Biosystematic comparison of species in the genus Agropyron Gaertn. with particular emphasis on serological studies

by Gordon Clifton Creel

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Genetics
Montana State University

Abstract:
Forty-five strains of 41 species of the genu Aqropyron were investigated to determine their serological relationships. A specially designed test known as the Four-way test was employed for these determinations. Each strain of the 41 species was compared with all others tested and a difference index determined. A difference index of less than 0.12 indicated a close serological affinity. In some cases different species have a difference index of zero. These were given additional test, all of which substantiated the accuracy of the Four-way test.

This investigation clearly shows that, on a serological basis, some of the 4 sections as proposed by Holmberg (1926) are unnatural. The crested wheatgrass complex was distinct from other species tested. An aberrant group composed of A. pycnanthum, A. tsukushiense, A. mayebaranum, and A. scabriglume was found to be distantly related to the other Agropyrons, especially the crested wheatgrass complex, Of all species tested A. pycnanthum was the most aberrant serologically.

The hybrid origin of A. sibiricum was tested and the resulting data rather strongly argue against its origin from a cross of A. repens and A. cristatiforme.

The recognition of A. cristatiforme Sarkar as a distinct species is substantiated. A detailed morphological study of the spike characteristics of the crested wheat grass complex was made. The glume, lemma, and palea were found to be smallest in the diploid, largest in the hexaploid, and intermediate in tetraploid species.

BIOSYSTEMATIC COMPARISON OF SPECIES IN THE GENUS AGROPYRON GAERTN. WITH PARTICULAR EMPHASIS ON SEROLOGICAL STUDIES

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ABSTRACT

Forty-five strains of 41 species of the genus *Agropyron* were investigated to determine their serological relationships. A specially designed test known as the Four-way test was employed for these determinations. Each strain of the 41 species was compared with all others tested and a difference index determined. A difference index of less than 0.12 indicated a close serological affinity. In some cases different species have a difference index of zero. These were given additional test, all of which substantiated the accuracy of the Four-way test.

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The hybrid origin of *A. sibiricum* was tested and the resulting data rather strongly argue against its origin from a cross of *A. repens* and *A. cristatiforme*.

The recognition of *A. cristatiforme* Sarkar as a distinct species is substantiated. A detailed morphological study of the spike characteristics of the crested wheat grass complex was made. The glume, lemma, and palea were found to be smallest in the diploid, largest in the hexaploid, and intermediate in tetraploid species.

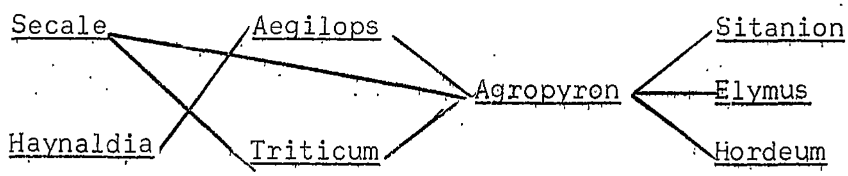
INTRODUCTION

The genus *Agropyron* of the Gramineae is cosmopolitan in distribution with about 100 of the 150 recognized species indigenous to Eurasia, about 30 species to North America, and the remainder to South America and the Australian region. The *Agropyrons* are of considerable economic importance, some being excellent forage grasses and others serious pests.

With the coming of modern transportation and an ever-increasing interest in forage crops, the more desirable *Agropyron* species were introduced and tested in areas other than their native habitat. Breeders in North America began to introduce foreign *Agropyron* grasses as early as 1898. A number of these have become well established in the United States and are now important crops. The crested wheatgrasses, native to Eurasia, are one of the important groups which have been introduced.

As knowledge of cytogenetics increased and investigators began to make interspecific hybrids, it became apparent that the wheatgrasses could contribute desirable genes, through introgressive hybridization, to important crop species such as wheat.

The genus *Agropyron* is the largest within the tribe Triticeae (Hordeae) of the Gramineae. In addition to the genus *Agropyron*, 9 other genera are classified as belonging to the tribe Triticeae. Clausen, Keck, and Hiesey (1945) stated that eight genera belong to the same comparium which Godley (1951) diagrammed as follows:



Stebbins (1961) recommended adding two other genera which are generally included in this tribe. These are the genera *Hystrix* and *Heteranthelium*. According to Godley (1951) and Stebbins (1961) *Agropyron* forms the important connecting link between the genera *Secale*, *Aegilops*, *Haynaldia*, *Triticum*, and *Heteranthelium* on the one hand, and *Sitanion*, *Elymus*, *Hordeum*, and *Hystrix* on the other.

