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Series on Harmonization of Regulatory Oversight in Biotechnology No. 9

CONSENSUS DOCUMENT ON THE BIOLOGY OF TRITICUM AESTIVUM (BREAD WHEAT)

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OECD Environmental Health and Safety Publications

Series on Harmonization of Regulatory Oversight in Biotechnology

No. 9

Consensus Document on the Biology of *Triticum aestivum* (Bread Wheat)

Environment Directorate

Organisation for Economic Co-operation and Development

Paris 1999

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FOREWORD

The OECD's Working[1] Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session, in June 1995, to focus its work on the development of **Consensus Documents** that are mutually recognised among Member countries. These Consensus Documents contain information for use during the regulatory assessment of a particular product. In the area of plant biosafety, Consensus Documents are being developed on the biology of certain plant species, on specific genes and resulting proteins that, when introduced into a plant, result in the expression of specific traits, and on biosafety issues arising from certain general trait modifications made to plants.

This document, which was prepared by Germany as lead country, addresses the biology of the species *Triticum aestivum*. It has been revised based on comments received from OECD Member countries and on subsequent comments from National Co-ordinators, following further rounds of review in 1997 and 1998.

As part of a joint project with the United Nations Environment Programme (UNEP) and the United Nations Industrial Development Organization (UNIDO) on centres of origin and diversity, the document was reviewed by experts in several countries that are centres of origin and diversity for wheat. The Joint Meeting of the Chemicals Committee and the Working Party on Chemicals has recommended that this document be made available to the public. It is published on the authority of the Secretary-General of the OECD.

1. In August 1998, following a decision by OECD Council to rationalise the names of Committees and Working Groups across the OECD, the "Expert Group on Harmonization of Regulatory Oversight in Biotechnology" became the "Working Group".

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Preamble

OECD Member countries are now commercialising and marketing agricultural and industrial products of modern biotechnology. They have identified the need for harmonization of regulatory approaches for the assessment of these products, in order to avoid unnecessary trade barriers.

In 1993, **Commercialisation of Agricultural Products Derived through Modern Biotechnology** was instituted as a joint project of the OECD's Environment Policy Committee and its Committee on Agriculture. The objective of this project is to assist countries in their regulatory oversight of agricultural products derived through modern biotechnology – specifically in their efforts to ensure safety, to make oversight policies more transparent and efficient, and to facilitate trade. The project is focused on the review of national policies, with respect to regulatory oversight, that will affect the movement of these products into the marketplace.

The first step of this project was to carry out a survey concentrating on national policies in regard to regulatory oversight of these products. Data requirements for products produced through modern biotechnology, and mechanisms for data assessment, were also surveyed. The results were published in *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (OECD, 1995).

Subsequently, an OECD Workshop was held in June 1994 in Washington, D.C. with the aim of improving awareness and understanding of the various systems of regulatory oversight developed for agricultural products of biotechnology; identifying similarities and differences in various approaches; and identifying the most appropriate role for the OECD in further work towards harmonization of these approaches. Approximately 80 experts in the areas of environmental biosafety, novel food safety and varietal seed certification, representing 16 OECD countries, eight non-member countries, the European Commission and several international organisations, participated in the Workshop. *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* was also published by the OECD in 1995.

As a next step towards harmonization, the Working Group on Harmonization of Regulatory Oversight in Biotechnology instituted the development of **Consensus Documents** that are **mutually recognised** among Member countries. The purpose of these documents is to describe common elements in the safety assessment of a new plant variety developed through modern biotechnology, to encourage information sharing and prevent duplication of effort among countries. These common elements fall into three general categories: the biology of the host plant species, or crop; the introduced genes and gene products conferring the novel trait; and biosafety issues arising from the introduction of certain general trait types into plants.

The safety issues identified in the Consensus Documents on the biology of specific crop plants are intended to address the potential for gene transfer within the crop plant species, and among related species, as well as the potential for weediness. They make no attempt to be definitive in this respect,

however, as the many different environments in which the crop species may be grown are not considered individually.

This Consensus Document is a "snapshot" of current information that may be relevant in a regulatory risk assessment. It is meant to be useful not only to regulatory officials, as a general guide and reference source, but also to industry and others carrying out research and product development.

Reference to two other OECD publications that have been published in recent years will also prove useful. *Traditional Crop Breeding Practices: An Historical Review to Serve as a Baseline for Assessing the Role of Modern Biotechnology* presents information concerning 17 different crop plants. It includes sections on phytosanitary considerations in the movement of germplasm and on current end uses of the crop plants. There is also a detailed section on current breeding practices. *Safety Considerations for Biotechnology: Scale-Up of Crop Plants* provides a background on plant breeding, discusses scale dependency effects, and identifies various safety issues related to the release of plants with "novel traits".[1]

To ensure that scientific and technical developments are taken into account, OECD countries have agreed that Consensus Documents will be updated regularly. Additional areas relevant to the subject of each Consensus Document will be considered at the time of updating.

Users are therefore invited to provide relevant new scientific and technical information, and to make proposals concerning additional areas that might be considered in the future. ***A short, pre-addressed questionnaire is included at the end of this document. The information requested should be sent to the OECD at one of the addresses shown.***

1. For more information on these and other OECD publications, contact the OECD Publications Service, 2 rue André-Pascal, 75775 Paris Cedex 16, France. Fax: (33) 01.49.10.42.76; E-mail: PUBSINQ@oecd.org; or consult **http://www.oecd.org**

Section I - General Description and Use as a Crop, Including Taxonomy and Morphology

Triticum aestivum, bread wheat, belongs to the order *Poales* (*Glumiflorae*), family *Poaceae* (*Gramineae*), tribe *Triticeae*, genus *Triticum*. The tribe *Triticeae* consists of 18 genera which are divided into two sub-groups, the *Triticinae* and the *Hordeinae*. The major genera in the sub-group *Triticinae* are *Triticum, Aegilops, Secale, Agropyron* and *Haynaldia* (Odenbach 1985, Zeller 1985, Körber-Grohne 1988).

Plants of the genus *Triticum* are annuals with spring or winter forms. They show the following morphological features: short ligule and spikelets that are sometimes hairy, and a smooth, bald, usually hollow culm, 0.7-1.6 metre in height. Pithy filling is less common than a hollow culm. The ears have a brittle or tough rachis. Generally they are four-sided. The spikelets have two to five florets. Each floret can produce one grain (caryopsis), i.e. is distichous. The glumes are keeled, on the upper side for example in *T. aestivum*, with serrated lemmas, long and either bearded or unbearded. Grains are loosely enclosed (naked wheat) and easily threshed. The rachilla has thin walls and does not disarticulate on maturity. In case of *T. aestivum* ssp. *spelta* (spelt wheat) the grains are hulled by the spelta. For this reason they cannot be dropped during the process of threshing (Garcke 1972, Geisler 1991).

T. aestivum is a cereal of temperate climates. The northern limit of wheat cultivation in Europe lies in southern Scotland (60° latitude) and occasionally beyond (central Scandinavia up to 64°). In North America wheat is grown to about 55° latitude. Wheat occurrence follows a similar pattern in the southern hemisphere. In the Alps, it is grown to an altitude of 1 500 metres above sea level (Körber-Grohne 1988, Geisler 1991).

The minimum temperature for germination of *T. aestivum* seeds is between 3 and 4°C. Flowering begins above 14°C. The vegetative period is 120 to 145 days for spring wheat and 280 to 350 days for winter wheat. Some varieties of *T. aestivum* need long photoperiods; some, especially those cultivated in southern Europe, are insensitive to day length. The harvested fruit, a grain with the botanical name caryopsis, contains approximately 80 to 84 per cent endosperm, approximately 60 per cent carbohydrate (starch), approximately 10 to 16 per cent protein, approximately 2 per cent fat, and approximately 13 per cent water (Hömmö and Pulli 1993). The starch granules of the *Triticeae* are botanically distinctive. Wheat meal is an important product. Meal from *T. durum* (macaroni wheat), for example, is used for the production of pastas such as spaghetti and semolina. Meal from *T. aestivum* (bread wheat) on the other hand contains a high proportion of gluten. For this reason it is very suitable for baking. Spelt wheat is rich in protein. Overlapping in protein content and high starch content can occur, as there is a wide range of difference due to both genetic variation and variable environmental conditions (Körber-Grohne 1988).

