant with fragile rachis, is widely distributed from Central Hungary eastward throughout the sandy steppes of Southern Russia and can be easily distinguished from other taxa by its long awned glumes (Stutz 1972). Khush and Stebbins (1961) showed that *S. sylvestre* is cytogenetically very distant from *S. cereale* and is geographically, ecologically, and reproductively isolated from *S. montanum* (Sencer and Hawkes 1980). In addition, *S. sylvestre* exhibits other unique characteristics, such as distinctive chloroplast DNA (Petersen and Doebley 1993), a spacer length variant of the ribosomal DNA (Reddy et al. 1990), and the internal transcribed spacer of the 18S-5.8S-26S rDNA (ITS-1) region. Given the strong distinction of *S. sylvestre* from other taxa for a wide assortment of characteristics, *S. sylvestre* has been considered a distinct species. In AFLP analysis, *S. sylvestre* demonstrated a distinct profile in all primer combinations (Chikmawati 2003). This taxon was well separated from others in all studies, and AFLP analyses confirmed that *S. sylvestre* was a distinct species.

The presence of a high degree of similarity among wild, weedy annual forms and cultivated rye was demonstrated by Khush (1963), who showed that there was no evidence of structural differences between the genome of cultivated rye and several weedy ryes (*S. cereale, S. vavilovii, S. ancestrale, S. afghanicum, S. dighoricum,* and *S. segetale*), which had previously been recognized as varieties, subspecies, or even species. These all readily crossed producing vigorous F_1s, which had similar chromosome arrangements, breeding habit, and periodicity, and also demonstrated geographical continuity. Khush (1963) proposed all annual forms to be subspecies of *S. cereale*. Morphometrical analyses concluded that it was impossible to recognize each annual taxon based on their morphology (Frederiksen and Petersen 1997). They proposed two intraspecific taxa within a single species (*S. cereale*), which are *S. cereale* subsp. *cereale* for cultivated rye and *S. cereale* subsp. *ancestrale* for weedy and wild taxa. Recently, de Bustos and Jouve (2002) found no differences between the weedy forms and cultivated rye in the ITS-1 region. Thus, previous studies have demonstrated that morphologically and genetically the annual taxa were too similar to be distinguished as separate species.

An AFLP analysis by Chikmawati (2003) showed that six accessions of *S. cereale* originating from different locations made a monophyletic group. *S. dighoricum* accessions also clustered together; however, those accessions originated from the same location. Other annual taxa represented by more than one accession did not make monophyletic clusters but intermingled with each other. Cluster analysis demonstrated that the inclusion of each annual taxon was not well supported. Except for cultivated rye, it is still difficult to discriminate wild and weedy rye using AFLP markers. Therefore, Chikmawati's (2003) AFLP analysis supports the results of Frederiksen and Petersen (1997).

6.3.2 Distinction Among Perennial Species

Among perennial species, *S. ciliatoglume* does not cluster together with the others. It stands alone between annual and perennial taxa according to cluster analyses (Chikmawati 2003), but the separation was intermediate (58% in phylogenetic and 74% in phenetic analysis). Principal coordinate analysis (Fig. 3) placed this accession in the same quadrant with the other perennial taxa. Information about *S. ciliatoglume* from the previous studies was very limited to morphological data, which showed that this species is morphologically similar to *S. montanum*, deviating only by having a dense cover of hairs over the internodes, leaf sheaths, and blades (Frederiksen and Petersen 1998). Frederiksen and Petersen (1997) suggested that *S. ciliatoglume* should be given an intraspecific rank.

Previous studies showed that several perennial forms (*S. anatolicum*, *S. africanum*, *S. dalmaticum*, and *S. montanum*) readily crossed to each other, and that crossing among them yielded normal chromosome configurations (Stutz 1972), indicating no re-