Agropyron has been regarded as an artificial genus by Nevski (1934), Parodi (cited by Conner, 1954) and Stebbins and Walters (1949). Stebbins and Walters concluded that there was not enough evidence to determine accurately the boundaries of the genus. They were of the opinion that the established species names should be retained until more adequate cytogenetic and morphological information would permit the development of a natural system.

It is generally agreed that much confusion exists regarding the status of the genus *Agropyron* and regarding many species within the genus. A number of workers are studying the cytogenetics and the breeding behavior of the members of the genus, while others are investigating the morphology and anatomy.

A research program at Montana State College has been concerned with the status of the Agropyron species. Schulz-Schaeffer and Jurasits (1962) and Schulz-Schaeffer, *et al*. (1963b) determined the karyotypes of 33 species of the genus. Schulz-Schaeffer, et al. (1963a) studied the nature of the polyploidy in tetraploid and hexaploid Agropyron species of the crested wheatgrasses. Lorenz and Schulz-Schaeffer (1964) used two-dimensional paper chromatography to study the flavonoid compounds of 23 strains of 14 Agropyron species and found a definite parallel with morphological classification. The present study is concerned with a comparison of 45 strains of 41 Agropyron species by serodiagnostic methods.

REVIEW OF THE LITERATURE

Nuttall laid the ground work for the serological approach when in 1901 he published on the phylogenetic relationships of some animals, using a "biological test" of blood components. Many workers adopted and modified his method and extended it to include many species of both plants and animals.

That same year Kowarski (1901) found that "heat-resistant albumose of wheat induces in rabbits the production of precipitins which react strongly with wheat albumin extracts, and weakly or not at all with the albumoses of rye, barley, oat and peas, although the reaction with pea extract was stronger than that with oat extract." Ballner and Burow (1910) showed by complement fixation that rye is serologically more closely related to wheat than to barley and oats. He also found that rice and maize were still more distantly related to rye.

Rives (1923a,b) found, while working with grapes, that "failure to graft successfully was accompanied by a marked serological difference, while varieties which grafted successfully were similar by the test." Green (1926), working with _Citrus_, Rosaceae, and Solanaceae came to the same conclusions as Rives.

The period 1920-30 was one of much serological activity and some began to believe that this technique would provide an almost completely objective approach to systematics. At Königsberg, Germany, there emerged a very prominent group of phytoserologists who patterned their methods after Gohlke's (1913). Later Mez was the leading investigator of this school. Mez and Ziegenspeck (1926) published their "Serodiagnostiche Stammbaum" as the culminating work of the Königsberg school. This work was greatly criticized by the Berlin school of serologists, primarily Gilg and Schurhoff (1927), who stated that "the serodiagnostic method is, for investigation of plant relationships, completely useless."

Barner and Helwig (1927) followed the procedure of serology which Mez used, but later drastically altered it. Working with Geraniales, Sapindales, Rhamnales, Malvales, Verticillatae, Ranales, and Primulales, they extracted the proteins (antigens) from powdered seeds by using physiological saline or 0.1 sodium hydroxide. They concluded from their work that "Serodiagnostic methods are ...., not suitable for studies in phylogeny of plants, but are suitable for identification of specific plant proteins or of individual plant species and even for demonstration of relationships of plants within a family."

Animal serologists led the field of biochemical systematics from the late twenties until today. In 1928 Saski published on serological relationships between wild and domestic ducks as determined by the precipitin reaction. He found that he could distinguish the Japanese duck, Anas domestica erecta, from the Chinese goose, Anser cygnoides, but could not distinguish the Japanese duck from the Muscovy duck, Cairina moschata.

Martin and Cotner (1934) made a serological study of moth proteins and attributed special importance to their phylogenetic significance. They used the classical precipitin test made popular by the Rutgers school of serologists, one of whom was Makino (1934), who worked with several species of mollusks. By means of several cross tests with the precipitin, complement fixation, and anaphylactic reactions, he determined their serological relationships. Levit, et al. (1936) had so perfected the serological method that the complement fixation test could be used to detect the presence of the Y chromosome in males and attached-X females of Drosophila. Cumley (1940), commenting on the work of Levit, et al., said "this suggests the possibility of applying immunological techniques to the study of the expression of individual chromosomes, or even genes."

Levine and Moody (1939) determined serological relationships of a number of rodents and found, with a single exception, agreement with taxonomy based on morphology. They found that the North American porcupine, Erethizon dorsatum (L.), and woodchuck, Marmota Monax (L.), are more closely related than previously thought and that the pocket gopher (Geomyidae), even though placed in the same superfamily as the woodchuck, was not as closely related to it as the porcupine, which is in a distant superfamily.