Section II - Agronomic Practices

In the Northern Hemisphere, depending on the location and the preceding crop, winter wheat can be sown from late August to late December. Sowing usually occurs between mid-September and late October. Seeds of winter wheat need 40 to 70 days vernalisation with a temperature between -1°C and +8°C (Geisler 1970, 1971, Kübler 1994). Hömmö and Pulli (1993) reported a maximum cold tolerance for winter wheat of about -25°C.

Seeds of spring wheat need only 3 to 5 days (Geisler 1970) or 0 to 14 days (Reiner et al. 1992) vernalisation. The commencement of growth of shoots is decisively influenced by the photoperiod in the case of spring wheat. The cold tolerance for seedlings of spring wheat is about -5°C (Hömmö and Pulli 1993). The sowing season for spring wheat is from January to May (Kübler 1994).

In normal agricultural practice *T. aestivum* is used in a crop rotation schedule. Sugar beet, grain legumes and corn (*Zea* mays) or fodder maize make good preceding crops (Kübler 1994). Oilseed rape and winter barley occupy large areas and are part of many crop rotation systems that include winter wheat. Wheat/fallow rotations are commonly used in the western Great Plains region of the United States. Problems with plant diseases (see Annex I) may arise from the frequent use of wheat as part of the crop rotation system.

As with all crops cultivated and harvested at the field scale, some seeds may escape and remain in the soil until the following season when they germinate either before or following seeding of the succeeding crop. In some instances these “volunteers” may give considerable competition to the seeded crop and warrant chemical and/or mechanical control. The problem of volunteer plants in succeeding crops is common to most field crop species. Much depends on the management practices used in the production of the crop, e.g. the speed of the harvesting operation which will determine whether more or less seed is lost by the harvester. A suitable soil treatment after the harvest can considerably reduce the volunteer problem.

A great number of dicotyledonous and fewer monocotyledonous weeds have been reported to occur in fields used for wheat production. Seeds of some of these, when harvested and mixed with the wheat grain, can reduce flour quality (Wolff 1987).

Isolation of wheat plants for seed multiplication within the context of plant breeding can be done with greaseproof paper or cellophane bags placed over the heads (Mandy 1970, Saatgutverordnung/BGbl 1986). Without these, modest spatial isolation may be required to prevent outcrossing. In Germany, for example, there is no minimum isolation distance for wheat breeding, but there is a requirement for separation from all neighbouring plants that can be threshed, and for a buffer zone of a minimum of 40 cm to prevent mechanical mixing of the seeds (Saatgutverordnung 1986).

Section III - Centres of Origin/Diversity, Geographic Distribution

History of Wheat

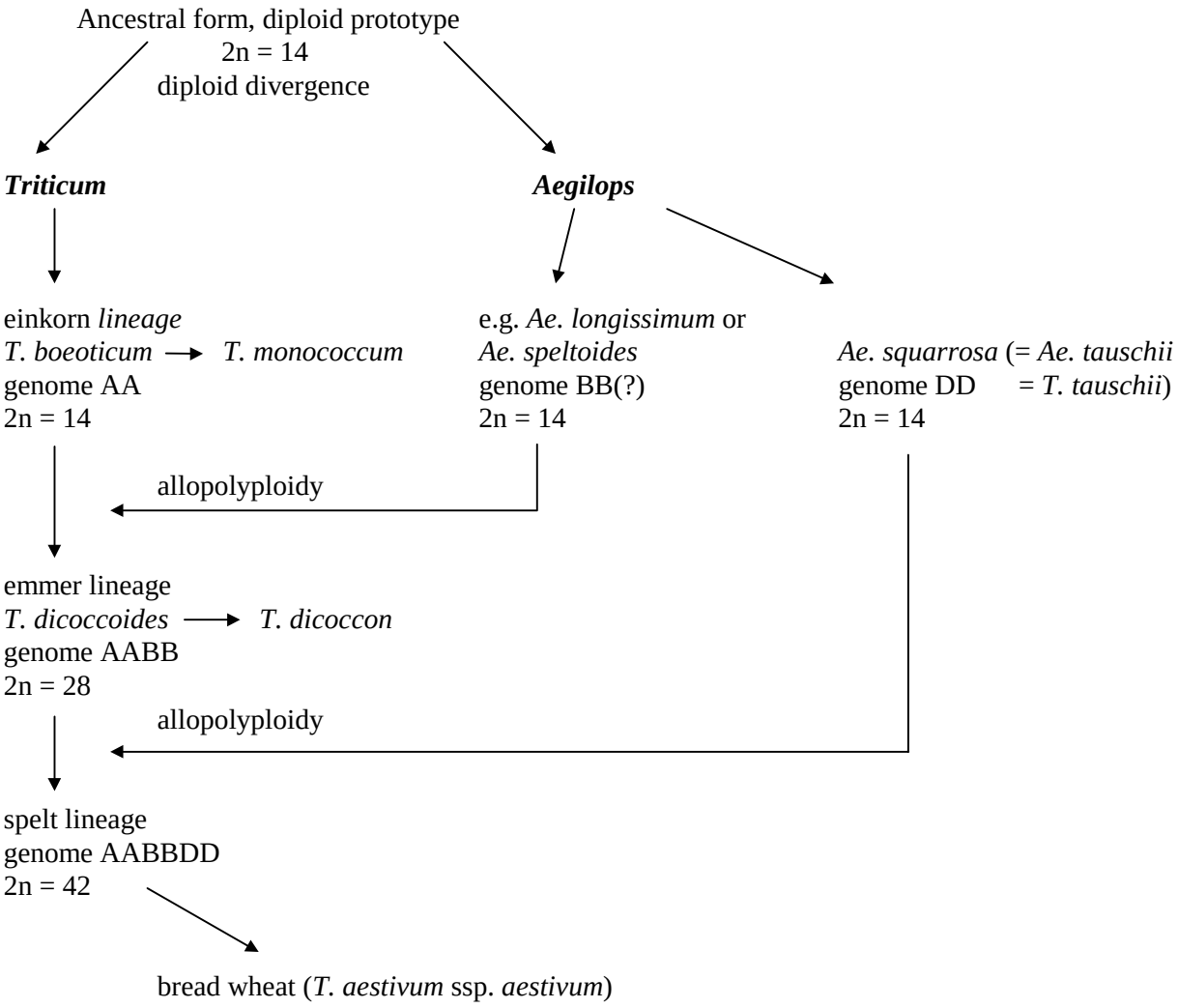
The oldest archaeological findings of naked wheat (6800 to 5200 B.C.) come from southern Turkey, Israel, Syria, Iraq, Iran and south of the Caucasus Mountains in Georgia. At that time, einkorn, emmer and barley were the staple cereal crops in Asia Minor. Wheat was only grown on a regional basis. There is evidence that naked wheat was cultivated in the southern Caucasus in neolithic settlements between the late fifth and early fourth millennium B.C. Late Bronze Age specimens (approximately 1000 to 900 B.C.) of naked wheat have been found at several sites in the Crimea, which was an early and significant wheat-growing area. Archaeological findings of wheat in Israel date from the same period (Körber-Grohne 1988).

In Central Europe, the oldest dated findings of wheat grains (a mixture of *T. aestivum*, *T. dicoccon* and *T. monococcum*) were in soil samples from the New Stone Age (4600 to 3800 B.C.). When the late neolithic period began, naked wheat was gaining importance as a crop in some areas along the River Neckar and around riverside and moorland settlements in the northern foothills of the Alps. It was not until the Roman Empire that wheat spread to the lower Rhine regions, the lower Meuse and the Scheldt Estuary, where it became the main cereal crop. Further south, spelt was favoured. Wheat farming declined north of the Alps between the fall of Rome and the Middle Ages. Evidence from excavated sites shows that little wheat was grown in the period 800 to 1200 (Körber-Grohne 1988).