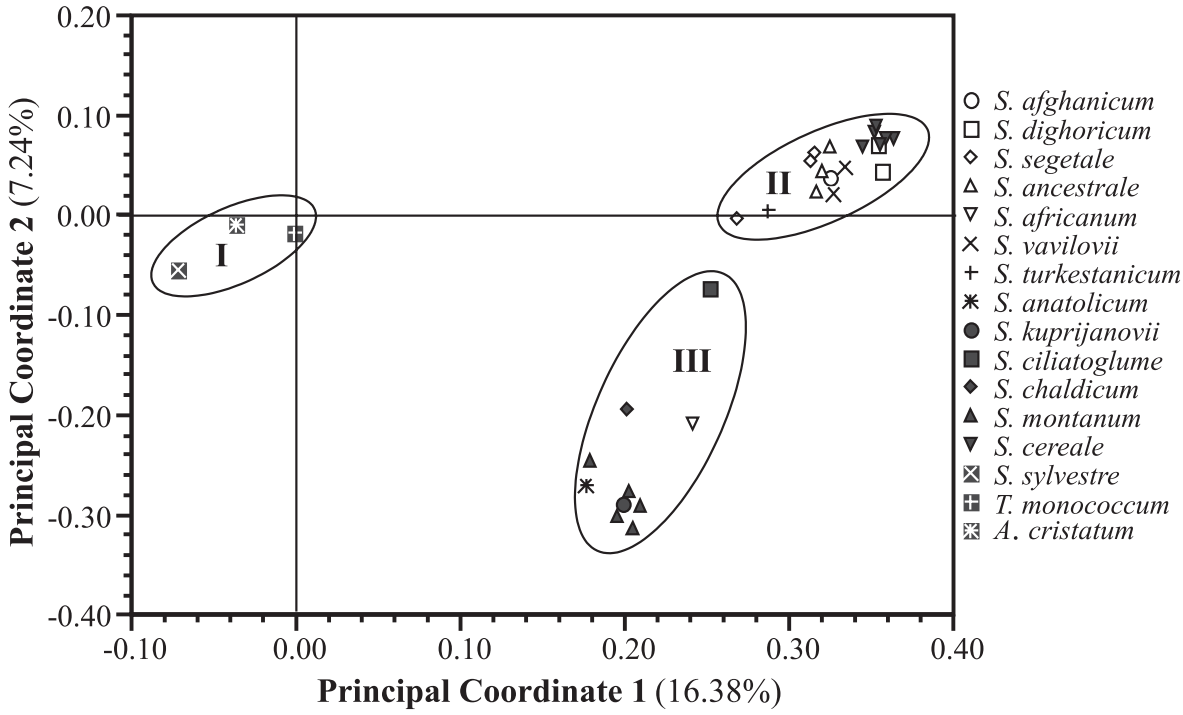

Fig. 3. Principal coordinate analysis of *Secale* species based on AFLPs. *I* = *S. sylvestre* and two outgroups. *II* = annual forms. *III* = perennial forms

productive barrier. Khush (1962) proposed all perennial taxa to be subspecies of *S. montanum*. Furthermore, Sencer and Hawkes (1980) showed that all the wild perennial forms had high morphological resemblance. The phylogenetic analysis based on AFLPs clustered six accessions of *S. montanum* together with low bootstrap support (36%), with the separation of the other perennial taxa showing only intermediate support (44 to 69%) (Chikmawati 2003). However, the genetic differentiation level among perennial taxa was very high (Gst = 0.90). This result suggests that the polymorphism of AFLP markers within perennial taxa were sufficient to discriminate and place them in an intraspecific rank, instead of a specific rank.

6.4 Genetic Diversity Among Cultivated Rye Genotypes

Morphologically, cultivated rye shows variation in a number of characters especially related to the color and hairiness of the bracts and color of the caryopsis; however, these characters also demonstrate overlapping and continuous variation (Tumania 1929). In addition to morphological variation, *S. cereale* was thought to be extensively variable; however, a comparative study of four populations of *S. cereale* based on allelic frequencies and heterozygosity of allozymes revealed no significant differences among rye populations with different geographical origins (Peres de la Vega and Allard 1983). Persson and von Bothmer (2000) showed higher diversity and larger within-population isozyme loci variation compared to the variation among populations.

6.5 Utilization of Molecular Markers in Rye Systematics

Molecular phylogenetics is expected to clarify many patterns of rye evolution that have been hard to resolve by classical approaches. There are several reasons why molecular data are much more powerful than morphological and physiological data for evolutionary studies. First, DNA and protein sequences generally evolve in a much more regular fashion than do morphological and physiological characters and therefore can provide a clearer picture of organism

relationships. Second, molecular data are often much more amenable to quantitative treatments than are complex morphological data. Third, molecular data are becoming much more abundant (Nei and Kumar 2000). Avise (1994) pointed out several special advantages of molecular data for use in phylogeny estimation. First, molecular data are genetically inherited. Since phylogeny is the stream of heredity, only genetically transmitted traits are informative to phylogeny estimation. Second, molecular methods open the entire biological world for genetic scrutiny. Various molecular assays provide direct structural evidence for genes or their products and can be applied to the genetics of any organism from microbes to whales. And third, molecular data can distinguish homology from analogy.