Cumley (1940) compared the serological and taxonomic relationships of Drosophila caribbea, D. melanogaster, D. mulleri, and D. virilis. He found, by both techniques, that D. melanogaster and D. caribbea were closely related, and likewise D. mulleri and D. virilis.

Cumley, et al. (1943) working with several species of doves, their hybrids, and backcrosses concluded that "the species specific antigens of the serum of these different species are controlled by genes. The inference may well be drawn that all the antigens of the serum of animals, except those antigens which may be metabolic or other products being transported by the serum are gene-determined. Thus a genetic basis of classification is given the systematist who uses serology in his work."

Wilhelmi (1944) made a serological comparison of the Mollusca with other invertebrate phyla and found them most closely related to the Annelida. Leone (1947), using serological methods, was able to determine the relationships of 5 families of Orthoptera. Bacci and Oddo (1948) worked out the relationships of several species of mollusks using serological procedure and their conclusions agreed with the findings of classical taxonomy.

It had long been supposed that the Lagomorpha were more closely related to the Rodentia than to other groups. In 1949 Moody, Cochran, and Drugg performed serological tests which indicated that the lagomorphs are more closely related to the artiodactyls than other groups tested. This work confirmed the separation of lagomorphs and rodents into distinct orders and showed that these orders are not closely related.

Cather (1955) applied serological methods to determine the relationship of some gastropods collected in Dallas County, Texas. The results of his research support the theory that Pulmonata was derived from the Monotocardia. He also concluded that Limax has apparently undergone considerable protein change since it originated.

The relationship of the different groups of poisonous snakes has been a subject of much concern to herpetologists and evolutionists for a long time. Kuwajima (1953) worked with the venoms and blood sera of the Formosan poisonous snakes as antigens and demonstrated differences and similarities.

Leone (1950a) made serological comparisons of several species and families of European Brachyura and found that his results confirmed the existing classification based upon morphological characters. He was also able to show that sera of the same species of Brachyura collected at widely separated geographical localities showed no difference in their serological activities. In another paper on Crustacea, Leone (1950b) concluded that:

1. Sera of species within a genus react more strongly with an antiserum made against one of them than with any other antiserum.

2. Sera of different species within the same genus may show different degrees of relationship to an antiserum made against the serum of one member of that genus.

3. Sera of representatives of different genera in the same family show different degrees of relationship.

4. Sera of species within a family react to a greater extent with antisera made against the sera of members of that family than with any other antisera.

He also found from this work that freezing, Seitz filtration, centrifugation, and storage for periods up to 15 years caused no change in the serological activity of the hemocyanins.

Leone and Pryor (1954) made a serological study of the 4 Mollusca classes, Amphineura, Gastropoda, Cephalopoda, and Pelecypoda, and found that only extremely slight cross-reactions occurred between classes. They concluded that the 4 groups should be retained as distinct classes.

A study of the comparative serology of the carnivores was conceived and undertaken by Leone and Wiens (1956) in which they compared 7 families of fissipeds and one family of pinnipeds. From this work they came to the conclusion that the serological divergence between canoid and feloid carnivores was sufficient to justify making each group a suborder. They also found that classifying the Pinnipedia as a separate suborder was not justified on the basis of their serological data which placed them within the Canoidea.

Mainardi (1959) undertook a serological study of the relationship of several gallinaceous birds. He found that the ring-neck pheasant (Phasianus colchicus L.), guinea (Numida meleagris), and turkey (Meleagris gallopavo L.) have a close immunological affinity in spite of the fact they belong to three different families (Phasianidea, Numidinae, Melleagridae). He also found that the domestic chicken (Gallus gallus) and Japanese quail (Coturnix) which belong to the same family as the ring-neck pheasant are rather remote serologically from one another and from all other species studied.

Moody and Doniger (1956) made a serological comparison of old and new world porcupines, Hystrix and Erethizon dorsatum (L.) respectfully, and found them to be rather remotely related as indicated by their slight serological similarity. Sindermann and Mairs (1960), working with both agar diffusion and turbidometric methods, studied the serological relationship of several fishes. They found that alewives, Alosa pseudoharengus (Wilson), and blueback salmon, Oncorhynchus nerka (Walbaum), have a high degree of serological similarity. Common shad were found to be closer to the alewife-blueback complex than to either menhaden, Brevoortia tyrannus, or herring, Clupea harengus; and menhaden and herring were remote from all others tested and from each other. Herring showed the greatest serological difference.

Although most serologists have worked with animals, a scattered few plant taxonomists have maintained a confidence in the method for their purposes, and in the last 15 years investigations have been made with results apparently as useful as those of the animal systematists.