The origin of Wheat has been well known since the 1940s, mainly through the work of E. R. Sears at the University of Missouri, Columbia (USA) from 1939 to 1980 (MacFadden and Sears 1946). The evolution of wheat began with an unknown diploid prototype, from which the genera *Triticum* and *Aegilops* were formed by diploid divergence. The development of the genus *Triticum* (see Figure 1) began with the einkorn lineage (*T. monococcum* line, genome AA), which developed into the cultured form *T. monococcum* from the wild form *T. boeoticum*. Allopolyploidization with an *Ae. speltoides* descendant (genome BB) led to the tetraploid emmer lineage (*T. turgidum* line, genome AABB) with the wild form *T. dicoccoides* from which the cultured form *T. dicoccon* developed. The origin of the B-genome is more uncertain; *Ae. speltoides*, *Ae. longissimum*, *Ae. bicornis*, *Ae. searsii*, *Ae. sharonense* are suggested as possible progenitors. The spelt lineage[1] with the genome AABBDD resulted from further allopolyploidization with the species *Ae. squarrosa* (= *Ae. tauschii*; genome DD) (Körber-Grohne 1988, Sitte et al. 1991, Zeller and Friebe 1991). For the current classification of the genus *Triticum* see the monograph of van Slageren (1994), also available on the home page of the Wheat Genetics Research Center, Kansas State University (**http://www.ksu.edu/wgrc**, under "Triticum" accessions). More recent references in regard to the issue of wheat origin are Cauderon (1994), Zohary and Hopf (1994) and Feldman et al. (1995).

1. Note that the term "lineage" is used to indicate that descendants are related.

Figure 1 An overview of the diploid einkorn lineage
(Körber-Grohne 1988, Sitte et al. 1991, Zeller and Friebe 1991)



A. Origin of einkorn lineage

The einkorn lineage includes the wild species of *T. boeoticum* and various goat grasses (see Table 1). The latter were formerly considered to belong to the genus *Aegilops,* but many geneticists now classify them as belonging to the genus *Triticum.* The only domesticated species in this group is einkorn *(T. monococcum). S*pecies have only one grain per floret; however, they may have one or two florets per spikelet. They are diploid (2n = 14, genome AA) (Körber-Grohne 1988, Sitte et al. 1991, Zeller and Friebe 1991).

Table 1 Geographic distribution of the diploid einkorn lineage (Körber-Grohne 1988)

Hulled grain
Wild einkorn *T. boeoticum* (AA)
Single-grain var. *aegliopoides* (AA) Balkans, N. Greece, W. Turkey
Double-grain var. *thaoudar* (AA) E. Turkey, N. Iraq, Iran
Progeny of the two varieties (AA) Central Turkey, Transcaucasia
Goat grass *T. tauschii (Aegilops tauschii = Aegilops squarrosa)* (DD) Mediterranean, Central Asia, Iran, Iraq, Transcaucasia
Another five species of *Aegilops* (similar to B) Asia Minor and Central Asia
Einkorn *T. monococcum* (AA)

B. Origin of emmer lineage

The emmer lineage includes only tetraploid hybrids with the genome AABB (see Table 2). The cultivated form *T. dicoccon* developed from the wild form *T. dicoccoides*. Three forms of wild emmer are found today in various parts of Asia Minor and Central Asia. Of the six domesticated species, only emmer retains its hull as a mature grain. Species have two to three florets with two grains each (Körber-Grohne 1988, Sitte et al. 1991, Zeller and Friebe 1991).

Table 2 Geographic distribution of the tetraploid emmer lineage (Körber-Grohne 1988)

Hulled grain	Naked grain
Wild emmer *T. dicoccoides* (AABB) S.E. Turkey, Israel, S. Syria, N. Iraq, W. Iran	
Wild emmer *T. timopheevi* (AAGG) Transcaucasia, Armenia, N. Iraq, W. Iran	
Wild emmer *T. araraticum* (AAGG) Transcaucasia	
Emmer *T. dicoccon* (AABB)	Durum wheat *T. durum* (AABB) N.E. Africa, Mediterranean, Spain
	Rivet/cone wheat *T. turgidum* (AABB) Portugal, UK, Spain
	Persian wheat *T. carthlicum* (AABB) Caucasia, Iraq, Iran
	Oriental wheat *T. turanicum* (AABB)
	Polish wheat *T. polonicum* (AABB) S. Europe, Turkey, Iraq, Iran, Armenia, N.W. India

C. Origin of spelt lineage

It is assumed that genome A derives from einkorn (*T. monococcum*) and genome D from goat grass (*T. tauschii = Ae. squarrosa = Ae. tauschii*). The origin of the third genome (B) is still unclear. It possibly belongs to *Ae. speltoides* descendants or ancestors (see Section II: History of Wheat).

The hexaploid wheat group (2n = 42, genome AABBDD) is closely related to spelt, macha and the naked wheats (see Table 3). The genetic differences in the gene pool of hexaploid wheat are small, although they exert a considerable influence, yielding both hulled grain (e.g. spelt) and naked grain (wheat).

The entire hexaploid lineage (AABBDD) is regarded as a single species. The various grains (e.g. bread wheat *T. aestivum* ssp. *vulgare*, spelt *Triticum aestivum* ssp. *spelta*) are considered as subspecies. In practical usage, however, the earlier categories are still frequently applied (Körber-Grohne 1988).

Table 3 Geographic distribution of the hexaploid spelt lineage (Körber-Grohne 1988)

Hulled grain	Naked grain
Macha wheat *T. macha* (AABBDD) Georgia/Transcaucasia	
T. vavilovii (AABBDD) Armenia	
Spelt/dinkel *T. spelta* (AABBDD)	Dwarf/club wheat *T. compactum* (AABBDD) mountains of Afghanistan, Alps
	Cake wheat *(Kugelweizen) T. sphaerococcum* (AABBDD) Afghanistan, Bukhara, N.W. India
	Bread wheat *T. aestivum (aestivum)* (AABBDD) Temperate zones

Section IV - Reproductive Biology

Reproduction of *T. aestivum* is only known in the context of cultivation (Garke 1972). Harvesting and propagation of its seed are entirely dependent on man. Wheat is predominantly self-pollinating. The cross-fertilisation rate may be as high as 1 to 2 per cent, although it can be less than 1 per cent (Poehlmann 1959). Wind-borne cross-fertilisation depends heavily on physical factors. It is minimal (0.1 per cent) where there is high humidity, but higher when there is warm, dry weather. Under such conditions, it has been claimed that the cross-fertilisation rate may be between 3.7 and 9.7 per cent. Cross-fertilisation is considerably more likely in the ears of stem branches (also called tillers) (Mandy 1970). The rate of cross-fertilisation may also depend on the variety (e.g. Stoner 24 to 37 per cent). Hucl (1996) shows for 10 Canadian spring wheat cultivars that the cross-pollination frequency varies according to the genotype. The frequency was always lower than 9 per cent. Apomixis is very rare (Mandy 1970).

Wheat's flowering season depends on geographical location. For example, in Germany and Sweden it flowers from late May to late June (Mandy 1970, Garke 1972). Flowering times for Mediterranean Europe and the centres of origin and diversity of wheat are late winter, and early spring (Galun, personal communication). Sunny weather and temperatures of at least 11 to 13°C are propitious for flowering (Mandy 1970). The influorescence of wheat is a spike, and the ear on the main culm flowers first. The process begins in the middle third of the ear, spreading towards the tip and base. The spikelets at the top and bottom of the ear are the last to bloom (Mandy 1970). In cultivated wheat fields, the number of ears is usually between 400 and 650/m². Depending on the proportion of well-developed ears, the average grain count per ear varies between 35 to 40 and 20 to 25. However, the standard number of seeds per head is 30 to 35 (one ear carrying an average of 80 florets) (Kübler 1994; average data in Germany).