A variety of molecular techniques have been developed that provide genetic diversity and genetic relationship information that can be used as DNA fingerprinting strategies. Each method has its own benefits and constraints. The most common techniques are RFLPs and numerous PCR-based genetic marker assays, such as randomly amplified polymorphism DNA (RAPDs), SSRs, and AFLPs. Many studies have shown that both SSRs and AFLPs are suitable tools for assessing genetic diversity and the genetic relationship among accessions within species.

6.6 A Review of Linkage Mapping in Rye

The gateway for genome studies was the development of large-scale genome sequencing technology, which has already been used to sequence the entire genomes of a number of plants and animals. However, most of the complete sequencing efforts in plants have focused on a few model species with relatively small genome sizes. Thus, for most cereal crops with their relatively large genome sizes, genetic (linkage) and physical mapping are still the fundamental genomic studies. In addition, map-based cloning and marker-assisted selection have proven particularly important for crop improvement. As a result, much effort has been applied to the development of genetic maps in various cereal crops over the last 15 years.

As with other cereals, rye has experienced rapid progress in map development. Schlegel et al. (1997) have updated the rye mapping data, which are publicly obtainable at http://www.desicca.de/plant_breeding/Rye_map/rye_map.html. The substantial amount of data available makes a summary of rye mapping progress useful. Table 1 includes data from maps containing at least six linkage groups.

6.6.1 Mapping Population and Linkage Maps

Twelve major maps are listed in Table 1. These maps were developed from seven mapping populations in six laboratories. The E-line × R-line population was used by Loarce et al. (1996) to construct a map consisting of 89 loci spanning 339.7 cM on all the rye chromosomes except for 2R. The map generated from population UC90 × E-line (Ma et al. 2001) contains 184 loci, including seven genes of known function and one cytological marker, covering 727.3 cM with a relatively equal distribution of loci in each of the seven rye chromosomes. In addition, at least two thirds of the markers in this map were derived from other cereal crops, allowing for good integration and estimation of syntenic relationships with maps of other crops (Ma et al. 2001).

The Ds2 × RxL10 population was first used for restriction fragment length polymorphism (RFLP) mapping by Devos et al. (1993) and consists of 156 loci spanning about 1,000 cM. The map of Devos et al. (1993) gives the most detailed description of rye chromosomes relative to their wheat homoeologs but contains no rye genomic or cDNA markers. This map was later saturated with random amplified polymorphic DNA (RAPD) and isozyme markers, resulting in the largest rye linkage map containing 282 markers covering 1,140 cM (Masojć et al. 2001). One notable observation regarding this population is that the loci on the maps were heavily clustered around the centromeres.

The P87 × P105 is a pooled mapping population generated by combining F_2 individuals derived from a pair of reciprocal crosses of the two inbred parents. The population has been used for a series of mapping efforts from RFLP mapping with genomic and cDNA clones (Korzun et al. 1998) to simple sequence repeat (SSR) or microsatellite mapping (Korzun et al. 2001), and from general linkage development to locating genes and quantitative trait loci (QTL) (Börner et al. 2000). As a result, the final map contains the greatest number of known function genes and morphological traits, including 19 isozyme and protein markers, 10 known function sequences, and two mor-