Baldwin, et al. (1927) made a serological comparison of 29 species of cultivated legumes using precipitin and anaphylaxis reactions. They were interested in correlating serological relationship with cross inoculation. The results of their research showed "that all members of any cross inoculation group are closely related with respect to the protein characteristic of their seeds, and in the majority of cases all legumes which possess closely related seed protein complexes cross inoculate."

Hyun (1949) made a serodiagnostic investigation of the affinities of different species of the genus *Quercus*. He extracted proteins with physiological saline from seeds in the first stage of germination and injected them into rabbits to stimulate antibody production. His interpretation of the data was that serological relationships between the 15 species of *Quercus* coincided with the treatment of systematic botanists. The serology indicated that *Q. mangorica* and *Q. aliena* are between *Q. serrata* and *Q. dentata* in relationship. Of interest was his discovery that the evergreen *Q. phylliraeoides* is more closely related to the deciduous *Q. variabilis* and *Q. acutissima* than to other evergreen oaks.

Johnson (1954) made a serological comparison of several genera of Magnoliaceae by comparing them to *Magnolia*. He also compared several species of *Magnolia* in an effort to establish intrageneric relationships. His intergeneric serological comparisons confirmed the classical taxonomy grouping. He found that *Magnolia*, *Michelia*, and *Talauma* did indeed form a natural group, and that *Liriodendron* is relatively distant. He also found that *Illicium*, which had been removed from the Magnoliaceae by earlier workers, gave no reaction when tested with *Magnolia* antiserum. McLaughlin (1933) had placed the genus *Illicium* in the Hamamelidaceae, but Johnson (1954) found that *Disanthus*, also in the family Hamamelidaceae, gave no serological reaction with Illicum. This would perhaps support the treatment of some taxonomists of a monotypic family, Illiciaceae. Johnson's investigation at the species level revealed that the Asiatic species Magnolia obovata is more closely related to the American species M. tripetala than M. tripetala is to two other American species, M. acuminata and M. virginiana. His data indicated that Magnolia portoricensis is farthest removed of any species checked. Serological and morphological evidence supports the proposal that it be placed in a separate subgenus.

Hammond (1955) did some serological work with several genera of Ranunculaceae and as a result of this and a morphological study he produced a new systematic treatment of the genera. Hammond's work with this family led him to the conclusion that the family is serologically close-knit. One of the outstanding results of Hammond's work was his placement of Hydrastis into the Ranunculaceae. This action was based on a positive reaction with Aquilegia antiserum.

Gell, et al. (1960) with the application of immunological methods, studied the taxonomy of some species of the genus Solanum. They used the gel-diffusion technique developed by Elek (1948) and Ouchterlony (1948) with some modifications, notably the application of immuno-electrophoresis developed by Grabar and Williams (1953). They found that what appeared as a single line in the gel could often be separated into two or more by use of the immuno-electrophoretic technique. Using absorbed antisera, they were able to divide the species of Mexican potatoes into 4 well-defined groups. They concluded that "Morphological considerations then, indicate a rough correlation with the serological tests, though some exceptions can be noted. With the exception of the linking series Polydenia and the possibly linking series Bulbocastana, the serological results follow quite closely those obtained from crossability and morphological studies."

Tucker (1963) working with two subspecies of Marchantia polymorpha was able to show a serological difference between them. The procedure which he used was essentially that used by Gell, et al. (1960).

Serological techniques have made a significant contribution in research with hybrids. One of the classical papers on the serological study of animal hybrids was that of Irwin (1951) who worked with Columbidae and studied the serology of the Ring dove, Streptopelia risoria Linnaeus, Pearlneck dove, S. chiensis Scopoli, and their hybrids. He found that the hybrids had the antigens of both parents and some antigens not found in either parent. McGibbon (1944) was able to demonstrate the presence of antigens in hybrid ducks which did not occur in either parent. He found that after injecting this "hybrid antigen" into the parents, both produced antibodies in response, thus demonstrating its uniqueness in the hybrid.

Fox, et al. (1961) found that hybrids of the toads Bufo fowleri Hinckley and B. valliceps Wiegmann had all the components of the parents but no hybrid antigens.

Hybrids from species of several families of plants have also been studied serologically. Zade (1914) published on the serology of some legumes and one part of his work was a serological comparison of _Trifolium repens_, _T. pratense_, and _T. hybridum_. _Trifolium hybridum_ was considered to be of hybrid origin as the epithet indicates resulting from a cross of _T. repens_ and _T. pratense_. The serological data of this work led Zade to the conclusion that _T. hybridum_ was indeed of hybrid origin and derived from the supposed parents. Chester (1937a,b) wrote "Zade, with precipitin test, showed that _Trifolium pratense_ and _T. repens_ are related, but serologically distinct, their hybrid, _T. hybridum_ reacts so strongly with both as to demonstrate its hybrid nature."