When flowering, the lemmas and palaeas open to an angle of 20 to 35°. The pollen sacs appear about four to six minutes later adopting a horizontal position. Under favourable weather conditions a floret will complete the flowering cycle in 13 to 18 minutes. The reproductive organs are slightly protandrous (pollen sacs mature one to three days earlier). An unfertilised spikelet remains open for several hours or even days (Mandy 1970).

Flowering for a full ear takes between 101 and 120 hours, 23 florets a day blooming on average. Blooming begins in the early morning between 4 and 5 a.m. Peak flowering time is between 9 and 10 a.m., with a second peak between 2:30 and 3:30 p.m. By 7 p.m. flowering is usually completed. A wheat plant flowers for four to 15 days (Mandy 1970; average data in Germany).

The quantity of pollen produced by an anther is low, being approximately 2700 pollen grains per sac. It has been established that, on average, 80 per cent of pollen from an anther which protrudes from the spikelet is dispersed into the air. It was assumed from this that a wheat variety with a large number of protruding anthers would make enough pollen available to achieve cross-fertilisation. Under experimental conditions in the laboratory (moderate mass exchange of 10 g/cm per second and moderate wind speed of 3 m/sec), pollen travels about 60 m distance at a height of 1 m (D'Souza 1970). In field experiments Wilson (1968) found 10 per cent seedsetting on male sterile wheat plants that were 30 m from the pollen donor plants.

Pollen begins to germinate 15 minutes after deposition on the stigma (D'Souza 1970) and retains its fertilisation ability for only a very short period. Even under optimum conditions of 5°C and 60 per cent relative atmospheric humidity, this period will not exceed three hours. Under common field conditions of 20°C and 60 per cent relative atmospheric humidity it may remain viable for less than 30 minutes. With temperatures of about 30°C and low relative atmospheric humidity, the pollen is only able to achieve its function for 15 minutes. On hot days, therefore, this short fertilisation period can considerably reduce pollen germination in the event that cross-pollination does occur (D'Souza 1970).

Section V - Cross-fertilisation

A. Interspecific/genus

Selection breeding, which had been ongoing for centuries, and the more recent methods of classical hybridisation breeding, have led to an enormous improvement of bread wheat traits. Biotechnological methods offer the potential to complement these traditional techniques. It has been 20 years since *in vitro* methods were first used in wheat breeding (Picard and de Buyser 1973). At that time the first variety, "Jinghua", which was produced using anther culture techniques, was licensed in China. In 1985, "Florin" became the first variety developed using *in vitro* methodology to be licensed in Europe (France) (de Buyser et al. 1987, Henry and de Buyser 1990).

There are many examples of successful classical cross-breeding within the genome lineage of *T. aestivum,* and between *T. aestivum* and the other lineages described above (see Figure 1). Hybridisation is possible with any combination in the hexaploid lineage. The progeny are fertile because the genomes are homologous. Heterosis frequently occurs.

In general, *T. aestivum* has been used as the mother plant in inter-generic and inter-specific crossing. Many crosses have been successful, although techniques such as embryo rescue may be required to obtain viable progeny. Differences have been noted in the receptivity of different varieties of *T. aestivum* to accept cross-fertilisation by other species such as rye (Zeven 1987). One of the reasons for this is the potential control (or lack thereof) by genes Kr1 and Kr2 (Gale and Miller 1987). Wheat has been the subject of considerable work involving wide crossing, but much of this will have little relevance to crosses that might occur naturally in the environment.

Crosses such as (diploid x hexaploid, tetraploid x hexaploid) reduce the fertility of the F_1 generation substantially. Hybridisation is more successful if the parent with higher chromosome number is used as mother plant, although it should be noted that hybridisation between wheat x barley is efficient when barley (14 chromosomes) is used as the female parent. Most F_1 hybrids from hexaploid x diploid crosses are sterile. Only manual crossing of *T. aestivum* x *T. monococcum* produced F_1 hybrids with grains that germinated. Grains of the reciprocal hybrid did not germinate. When tetraploids were manually crossed with hexaploids, only the crossing of *T. aestivum* with *T. turgidum, T. durum, T. timopheevi* or *T. carthlicum* was successful (Mandy 1970, Sharma and Gill 1983). Hybrids from *T. aestivum* and *T. turgidum* are fertile. So while wheat may be crossed with many related species and some related genera, F_1 plants are often highly sterile, or the embryos abort. Gene transfer occurs only through man's intervention, e.g. hand pollination, and through rescue of F_1 embryos or through the use of male-sterile female plants. The chance of gene transfer occurring through such hybrids in nature is minimal. For production of genetically modified *T. aestivum,* and information about technical barriers that were overcome in achieving wheat transformation, see Appendix II.

Triticum species can be crossed by hand with the genera *Aegilops, Secale, Agropyron, Haynaldia Hordeum* and *Elymus* (see Table 4). Trigeneric hybrids are formed in some cases (see Table 5).

Cross-breeding with *Elymus* species has proved least successful (Poehlmann 1959, Sharma and Gill 1983, Zeller 1985, Maan 1987, Jiang et al. 1994). Natural wild crosses of *T. aestivum* with the following members of the genera *Aegilops* (*Ae. cylindrica*, *Ae. triticoides*, *Ae. neglecta*, *Ae. triuncalis*, *Ae. ventricosa*, *Ae. genicularia*, *Ae. bluncalis*, *Ae. crassa*, *Ae. juvenalis*, *Ae. speltoides*, *Ae. tauschii* and *Ae. umbellata*) have been reported (van Slagern 1994). Crosses of *T. aestivum* to tetraploid *Aegilops* species resulted in hybrid seeds from which addition, substitution and translocation lines with introgressed genes for disease resistance have been selected (Spetsov et al. 1997, Petrova and Spetsov 1997). For information about cross-breeding of wheat with *Elymus*, see Dewey (1984), Plourde et al. (1989) and Koebner et al. (1995); with *Thynopyrum*, see Dewey (1984) and Sharma and Baezinger (1986); with *Elytrigia*, see Dewey (1984) and Cauderon (1994); and with *Pseudoroegnaria*, see Dewey (1984). Wheat can also cross with *Sorghum* and *Setaria* (Laurie et al. 1990).

Most manual cross-breeding has been carried out with *Secale cereale*, in order to combine the high grain yield and protein quality of wheat with rye's disease resistance and tolerance of poor soil conditions. The resulting generic progeny is called "triticale." There are only a few reports on natural hybridisation between wheat and rye. Müntzing (1979) reports a massive natural hybridisation in 1918, resulting in up to 20 per cent male sterile F_1 wheat x rye hybrids within wheat plots isolated by surrounding rows of rye plants. This spontaneous hybridisation occurred with wheat cultivars exhibiting anemophilic flower characters under dry continental conditions. In most cases, the F_1 hybrids are completely male sterile and have to be pollinated by wheat, rye or fertile tricicale to obtain generic progenies. Another possibility to overcome pollen sterility of wheat x rye hybrids is to double their chromosome number. Modern triticale breeding based on recombination among hexaploid triticales has solved the most important problems with the crop, namely low fertility, poor grain filling, tall stem and late ripening (Wolski et al. 1996). Triticale can be exploited as a bridge for the introgression of valuable genes from *Secale cereale*, e.g. by the generation of 1B/1R translocation chromosomes. The first European cultivar of triticale was obtained in France [Clerical since 1982 and on open catalogues since 1983 (Bernard and Guedes Pinto 1980, Cauderon and Bernard 1980)].