Table 1. A summary of major mapping data in rye

Population	No. of loci	cM	Program	Marker type	Segregation distortion (%)	Reference
E-line × R-line	89	339.7	MAPMAKER 3.0	RFLP, RAPD	20.2	Loarce et al. 1996
UC90 × E-line	184	727.3	JoinMap 2.0	RFLP, SSR, cytology	72.8 ($P < 0.01$)	Ma et al. 2001
Ds2 × RxL10	156	~1,000.0	MAPMAKER 2.0	RFLP, protein		Devos et al. 1993
Ds2 × RxL10	282	1,140.0	MAPMAKER 3.0b	RAPD, isozyme		Masojć et al. 2001
P87 × P105	91	660.0	MAPMAKER 2.0	RFLP, isozyme, morphology	11.0 ($P < 0.05$)	Korzun et al. 1998
P87 × P105	113	1,018.0	MAPMAKER 3.0	RFLP, isozyme, morphology, QTL		Börner et al. 2000
P87 × P105	183	1,063.4	MAPMAKER 2.0	RFLP, SSR, isozyme, protein, morphology	12.0 ($P < 0.05$)	Korzun et al. 2001
Danko × Halo	60	~350.0	LINKAGE 1.0	RFLP, RAPD, isozyme, morphology	34.3 ($P < 0.05$)	Philipp et al. 1994
F_2*	127	~760.0	MAPMAKER JoinMap 1.4	RFLP, RAPD, isozyme, morphology	6.3	Senft and Wricke 1996
F_2*	102	#757.4	MAPMAKER 2.0	RFLP, RAPD, SSR, isozyme	Observed†	Saal and Wricke 1999
F_2*	182	1,062.0	MAPMAKER 3.0b	RFLP, RAPD, SSR, AFLP	5.0	Saal and Wricke 2002
9953**	56	685	JoinMap 3.0	SSR	67.9 (7R only)	Hackauf and Wehling 2001

* An F_2 mapping population originated from a cross between two inbred lines, which were selected from a self-fertile synthetic population based on their allelic constitution of 11 isozyme loci

** A BC_1 population

Two gaps are not counted

~ Approximate values

† Observed, but the value is not given

phological genes. In addition, 23 gene loci and 25 QTL were anchored on particular regions of the linkage frames (Korzun et al. 2001).

The map based on the population Danko × Halo was relatively small, but it was one of the earliest linkage maps covering all seven rye chromosomes (Philipp et al. 1994). Those mapping data were later integrated into those of another population created by Senft and Wricke (1996). The new mapping population originated from a cross between two inbred lines selected from a self-fertile synthetic population based on their allelic constitution of 11 isozyme loci. This population was the first to incorporate and extend the previous mapping data provided by Philipp et al. (1994) with more RFLP and RAPD markers (Senft and Wricke 1996). The map was then further extended with SSR (Saal and Wricke 1999) and amplified fragment length polymorphism (AFLP) (Saal and Wricke 2002) markers. The final map involving this population consists of 182 markers distributed through a mapping length of 1,062 cM, and it is the only rye map that contains AFLP markers (Saal and Wricke 2002).

Hackauf and Wehling (2001) developed a second-generation rye linkage map composed solely of expressed sequence tag (EST)-derived SSRs. The map covers 685 cM and it is the only map developed from a non-F_2 population. Since the SSR markers were derived from expressed sequences, the resulting map contains a number of markers directly related to known function genes; thus it provided the basic framework for a "functional map" (Hackauf and Wehling 2001). The utilization of EST markers opened up the possibility of constructing maps based solely on gene-rich regions of the rye genome. At present, there are well over 500,000 wheat ESTs in Genbank that can be used as PCR-based markers for high-saturation mapping in rye.

Similarly to other cereal crops, all the rye linkage maps have shown a nonuniform distribution of mapped loci, which are often typically clustered near the centromeres, suggesting that more recombination occurs in the distal regions of chromosomes. Centromere clustering results in inflated interval distances between distal loci, thus reducing overall map resolution and utility (Devos et al. 1993).

6.6.2 Markers

The maps showed that various kinds of markers, involving most of the major marker technologies including RFLP and PCR-based methods (RAPD, SSR, and AFLP), could be used for genetic mapping in rye. The mapping efficiency and the potential application could be very different for each kind of marker. Many RFLP markers have been used for mapping in rye and have been utilized as core markers for mapping frame establishment (Loarce et al. 1996; Ma et al. 2001; Masojć et al. 2001; Korzun et al. 2001; Saal and Wricke 2002). Most of the RFLP markers are genomic or cDNA clones with relatively low to moderate levels of polymorphism; however, the degrees of polymorphism (generally 30 to 70%) depend on the marker sources, populations, and the species involved. Since rye is an outcrossing species and is comprised of ca. 85 to 90% repetitive sequences, most of the genomic clones showed multiple bands, which increased the variability of mapping efficiency in different populations. In addition, any genomic DNA-derived loci mapped in each individual rye population could be different.