Moritz and vom Berg (1931) working with _Vicia leganyi_ (hybrid) and its supposed parents _Lens esculenta_ and _Vicia sativa_ decided that it was of hybrid origin in spite of the observation that some of the proteins of _Lens esculenta_ were absent in the hybrid. Moritz (1958), working with amphidiploid hybrids, _Aegilops ovata_ X _Triticum dicoccoides_ and _Triticum aestivum_ X _Secale cereale_, found them to be serologically intermediate to the parents.

Hall (1959) used the immuno-electrophoretic technique descried by Grabar (1955) to study the macromolecular composition of a rye-wheat hybrid and its parents. The macromolecules analysed were extracted from dry seeds, which were ground into a fine powder, with 0.85% sodium chloride. His conclusions were ---1) immuno-electrophoretic identity has been established between some soluble seed proteins from wheat and rye, 2) all proteins immuno-electrophoretically identified in the wheat could be traced in the rye-wheat, 3) indications have been found that rye-wheat is unable to produce some of the proteins specific for rye, and 4) specific rye-wheat proteins could not be found.

Alston and Turner (1963) wrote "Suppose, for example, that species A contains antigen complement a + b and species B contains b + c. Thus b, represents the common antigenic substances. The hybrid should, therefore, possess a complement a + b + c, and a hybrid antiserum, if absorbed with serum type A and then serum type B, should be completely neutralized. Presumably then, if a residual activity remained in the antiserum after absorption with sera A and B, one of three explanations might hold:

(1) The plant was not a hybrid.
(2) New hybrid-type antigenic substances were present.
(3) Genetic heterozygosity in one or both parents led to individual differences in antigenic complement.

However, if serum of the "hybrid" is completely neutralized by antisera of type A and type B, this result offers strong support for the true hybrid nature of the plant in question."

Troitskii (1932) and Tronickij (1959) indicated that A. sibiricum probably is of hybrid origin resulting from a hybrid between A. repens and A. cristatum (diploid A. cristatiforme). It seems possible that this could be true when the confusion regarding the statues of the genus Agropyron and some of its species is considered.

A conflict concerning the type species of this genus has complicated the work of taxonomists. Jones (1960) wrote that when Gaertner described the genus Agropyron, only two species were included (Novi Comm. Petrop. XIV p. 531, 1770). The first one treated was A. cristatum (L.) Gaertn. which was based on the perennial Bromus cristatus, and the second, A. triticeum, was described as a new species. The description of A. triticeum was accompanied by detailed drawings. Britton and Brown (1913) (cited by Jones, 1960) chose A. cristatum as the type species. Hitchcock (1920), unaware of the work of Britton and Brown, selected A. triticeum Gaertn. as the generic type. He selected this species because Gaertner included detailed drawings with his work in describing the genus.

Nevski (1934) amended the genus Agropyron and chose A. cristatum (L.) Gaertn. as the type species. In this amended work he placed A. triticeum Gaertn. into a new genus, Eremopyrum (Ledeb.) Jaub. and Spach., along with the other annuals. Recently Skalicky and Jirasek (1959) have returned the annual species of Eremopyrum to the genus Agropyron. They also have transferred the species of Agropyron Gaertn. em. Nevski (the crested wheatgrasses) to the genus Kratzmannia Opiz.

Nevski's (1934) generic and subgeneric names for the wheatgrass complex are generally substituted for by the sections of the genus Agropyron published by Holmberg in 1926. The sections are 1) Goulardia (Husnot) Holmberg, 2) Holopyron Holmberg, 3) Agropyron, and 4) Eremopyrum.

MATERIALS AND METHODS

Forty-five strains of 41 Agropyron species were analyzed in this study. Seeds of these species were obtained from research institutions, plant material centers, and botanic gardens. Accession numbers, names of collection locations, and observed chromosome numbers are reported in Table 1. Voucher specimens are located in the Montana State College Herbarium.

All plants used in this study were grown in the Montana State College grass nursery or in a greenhouse. The leaf material used for this study was from adult plants with new vegetative growth or from seedling plants allowed to reach a height of about 15 cm. before being cut. When seedlings were used, many plants were cut and squeezed as a composite lot. Otherwise a single plant was used as representative of the species except in the cases of A. elongatum, where three different plants were used, representing the three ploidy levels (each was treated separately. Two strains of A. desertorum and A. trichophorum were treated.