Through the use of *in vitro* methods, dihaploid plants have been produced from crosses between wheat and *Hordeum bulbosum* (Blanco et al. 1986, Cauderon and Cauderon 1956, Stich and Snape 1987) and wheat and *Zea mays* (Kisana et al. 1993). In these cases, the barley and maize chromosomes are eliminated in early stages of embryo development (Barcley 1975, Laurie and Bennett 1988, 1989). After diploidisation of the resulting haploid plants, the homozygous wheat material can be used for RFLP analysis, gene localisation and isolation.

Mandy (1970) reported the first manual intergeneric hybrid between ((*Triticum vulgare* x *Haynaldia villosa*) x *Secale cereale*), with the chromosome number (n = 35). Reciprocal hybridisation has had low success.

Interspecific hybridisation under natural conditions has been reported to occur only rarely (Gotsov and Panayotov 1972).

Table 4 Manual intergeneric crossing with *Aegilops* (*Ae.*), *Secale* (*S.*), *Agropyron* (*A.*), *Haynaldia* (*Ha.*), *Hordeum* (*H.*) and *Elymus* (*E.*) (Sharma and Gill 1983)

Wheat parent	Species of allied genera crossed
Diploid wheat: *Triticum monococcum*	*Ae. bicornis, Ae. caudata, Ae. columnaris, Ae. comosa, Ae. cylindrica, Ae. longissima, Ae. mutica, Ae. ovata, Ae. speltoides, Ae. squarrosa, Ae. triaristata, Ae. tripsaccoides, Ae. triuncialis, Ae. umbellulata, Ae. uniaristata, Ae. variabilis, Ae. ventricosa* *S. cereale* *A. elongatum, A. intermedium* *Ha. villosa* *H. vulgare*
Tetraploid wheat: *T. turgidum*, includes *durum, carthlicum, dicoccum and dicoccoides*	*Ae. bicornis, Ae. biuncialis, Ae. caudata, Ae. clylindrica, Ae. columnaris, Ae. comosa, Ae. crassa, Ae. dichasians, Ae. heldreichii, Ae. kotschyi, Ae. longissima, Ae. mutica, Ae. ovata, Ae. sharonensis, Ae. speltoides, Ae. squarrosa, Ae. triaristata, Ae. tripsaccoides, Ae. triunciales, Ae. umbellulata, Ae. uniaristata, Ae. variabilis, Ae. ventricosa* *S. africanum, S. ancestrale, S. cereale, S. montanum, S. vavilovii* *A. campestre, A. dasystachyum, A. distichum, A. elongatum, A. intermedium, A. junceum* 4x, *A. obtusiusculum, A. repens* *Ha. hordeace, Ha. villosa* *H. brevisubulatum, H. chilense, H. vulgare* *E. arenarius, E. giganteus*
Tetraploid wheat: *T. timopheevi*	*Ae. bicornis, Ae. caudata, Ae. comosa, Ae. cylindrica, Ae. dichasians, Ae. kotschyi, Ae. longissima, Ae. mutica, Ae. ovata, Ae. speltoides, Ae. squarrosa, Ae. triuncialis, Ae. umbellulata, Ae. uniaristata, Ae. ventricosa* *S. africanum, S. cereale, S. vavilovii* *A. campestre, A. cristatum, A. elongatum, A. intermedium, A. junceum* 4x, *A. repens* *Ha. villosa* *H. bogdanii, H. vulgare, H. vulgare* ssp. *distichon*
Hexaploid wheat: *T. aestivum*	*Ae. bicornis, Ae. biuncialis, Ae. caudata, Ae. columnaris, Ae. comosa, Ae. crassa, Ae. cylindrica, Ae. dichasians, Ae. juvenalis, Ae. kotschyi, Ae. longissima, Ae. mutica, Ae. ovata, Ae. sharonensis, Ae. speltoides, Ae. squarrosa, Ae. triaristata, Ae. tripsaccoides, Ae. truncialis, Ae. umbellulata, Ae. uniaristata, Ae. variabilis, Ae. ventricosa* *S. africanum, S. ancestrale, S. cereale, S. montanum, S. vavilovii* *A. caespitosum, A. distichum, A. elongatum, A. intermedium, A. junceum* 2x, *A. podperae, A. scirpeum, A. smithi, A. trachycaulum, A. yezoense* *Ha. villosa* *H. chilense, H. pusillum, H. spontaneum, H. vulgare, H. vulgare* var. *distichum* *E. giganteus*

Table 5 Trigeneric hybrids from manual crossing *Triticum* (*T.*), *Aegilops* (*Ae.*), *Hordeum* (*H.*), *Agropyron* (*A.*), *Haynaldia* (*Ha.*) and *Secale* (*S.*) (Sharma and Gill 1983)

Trigeneric hybrid	**Reference**
(*T. timopheevi* x *H. bogdanii*) x *S. cereale*	Kimber & Sallee 1979
(*H. vulgare* x *T. aestivum*) x *S. cereale*	Claus 1980; Fedak & Armstrong 1980
(*H. vulgare* x *T. aestivum*) x *S. montanum*	Claus 1980
(*H. vulgare* x *A. elongatum*) x *Ae. crassa*	Pedigree of Sando's collection, USDA, Beltsville
(*T. aestivum* x *S. cereale*) x *T. aestivum* x *A. elongatum*	USDA, Beltsville
Triticale (6x) x (*T. durum* x *A. intermedium*) amphidiploid	Nowacki et al. 1979
(*Ae. ventricosa* x *S. cereale*) x *T. aestivum*	Dosba & Jahier 1981
(*Ae. crassa* x *T. persicum*) x *S. cereale*	Knobloch 1968
(*Ae. ventricosa* x *T. dicoccum*) x *A. intermedium*	Knobloch 1968
(*Ae. ventricosa* x *T. turgidum*) x *S. cereale*	Knobloch 1968
(*Ae. ventricosa* x *T. dicoccum*) x *S. cereale*	Siddiqui 1972
(*T. aestivum* x *Ha. villosa*) x *S. cereale*	Knobloch 1968
(*T. dicoccum* x *Ha. hordeacea*) x *S. cereale*	Knobloch 1968
(*T. dicoccum* x *S. montanum*) x *Ha. villosa*	Knobloch 1968
(*T. turgidum* x *Ha. villosa*) x *S. cereale*	Knobloch 1968

B. Introgression

Interspecific hybridisation under natural conditions has rarely occurred (Gotsov and Panayotov 1972), and the role of environmental conditions must be taken into consideration. For example, weather abnormalities may in some instances contribute to male sterility or in others to overlapping of flowering periods. Both of these factors can result in the breaking down of effective isolation barriers between species. The introgression of a new gene will also be dependent on whether or not that gene confers an ecological advantage on the recipient in specific environments. Even so, data on potential hybridisation events are helpful in assessing the potential for introgression of "novel traits" of transgenic *T. aestivum* into wild relatives. If potential "mates" of *T. aestivum* are occurring in the geographic region of interest, introgression has to be taken into consideration.

Rimpau reported observing volunteer crosses between *T. aestivum* x *S. cereale* in his wheat nursery at the beginning of this century. He called the bastard plants "mule-wheat" because they were infertile and he was not able to collect seed from them. Nevertheless, he continued to make artificial crosses (von Broock, personal communication).

Intra- and interspecific variation exists within the cytoplasms of wheat and related species, and this is important for wheat breeders. Cytoplasmic male sterility (CMS) systems are used successfully in several crops. CMS has been introduced into common wheat through interspecific and intergeneric hybridisation. Today, chloroplasts and mitochondria are subjects of molecular genetic studies and of genetic manipulation, and these techniques may in the future be used in wheat. All genetic information present in the DNA of cytoplasmic organelles is maternally inherited, and therefore the chance for gene transfer in nature is less than for nucleic genes.

C. Interactions with other organisms

Wheat grain yield is decreased by some 50 major diseases which can produce overall crop damage (including storage damage) of 20 per cent (Spaar et al. 1989). Fungal diseases are the greatest problem. Animals, e.g. pigeons, crows and pheasants, feed on seeds, dig and tear out plants, or otherwise damage them. Mice, rabbits and deer can also cause considerable damage to wheat plants.