The PCR-based methods (RAPD, SSR, and AFLP) are getting more important and popular because they are straightforward and can be carried out with a small amount of DNA. One of the earliest PCR-based markers involved the utilization of RAPDs, which have already been used to saturate rye maps in many of the rye populations (Table 1). Compared to RFLP markers, RAPD markers often show low reproducibility from population to population and laboratory to laboratory due to their randomness of amplification. Therefore, RAPD primers were not only screened for mapping polymorphism but also selected for reliability to ensure reproducible patterns of amplicons generated; thus the overall mapping efficiency using RAPDs was decreased (Senft and Wricke 1996; Masojć et al. 2001). SSR markers have been shown to be superior to other markers due to their levels of polymorphism (Saal and Wricke 1999) and to the fact that they are codominant; they have also been used to increase overall mapping coverage in several populations (Saal and Wricke 1999; Korzun et al. 2001; Ma et al. 2001). However, SSR marker primer design needs sequence information, and the amplicons usually need to be sequenced again for confirmation. In one population, AFLP markers have been used to extend rye linkage maps (Saal and Wricke 2002). This is one of the most

efficient methods because primers used do not need prior knowledge of DNA sequence and multiple loci always could be mapped from a single pair of primers. However, AFLP markers are dominant markers, and the AFLP amplicons are anonymous sequences.

Overall, the rye mapping coverage has been significantly increased with the development of new marker technologies. Meanwhile, the mapping tendency has changed, with the most time-consuming method, RFLP and the low-reproducible method, RAPD, being replaced by SSR and AFLP methods.

6.6.3 Mapping Programs

The major mapping programs used for rye linkage establishment are MAPMAKER (Lander et al. 1987) and JoinMap (Stam 1993). MAPMAKER is widely used and is a very reliable program for codominant marker incorporation. JoinMap is particularly useful for mapping data, which involves mixing codominant and dominant markers on the same map, and for the integration of mapping data involving two or more different mapping populations. This map-integration feature had been used by Senft and Wricke (1996) to combine their mapping results with data originated from another population (Philipp et al. 1994). Remarkably, using the JoinMap Gustafson and Snape (2001) have integrated the mapping data from five rye linkage maps (Devos et al. 1993; Philipp et al. 1994; Loarce et al. 1996; Korzun et al. 1998; Ma et al. 2001), which has resulted in a comprehensive map that contains more than 500 markers within a mapping distance of only about 760 cM. The data allowed for the establishment of a higher-resolution map with an average distance of only about 1.5 cM between adjacent markers. JoinMap also turned out to be powerful for incorporating dominant with codominant rye markers, as well as markers showing a high degree of segregation distortion (Ma et al. 2001).

6.6.4 Segregation Distortion

Segregation significantly different from the expected Mendelian ratios, 1:2:1 for codominant alleles and 3:1 for dominant alleles is defined as segregation distortion. In general, most of the existing rye mapping populations suffer significantly from segregation distortion, and the phenomenon has been observed in all seven rye chromosomes (Philipp et al. 1994; Loarce et al. 1996; Senft and Wricke 1996; Korzun et al. 1998, 2001; Saal and Wricke 1999, 2002; Ma et al. 2001). However, one population showed segregation distortion occurring mainly in chromosome 7R (Hackauf and Wehling 2001). Rye, an out-crossing species, suffers from variable amounts of inbreeding depression, and there is a strong reduction in viability following selfing. Distorted segregation ratios are known to result from competition among gametes for preferential fertilization. Selection operating at any stage of development from zygote to seedling may introduce a bias to the progeny (Loarce et al. 1996; Ma et al. 2001). One population, UC90 × E-line, demonstrated a considerable degree (72.8%) of segregation distortion in all seven rye chromosomes skewed in two directions (Ma et al. 2001). It is unclear why such a high number of alleles deviated from the normal segregation in the UC90 × E-line population compared to other rye populations. However, this demonstrates that each population of a highly out-crossing species such as rye can be significantly different.

The key to productive mapping in rye depends upon several factors. First, good quality mapping populations that have been derived from highly inbred rye parents (doubled haploids if possible) are required. The difficulty is that rye suffers greatly from inbreeding depression. Consequently, the selection of suitable parents will be the primary challenge. Second, the development of a large mapping population suitable for high-resolution mapping (a minimum of 1,000 lines) is required for marker-assisted selection programs or for map-based cloning in rye. Third, suitable markers, preferably PCR-based markers, are required. Fourth, EST sequences from all of the cereals (wheat, barley, rice, etc.) can be used as sources of markers.

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