The leaf material from a species was immediately placed in marked paper bags to eliminate error. The cut leaves were taken to the laboratory where they were washed at least twice. The adhering water was absorbed as completely as possible by blotting to prevent dilution of the extracts. After blotting, the leaves were placed in a metal cylinder which had a one inch inside diameter and was 4 inches long (Figure 1). The leaves were chopped firmly by hand

Table I. Sources of 45 strains of 41 Agropyron species, used as antigens in this study.

Species	Section	MSC Field No.	Source
A. acutum Roem. et Schult.	Hol.	0.5-2	P.I. 202,727 Botanic Gardens, Brussels, Belgium. 2n=30, 34, 35, 36, 37, 42 (Schulz-Schaeffer and Jurasits, 1962). MONT. 58,418.
A. agroelymoides (Hicken) Hunziker	Gou.	115-16	Hunziker 021. Baleares Province, Buenos Aires, Argentina.
A. albicans Scribn, et Smith	Hol.	111-12	Jenkins 4E6, Central Exp. Farms, Canada
A. angustiglume Nevski	Gou.	141-S	Seed received from Botanical Gardens, Moscow, USSR, No. 1254, via Dr. Christian Lehmann, Gatersleben, Germany, March 2, 1962.
A. arizonicum Scribn. et Smith	Gou.	2-8	Received from seed collection of the Institut für Pflanzenbau und Pflanzenzüchtung Göttingen, Germany, in January, 1960, as field Nos. 52-1957 and 165-1958. 2n=28, determined by Jurasits in 1960. MONT. 59,277.
A. brachyphyllum Boiss. et Haussk.	Gou.	3-2	Collected by H.S. Gentry, east base of Kuhe Zard, Charmahal, Iran, November 9, 1955. 2n=42 (Schulz-Schaeffer and Jurasits, 1962), n=21. MONT. 59,278.
A. caespitosum Grossh.	Hol.	4a(61)-1	P. I. 228,276 (Pullman, Wash.) Collected by H.S. Gentry, 25 miles NW of Shar Kard, Charmahal, Iran, on Oct. 3, 1955. 2n=42 (Schulz-Schaeffer and Jurasits, 1962).

Table I. (continued)

Species	Section	MSC Field No.	Source
A. ciliatiflorum Roshev.	Hol.	9-9	P.I. 207,452. Collected by H.S. Gentry at Paghman, Kabul Province, Afghanistan, Sept. to Oct. 1953. 2n=28 (Schulz-Schaeffer and Jurasits, 1962). MONT. 59,280.
A. cristatiforme Sarkar	Agr.	10-3	Seed from Hungary via Gatersleben, Germany. Received through Institut für Pflanzenbau und Pflanzenzüchtung, Göttingen, Germany, in January, 1960, as field Nos. 1-1957 and 126-1958. Received as A. cristatum. Reidentified by Dr. J.R. Schaeffer, J. Ericson, and Gordon Creel. 2n=14, (Creel, Ericson, and Schulz-Schaeffer, 1964).
A. cristatum (L.) Gaertn.	Agr.	80a-2	P. I. 223,323. Collected by E.E. Smith on road from Meshkinshahr to Ahar, Azerbaijan, Iran, on January 18, 1955. originally introduced as A. squarrosum. Reidentified by Dr. J.R. Swallen in 1962 as A. cristatum. 2n=42 (Schulz-Schaeffer and Jurasits, 1962). MONT. 58,420.
A. dasystachyum (Hook.) Scribn.	Hol.	147-S	P. I. 236,663. Received through Dr. Douglas Dewey, Agricultural Research Service, USDA, Logan, Utah. 2n=28.
A. desertorum (Fisch.) Schult.	Agr.	25-9	P. I. 19,538. Presented by Mr. Vasili S. Boydan, through Prof. M. Golenkin, director of Moskow Botanic Gardens, on December 12, 1906. Collected from Valuiki, Samara Government, Russia. 2n=28 (Creel, Ericson, and Schulz-Schaeffer, 1964) n=14. MONT. 59,291.

Table I. (continued)

Species	Section	MSC Field No.	Source
		30-9	Alma-Ata, Russia. 2n=28 (Creel, Ericson, and Schulz-Schaeffer, 1964).
A. donianum Buch.-White	Gou.	32-1	Received from Botanic Gardens, Cambridge, England, during March, 1960. MONT. 59,296.
A. elongatiforme Drob.	Hol.	33-3	P. I. 222,960. Collected by E.E. Smith, 16 miles east of Tabriz, road to Sarab, Azerbaijan, Iran, January 4, 1955. 2n=58 (Schulz-Schaeffer and Jurasits, 1962).
A. elongatum (Host.) Beauv.	Hol.	35a-2	Collected by Augustin Labbe in the vicinity of Saint Germain near Tunis, Tunisia, November, 1947. 2n=14 (Schulz-Schaeffer and Jurasits, 1962) MONT. 59,297.
		37-5	P. I. 119,603. Collected by H. L. Wellman from the ruins in Troy, Turkey, September 18, 1936. Original seed sample labeled A. intermedium (Host.) Beauv. Reidentified by Dr. J. R. Swallen. P-5330. Strain "Green". 2n=42 determined by Jurasits in 1960. MONT. 59,299.
		36-5	P. I. 98,526. Introduced by N. I. Vavilov from the Saratov Institute, Russia, on April 8, 1932. P-2326. Strains "Nebraska 98,526" and "Blue Type". 2n=71. Determined by Ericson in 1964. MONT. 59,298