The tables in Appendix I are intended as an identification guide for categories of organisms that interact with *T. aestivum*. Clearly the organisms listed are examples, with their occurrence depending upon the geographic region where *T. aestivum* is grown.

Section VI - Weed Characteristics/Weediness

Wheat is a crop plant species with low competitive ability. It has no natural habitat outside cultivation (Garcke 1972, Tutin et al. 1980). Wheat does not have high potential for weediness (Keeler 1989). Wheat plants may sometimes be found in “disturbed” areas where there is little or no competition from other “weed” species (e.g. waste places, fallow fields, along roadsides), but their survival at such sites is limited to short periods (Janssen et al. 1995). There are no indications that wheat can become established as a self-sustaining population on a long-term basis (Sukopp and Sukopp 1993, Newman 1990).

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Appendix I

Most Common Diseases and Pests in *Triticum aestivum*

Potential interactions of *T. aestivum* with other life forms during its life cycle (Wiese 1987, Spaar et al. 1989, Wolff and Richter 1989, Chelkowski 1991, Cook and Veseth 1991, Wolff 1992):

Viruses, Mycoplasms

See Brunt et al. 1996. For more information, also see the VIDE database:
http:\\www.csu.edu.au/viruses/virus.html

Disease	Agent
Agropyron mosaic virus	Agropyron mosaic virus (AgMV), geographic occurrence e.g. in Eurasia, Canada and the USA
Barley stripe mosaic hordeivirus	Barley stripe mosaic hordeivirus (BSMV), geographic occurrence e.g. in Eurasia, Northern America, Pacific
Barley yellow dwarf virus	Barley yellow dwarf virus (BYDV), geographic occurrence world-wide; wheat varieties show different tolerance level (Baltenberger et al. 1987); tolerance level had been increased through cross breeding with resistant *Agropyron* varieties (Ohm et al. 1989, Gonlart et al. 1993)
Barley yellow streak mosaic virus	Barley yellow streak mosaic virus, geographic occurrence e.g. in Canada and USA
Barley yellow striate mosaic cytorhabdovirus	Barley yellow striate mosaic cytorhabdovirus (BYSMV), geographic occurrence e.g. in Africa, Eurasia, Middle East and Pacific
Brome mosaic virus	Brome mosaic virus (BMV), geographic occurrence e.g. in Eurasia, Australia, South Africa and USA
European striped wheat mosaic	Probably mycoplasms

Disease	Agent
Wheat American striate mosaic nucleorhabdovirus	Wheat American striate mosaic nucleorhabdovirus (WASMV), geographic occurrence e.g. in Canada and USA
Wheat dwarf virus	Wheat dwarf virus (WDV), geographic occurrence e.g. in Bulgaria, former Czechoslovakia, Hungary, former USSR, France and Sweden
Wheat European striate mosaic tenuivirus	Wheat European striate mosaic tenuivirus (EWSMV), geographic occurrence e.g. in Czech Republic, Poland, Romania, Denmark, Finland, Sweden, Germany, UK and Spain
Wheat soilborne mosaic virus	Wheat soilborne mosaic virus, geographic occurrence e.g. in China, Japan, Italy and USA
Wheat spindle streak mosaic virus	Wheat spindle streak mosaic virus, (WSSMV), geographic occurrence e.g. in France, Germany, Italy, India, Japan, China, and USA
Wheat spindle streak virus	Wheat spindle streak virus
Wheat streak mosaic virus	Wheat streak mosaic virus (WSMV), geographic occurrence e.g. in Canada, USA, Romania and Jordan
Wheat striate mosaic virus	Wheat striate mosaic virus
Wheat yellow leaf virus	Wheat yellow leaf virus (WYLV), geographic occurrence e.g. in Japan and Italy
Wheat yellow mosaic brymovirus	Wheat yellow mosaic brymovirus, geographic occurrence e.g. in China, Japan, Korea, Canada and France
Wheat yellow mosaic virus	

Bacteria

Disease	Agent
Basal glume blotch	*Pseudomonas syringae* pv. *atrofaciens* (McCulloch)
Black glume	*Xanthomonas campestris* pv. *translucens* (Jones, Johnson et Reddy) dye Various known forms which differ only in host specificity: *undulosa, cerealis, hordei, secalis, orycicola* and *phleipratensis*

Fungi

Disease	Agent
Ergot	*Claviceps purpurea*: infects florets and produces grain-like sclerotia containing mycotoxins (ergot alkaloids). The fungal grains are harvested with the wheat grains and, if not removed, mycotoxin contamination of products occurs.
Eyespot, stembreak, straw breaker	*Pseudocerosporella herpotrichoides* (Fron.) Deight., Syn.: *Cerosporella herpotrichoides* (Fron.), breeding for resistance; wheat genotypes with short shoot and good steadiness
Fusarium diseases of shoots (root and culm rots, partial head blight)	Numerous *Fusarium* species play a part in the pathology of the cereal fusaria. The major species are: – *Fusarium nivale* (Ces., Syn.: *Gerlachia nivalis)* – *Fusarium culmorum* (W.G. Smith) Sacc. var. *culmorum* – *Fusarium avenaceum* (Fr.) Sacc. var. *avenaceum* – *Fusarium graminearum* Schwabe (perfect form: *Gibberella zeae* (Schw.) Petch): widespread, especially harmful not only to wheat but also to maize – *Fusarium poae* (Peck) Wollenw.: occurs sporadically, often in conjunction with the grass mite (*Siteroptes graminum* [Reuter]), which feeds on the fungus and helps it to proliferate. – Other species found in wheat include: *Fusarium acuminatum* Ell. et Kellerm. *(Gibberella acuminata* Wollenw.), *Fusarium dimerum* Penzig, *Fusarium equiseti* (Corda) Sacc. *(Gibberella intricans* Wollenw.), *Fusarium porotrichoides* Sherb., *Fusarium tricinctum* (Corda) Sacc. and *Fusarium moniliforme* Sheldon sensu Wollenw. et Reinking, increased resistance breeding in wheat; chemical treatment led to unsatisfactory results (Maurin et al. 1996).

Disease	**Agent**
Glume blotch (Septoria disease)	*Leptosphaeria nodorum* (E. Müll.), conidial form *Septoria nodorum* Berk., Syn.: *Phaesopheria nodorum* (E. Müll.) Hejarude, only partial resistance in wheat found (Jeger et al. 1983, Bostwick et al. 1993).
Helminthosporium yellow blotch disease	*Drechslera tritici-repentis* (Died.) Shoem., perfect form: *Pyrenophora trichostoma* (Fr.) Fckl., Syn.: *Pyrenophora tritici-repentis* (Died.) Drechsl.
Mould	*Aspergillus* ssp./*Penicillium* ssp. can proliferate during storage. Both are potential mycotoxin producers (Ochratoxin A).
Phoma leaf spot	*Phoma glomerata* (Cda.) Wr. et Hochaf.
Pointed eyespot (stembreak, straw breaker)	*Rhizoctonia* spp., *Thanatephorus cucumeris* (Frank) Donk.
Powdery mildew of cereals	*Erysiphe graminis* DC. f. sp. *tritici* March, resistance genes, e.g. Mlk, Pm1 to Pm9, M1Ax, U1 and U2, can be found in different wheat varieties and related species (Heun and Fischbeck 1987, 1989, Hovmoller 1989, Zeller et al. 1993).
Rusts	
Yellow/stripe rust	*Puccinia striiformis* (West., Syn.: *Puccinia glumarum* Erikss. et Henn). Formation of pathotypes which specialise in wheat or barley. In exceptional cases wheat stem rust strains may attack highly susceptible barley varieties or vice versa.
Leaf rust of wheat	*Puccinia recondita* Rob. ex Desm. f. sp. *tritici,* Syn.: *Puccinia triticina* Erikss., Syn.: *Puccinia rubigovera* Wint. Formation of pathotypes, alternate host *Thalictrum* spp.
Black stem rust of wheat	*Puccinia graminis* Pers. f. sp. *tritici* Development of formae speciales specialised in rye, barley, oats, wheat and grasses. Numerous pathotypes formed.
Septoria leaf blotch	*Mycosphaerella graminicola* (Fckl.) Sanderson, conidial form: *Septoria tritici* Rob. ex Desm.