Table I. (continued)

Species	Section	MSC Field No.	Source
A. fibrosum (Schrenk) Nevski	Gou.	112-5	Seed sent from All Union Institute of Plant Industry, U.S.S.R.. Collection in Lineck region, Russia (33869).
A. hirsutum (Bertol.) Nevski	Ere.	A1-S	P. I. 229,425. From Pullman, Washington. Received as A. orientale. Reidentified as A. hirsutum by Creel and Ericson in 1964. Collected in Iran.
A. imbricatum (M.B.) Roem. et Schult.	Agr.	28-9	Seed from Alpine Gardens Kirovsk, region Murmansk, Russia. Received through Dr. Christian Lehmann, Institut für Kulturpflanzenforschung, Gatersleben, Germany. Received as A. desertorum, reidentified by Creel, Ericson, and Schulz-Schaeffer. 2n=28, 30, 32, 34, 36 (Creel, Ericson, and Schulz-Schaeffer, 1964) MONT. 59,293.
A. intermedium (Host.) Beauv.	Hol.	44-6	Collected in the Kopet-Dag Mountains, Turkmen S.S.R., Russia. Seed received through Botanic Gardens, Turkmen Scientific Academy, Ashkhabad, Russia, via Dr. Christian Lehmann, Institut für Kulturpflanzenforschung, Gatersleben, Germany on May 27, 1960. 2n=42. Determined by Jurasits in 1960. MONT. 59,305.
A. junceum subsp. mediterraneum (L.) S.	Hol.	140-3	France. Origine Boucherdi Rhone i62. 2n=42 (Cauderon, 1958).

Table I. (continued)

Species	Section	MSC Field No.	Source
A. kosanini Nab.	Hol.	46a-2	P. I. 237,636. Collected by R. K. Godfrey near Zara, Sivas, Turkey, January 6, 1953. Separated from P. I. 204,425 (Bromus) and assigned new P. I. number by Regional Plant Introduction Station, Ames, Iowa. 2n=56 (Schulz-Schaeffer and Jurasits, 1962). n=28. MONT. 59,308.
A. latiglume (Scribn. et Smith) Rydb.	Gou.	47-3	P. I. 236,673. Collected by F. J. Herman and B. M. Leese at Sentinal Peak, Coleman, Kananaskes Road, Canada. 1956 2n=28 (Schulz-Schaeffer and Jurasits, 1962). n=14. MONT. 59,309.
A. littorale (Host) Dum.	Hol.	137-5	France. No. 643, Bases Pyrinees, 2n=42 (Cauderon, 1958).
A. mayebaranum var. mayebaranum Honda	Hol.	149-S	From S. Sakamoto (6387). National Institute of Genetics Misima, Sizuoka-ken, Japan. 2n=42, n=21 (Sakamoto, 1958).
A. mongolicum Keng	Agr.	48b-1	Collected during the Asiatic Expedition in the Suiyuan Province, China, during the period Aug. 9 to Sept. 2, 1935. USDA plant intro. 2n=28, 30, 31, 32, 34, 36. (Creel Ericson, and Schulz-Schaeffer, 1964).
A. panormitanum	Gou.	50a-7	P. I. 197,569. Received from Colonial Botanic Gardens, Palermo, Italy, July, 1951. 2n=28 (Schulz-Schaeffer and Jurasits, 1962). n=14. MONT. 58,421.