Disease	Agent
Smuts	
Loose smut of wheat	*Ustilago tritici* (Pers.) Rostr.
Covered smut of wheat	Various *Tilletia* species with different sori, including: – *Tilletia caries* (DC.) Tul. Syn.: *Tilletia tritici* (Bjerk.) Wint. – *Tilletia foetida* (Wallr.) Liro, Syn.: *Tilletia laevis* Kühn or *Tilletia foetens* (Bjerk. et Curt.) Schroet. – *Tilletia intermedia* (Gassner) Savul. Syn.: *Tilletia tritici f. sp. intermedia* Gassner
Dwarf bunt of wheat	*Tilletia controversa* Kühn
Carnal smut	*Neovossia indica* (Mit.) Mund.
Stripe/flag smut	*Urocystis agropyri* (Preuss.) Schroet.
Take-all	*Gaeumannomyces graminis* (Sacc.) v. Arx. et Olivier var. *tritici* Walker Several varieties with overlapping hosts, var. *tritici* attacks wheat, triticale, barley and rye, no resistant varieties in wheat found.

Animals

Pest	Agent
Apart from the above-mentioned species of aphid, the following species may cause damage to cereals, maize and grasses:	Bromegrass aphid *(Diuraphis bromicola* [H.R.L.]), cat's-tail aphid *(Diuraphis mühlei* [Börn.]), corn leaf aphid *(Rhopalosiphum maidis* [Fitch.]), yellow cherry/reed canary grass aphid *(Rhopalomyzus lonicerae* [Siebold], *Rhopalomyzus poae* [Gill.], cocksfoot aphid *(Hyalopteroides humilis* [Walk.], *Laingia psammae* (Theob.), *Schizaphis nigerrima* H.R.L., *Metopolophium festucae* (Theob.), green grain aphid *(Schizaphis graminum* [Rond.]), grain aphid *(Sitobion granarium* [Kirby]), cob aphid *(Sipha maydis* [Pass.], *Sipha glyeriae* [Kalt.]), black (bean) aphid *(Aphis fabae* Scop.), green peach aphid *Myzus persicae* [Sulz.])
Aphids:	Aphids arrive from early May (when wheat is shooting), settling first on leaf blades and sheaths, transferring to influorescence as ears extend. Warm and dry conditions encourage generations. The generation cycle lasts 8 to 10 days. Each aphid can lay 30 to 50 larva (parthenogenesis). Around mid-July mass proliferation is briefly interrupted due to poor feeding conditions and the appearance of parasites and predators (ladybirds/ladybugs). The grain aphid undergoes a holocycle, i.e. sexual differentiation takes place in autumn, and winter eggs are laid on grasses. More than 10 generations occur in the space of a year.
Grain aphids	*Macrosiphum avenae* (Fabr.), Syn.: *Sitobion avenae* (Fabr.) Also in barley, oats, rye, maize, fodder grasses
Oat or bird cherry aphid	Aphid species which does not alternate hosts *Rhopalosiphum padi* (L.) Alternate-host aphid with broad host plant profile among cereal and grass species, e.g. barley, oats, maize, fodder grasses.

Rose grain aphid	*Metopolophium dirhodum* (Walk.) Alternate-host aphid (also in barley, oats, rye, maize, fodder grasses).
Cereal cyst nematodes, cereal stem eelworm	*Heterodera avenae* Woll. Also attacks barley, oats, rye, fodder grasses. Several biotypes distinguished by their host profile. Cysts drop from roots and survive in soil. Larvae hatch in spring and infect roots. Sexual differentiation occurs in the root. Females carry up to 600 eggs. When a female dies, its body turns brown and is transformed into a lemon-shaped cyst, only limited resistance (Cre 1 gene on chromosome No. 2B) found in wheat (Slootmaker et al. 1974).
Cereal leaf beetle	Red-throated cereal leaf beetle *(Oulema melanopus* [L.], Syn.: *Lema melanopa* [L.]), blue cereal leaf beetle *(Oulema lichenis* [Voet], Syn.: *Lema lichenis* [Voet]) Beetles leave winter quarters in mid-April and migrate into cereal fields. Eggs are laid in late May on upper side of leaves. This takes 6 to 8 weeks. Each female lays 50 to 100 eggs. Egg development lasts 7 to 14 days.
Corn beetle	*Zabrus tenebroides* Goeze (corn ground beetle) Beetles appear in late June to early July. Eggs are laid in August and September. Each female lays 80 to 100 eggs in the soil. The first larvae hatch after 14 days and undergo three stages. Overwintering is in the 1st or 2nd larval stage. At soil temperatures of -1°C in spring they resume feeding. The bulk of damage now occurs. Soil pupation takes place in May. The generation cycle of the corn ground beetle lasts one year. Also found in barley, oats, rye, maize, fodder grasses.

Crane-fly larvae	Larvae of the marsh crane-fly *(Pales (Tipula) paludosa* Meig.), common crane-fly *(Pales (Tipula) oleracea* L.), autumn crane-fly *(Pales (Tipula) czizeki* de Jong). Biggest factor: *Pales paludosa*. Also in barley, oats, rye, maize, fodder grasses.
March fly larvae	*Bibio hortulans* (L.), *Bibio marci* (L.), *Bibio johannis* (L.), *Bibio clavipes* (Meig.) Also in barley, oats, rye, maize, fodder grasses.
Myriapods	Various species of myriapods, notably the common millipedes *Cylindroiulus teutonicus* (Pocock) and *Blaniulus guttulatus* (Bosc.) Also in barley, oats, rye, maize, fodder grasses.
Root aphids	*Anoecia corni* (Fabr.), *Anoecia vagans* (Koch), *Aploneura graminis* (Buckt.), *Aploneura lentisci* Pass., *Byrsocrypta personata* Börner, *Forda marginata* Koch, *Forda formicaria* V. Heyden, *Geoica discreta* Börner, *Tetraneura ulmi* (L.) Also in barley, oats, rye, maize, fodder grasses
Slugs	Various species of slug, notably the field slug *(Deroceras reticulatum* O.F. Müll., *Deroceras agreste* L.), the garden/blackfield slug *(Arion hortensis* [Fér.], *Arion rufus* [L.]). Also in barley, oats, rye, maize, fodder grasses.
Wheat and grass bugs	Wheat and grass bugs are a non-homogeneous group of pests. The greatest economic damage is caused by wheat bugs *(Eurygaster* spp.). Also in barley, oats, rye, maize, fodder grasses.
Wheat nematodes	*Anguina tritici* (Steinbuch) Filipjev The larvae which live in the galls can be preserved for years in dried state.

NOTE: A complete list of US wheat pests can be found on the American Phytopathology Society home page: **http://www.scisoc.og/resource/common**

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Appendix II

Transformation of *Triticum aestivum*

The genetic improvement of cereals, including wheat, has been a major focus of plant breeding efforts during the past 50 years. It has resulted in remarkable increases in yield as well as improvements in quality. Nonetheless, plant breeding is a slow process and has biological limitations. In this context the rapidly emerging technologies of plant cell and molecular biology, by permitting access to a much wider gene pool, have attracted much attention, for they provide powerful and novel tools to supplement and complement the traditional methods of plant breeding.

Modern plant biotechnology is based on the delivery, integration and expression of defined foreign genes into plant cells which can then be grown *in vitro* to regenerate plants. The efficient regeneration of normal fertile plants from protoplasts is a basic prerequisite for this technology. For gramineous species, the *in vitro* regeneration of fertile phenotypically normal plants has been very difficult (Vasil and Vasil 1992). The greatest problem to overcome was that of culturing immature and undifferentiated tissue and organ explants at defined development stages in special nutrient media. Now all important cereals, e.g. wheat, barley, rice, can be regenerated from cultured tissue as well as single cells (Vasil 1994). Most early attempts to transform cereals were limited to the use of totipotent embryogenic protoplasts, but embryogenic protoplast cultures are difficult to establish and maintain. For wheat, *in vitro* regeneration from immature embryos from young influorescences and microspores (somatic and gametic embryogenesis) has been possible for some time. However, to provide the cells with the greatest access to the transgenes, and in order to obtain cell culture homogeneity, it seems necessary to achieve genetic transformation of cereals using isolated single cells. In this way, it has been thought that the occurrence of chimaeric transformants would also be avoided. This strategy has been successful with many plant species (both dicots and monocots such as rice and maize). Today, normal and fertile plants can be regenerated from all major species of cereals, including wheat (Vasil et al. 1990). However, it is still an inefficient, time-consuming procedure (Vasil and Vasil 1992).

There are different methods of delivering foreign genes into plants (see review: Nehra et al. 1995). The well known, and often preferred method of *Agrobacterium*-mediated transformation does not work very well with cereals. Like most monocotyledonous species, wheat is generally considered to be outside the natural host range of the *Agrobacterium* pathogen. Experiments with wheat and maize have shown that *Agrobacterium* can transfer viral genomic sequences to cereal cells, resulting in a systemic viral infection called “agroinfection” (Smith and Hood 1995). For this to occur, it is not necessary to achieve integration of the viral genes into the plant genome. Thus it seems that the main difficulty is not the delivery of DNA, but rather its integration (Grimsley et al. 1987, Dale et al. 1989). Recent data from experiments with rice (Hiei et al. 1994), maize (Ishida et al. 1996), barley (Tingay et al. 1997) and also wheat (Chen et al. 1996) showed efficient transformation mediated by *Agrobacterium*, with stable integration, expression and inheritance of the transgenes (Chen et al. 1997).

Two methods, involving osmotic (polyethylene glycol treatment) or electric (electroporation) shock, have been used for transformation and have resulted in transient as well as stable expression of the introduced gene (review: Lörz et al. 1985), e.g. of maize (Fromm et al. 1986). For wheat transformation the biolistic method was used (Vasil et al. 1992, Weeks et al. 1993, Becker et al. 1994, Nehra et al. 1994). This procedure is based on the high-velocity bombardment of plant cells with DNA-coated microprojectiles, accelerated by gunpowder discharge or pressurised helium gas (Sanford et al. 1991, Klein et al. 1992). The main advantage of this method is its ability to deliver DNA into intact regenerable (via the formation of somatic embryos) plant cells, eliminating the need for protoplasts, which thus minimises the potential for tissue culture effects and the resulting abnormalities (Vasil et al. 1993, Vasil 1994).

Optimum expression of genes in the target cell is important for achieving a high frequency of stable transformation. In wheat, considerable efforts have been made in developing suitable gene expression vectors for transformation (Nehra et al. 1995). The inclusion of an intron between the promoter and the coding region proved useful to achieve enhanced transient gene expression in wheat (Chibbar et al. 1991). Furthermore, the isolation of monocot gene promoters, such as the rice actin (Act1) promoter (McElroy et al. 1991) or the maize ubiquitin (Ubi1) promoter (Christensen et al. 1992) sometimes resulted in higher expression frequency. Transgenic wheat has been produced using both promoters (Weeks et al. 1993, Nehra et al. 1994).

To obtain transgenic plants from the few stably transformed cells achieved through these transformation techniques, a suitable selection system is required. Selectable marker genes that confer resistance to antibiotics or herbicides are usually used. Among the various antibiotic resistance marker genes in use, the kanamycin resistance gene has proven ineffective for selection of transformed wheat cells because these cells and the wheat tissue itself both have a high level of endogenous tolerance to kanamycin. Another problem is that using this antibiotic as the selection agent interferes with plant regeneration (Hauptmann et al. 1988, Peng et al. 1992). Geneticin (G 418), however, another member of the aminoglycosides, can be effectively used (Nehra et al. 1994). Hygromycin was used by Hauptmann et al. (1988) with a positive result, but experiments conducted by Nehra et al. (1995) were not successful. As an alternative to antibiotic resistance marker genes, genes conferring resistance to herbicides such as glufosinate ammonium (l-phosphinothricin) can be used (Nehra et al. 1995). Detailed descriptions of the available monocot selection marker systems were presented in the following reviews: Wilmink and Dons 1993, McElroy and Brettell 1994.

In recent years there have been releases of transgenic wheat plants (see Table II-1). For more information about this topic in Europe, see RKI, the SNIF database (**http://www.rki.de**) and the list of "SNIF circulated under article 9 of Directive 90/220/EEC XI/559/94-Rev 6". For the United States, the reviews of James and Krattinger 1996 and de Kathen 1996, and the APHIS ISB environmental release database (**http://www.aphis.usda.gov/bbep/bp**) provide similar information. The OECD BioTrack database includes information on experimental releases to the environment of genetically modified plants and microorganisms (**http://www.olis.oecd.org/biotrack.nsf**).

Future advances in the molecular improvement of wheat, as in that of other plants, will depend upon the limited availability of agronomically important genes more than on any other factor. Attention is being directed to the development of DNA-based maps of wheat for identifying, and then characterising and cloning, genes of importance and interest. Gill et al. (1991), for example, provided a standard karyotype and nomenclature system for describing chromosome bands in bread wheat, while Hohmann et al. (1994) prepared a genetic/physical map of group 7 chromosomes. Devos and Gale (1992) tested the use of random amplified polymorphic DNA (RAPD) markers. They were unsuccessful because of the non-homologous, non-dose responsive and dominant behaviour of RAPD products. Vaccino and

Metakovsky (1995) used RFLP patterns of wheat gliadin alleles as markers, and Devos et al. (1995) used microsatellite sequences. Genetic maps, gene markers and QTL are now becoming available or are being developed. This work started in 1985 at the Plant Breeding Institute and the John Innes Centre in the UK, at universities in the United States, and at the INRA in France (Nelson et al. 1995a, 1995b, Cadalent et al. 1996).

Molecular improvement of wheat for multigenic traits, such as yield, will be a difficult and lengthy process (Vasil 1994). However, the conservation of gene order along chromosomes, as well as the similarity of gene composition and map collinearity in cereals, should be a great advantage in regard to the identification and cloning of important genes (Bennetzen and Freeling 1993, Kurata et al. 1994).

Table II-1 Deliberate releases of transgenic wheat

Country	First release	Main trait
UK	1994	marker
UK	1994	herbicide resistance (glufosinate)
UK	1995	herbicide resistance (glufosinate)
UK	1995	improved starch quality
UK	1996	pest resistance (tolerance to leaf fungal disease)
Spain	1996	herbicide resistance (glufosinate), improved starch quality
UK	1997	alteration in baking quality
Belgium	1997	male sterility/restorer
Argentina	1993	improved quality, male sterility, marker
Argentina	1995	herbicide resistance
Chile	1995	herbicide resistance
USA	1994	herbicide resistance
USA	1994	herbicide resistance (glufosinate)
USA	1994	herbicide resistance (glyphosate)
USA	1995	fungal resistance
USA	1995	herbicide resistance
USA	1995	virus resistance
USA	1995	improved quality
USA	1996	fungal resistance
USA	1996	improved quality
USA	1996	fungal resistance
USA	1996	fungal resistance (glyphosate)
USA	1996	improved quality
USA	1996	herbicide resistance
USA	1996	virus resistance (glyphosate)
USA	1996	herbicide resistance
USA	1996	fungal resistance (glyphosate)
USA	1996	fungal resistance

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