Table I, (continued)

Species	Section	MSC Field No.	Source
A. pauciflorum (Schwein.) Hitchc.	Gou.	121-4	Received from Dr. Jenkins, 4E47. Central Experimental Farms, Canada.
A. pungens (Pers.) Roem. et Schult.	Hol.	145-S	Seed received from Botanic Gardens, Taschkent, Russia, via Gatersleben, Germany, on Jan. 8, 1959. 2n=42. Determined by Jurasits in 1960.
A. pycnanthum Gren. et Godr.		128-S	France. Rie sur Belon (Finistere). Received via Dr. Christian Lehmann, Gatersleben, Germany.
A. repens (L.) Beauv.	Hol.	146-S	Received from All Union Institute of Plant Industry Leningrad, U.S.S.R. Collected in the region of Cheliabinsk, Russia. 2n=42. Determined by Jurasits in 1960.
A. riparium Scribn. et Smith	Hol.	62a-2	Collected by R. G. Johnson as A. smithii Rydb. near Canyon City, Grant County, Oregon, in area of 12 inch rainfall at elevation of 3000 feet in 1933. P-2415. 2n=42 (Schulz-Schaeffer and Jurasits, 1962). MONT. 59, 318.
A. scabrifolium (Doell) Parodi	Hol.	138-3	Received from Dr. Hunziker. Argentina, Province Cardoba, La Calera Cutl. Castelar. 4-I-1962. Hunz. iiiY025. 2n=42. Determined by Jurasits in 1960.
A. scabriglume (Hack.) Parodi	Hol.	1-5	P. I. 202,147. Collected by Alan A. Beetle, in the Parodi Grass Garden, Facultad Agronomia Buenos Aires, Argentina, from

Table I. (continued)

Species	Section	MSC Field No.	Source
			individual plant No. 16, February 19, 1952. 2n=42 (Schulz-Schaeffer and Jurasits, 1962). MONT. 59,319.
A. semicostatum (Steud.) Nees	Gou.	64-8	P. I. 203,242. Seeds presented by K. Ehara, Department of Agriculture, Kyushu University, Fukuoka, Japan, on October 22, 1952. Introduced from Manchuria. 2n=42. n=21. MONT. 59,320.
A. sibiricum (Willd.) P.B.	Agr.	68-5	Collected in the Kopet-Dag Mountains Turkmen S.S.R., Russia. Seeds received through Botanic Gardens, Turkmen Scientific Academy. 2n=28, n=14 (Creel, Ericson, and Schulz-Schaeffer, 1964). MONT. 59,322.
A. spicatum (Pursh.) Scribn. et Smith	Hol.	75-3	Grass and Legume Standard Accession List. USDA Soil Conservation Service, Pullman, Washington, Feb. 1955. P-6409. 2n=28. Determined by Jurasits in 1960. MONT. 59,327.
A. stipaefolium Czern.	Hol.	122-2	Received from All Union Institute of Plant Industry. Leningrad, U.S.S.R. Collected in Czern Lineck region, near Leningrad, Russia.
A. trachycaulum (Link) Malte.	Gou.	93-4	Collected by the USDA Forest Service near Beebe, Montana, 1933. Variety "Primar". P-2535. 2n=28. MONT. 59,338.

Table I. (continued)

Species	Section	MSC Field No.	Source
A. trichophorum (Link) Richt.	Hol.	57-5	P. I. 106,831. Introduced as A. popovii. Seed received from the Botanic Gardens, Turkmen Scientific Academy, Ashkhabad, Turkmen S.S.R., Russia, October, 1934. 2n=42. Determined by Jurasits in 1960. MONT. 59,315.
		104-3	Seed received from seed collection of the Institut für Pflanzenbau und Pflanzenzüchtung, Göttingen, Germany, January, 1960, as field Nos. 40-1957 and 138-1958. B-80. 2n-42. MONT. 59,344.
A. triticeum Gaertn.	Ere.	125-S	Collected by Dr. W.E. Booth, near Gardiner, Montana, in 1952. 2n=14. Determined by Ericson in 1964.
A. tsukushiense var. transiens (Honda)		148-S	Received from D.S. Sakamoto, National Institute of Genetics, Misima, Sizuoka-ken, Japan. 6380. 2n-42, n=21 (Sakamoto, 1958).

Explanation of Accession Numbers and Abbreviations:

Agr. Section Agropyron

B. Seed collection of the Department of Plant and Soil Science, Montana State College, Bozeman, Montana

Ere. Section Eremopyrum

Gou. Section Goulardia

Gr. Seed collection of the Institute für Kulturpflanzenforschung, Gatersleben, Germany

Hol. Section Holopyron

MONT. Herbarium of Montana State College, Bozeman, Montana, U.S.A. Lanjouro, J. and Stafleu, F. A. Index Herbariorum. Part I. The herbaria of the world. International Bureau for Plant Taxonomy and Nomenclature, Utrecht, 1954

P Seed collection of the USDA, Soil Conservation Service, Pacific Region, Plant Material Center, Washington State University, Pullman, Washington

P.I. Seed collection of the USDA, ARS, New Crops Research Branch, Crops Research Division, Plant Introduction Stations, Western Region, Pullman, Washington and North Central Region, Ames, Iowa

S Indicates that several seedlings were used rather than a single plant